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Research Article

Study of Genetic Diversity of Aromatic Rice Genotypes Using Random Amplified Polymorphic DNA Markers

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ABSTRACT

The study of genetic variation in aromatic rice is necessary for enhancing and preserving its genetic makeup. In this study, we amplified four Random Amplified Polymorphic DNA (RAPD) markers from the genomes of 11 different aromatic rice genotypes in order to evaluate the genetic variation and relatedness in aromatic rice. The genotypes were collected from different sources- five of which were obtained from the Bangladesh Rice Research Institute, one from the Bangladesh Institute of Nuclear Agriculture, and the remaining five were local genotypes. In RAPD profiling, twenty-seven of the thirty-six bands that were generated by the four primers were polymorphic, resulting in an estimated average polymorphism of 47.22 percent. The average value of the polymorphic information content was 0.309 with a range from 0.165 to 0.456, indicating that the primers were effective to detect genetic diversity between the genotypes. The genetic diversity and Shannon information index across all of the genotypes and primers were 0.176 and 0.260, respectively, which indicated that there was a certain amount of genetic variation in the studied aromatic rice genotypes. Kalizira and Binadhan-9 genotypes had the smallest genetic distance (0.057), while it was the highest (0.406) between the BRRI dhan 34 and BRRI dhan 50. The genotype pairs BRRI dhan 38 vs BRRI dhan 34, BRRI dhan 38 and Kalizira, and Zirakatari and Kalizira had also a higher level (0.365) of genetic distances. The genotypes were grouped into two main clusters in the UPGMA dendrogram. Cluster I included five genotypes: BRRI dhan 34, Basmoti, Atashail, Kalizira, and Binadhan-9. Cluster II included remaining six genotypes: BR5, BRRI dhan 38, BRRI dhan 50, Uknimodhu, BRRI dhan 37, and Zirakatari. Documentation of the genetic variation and relatedness among the aromatic rice genotypes under this investigation could be useful for future research into the development of aromatic rice and related fields.

Introduction

Rice (*Oryzae sativa* L.) belongs to the family *Graminae*. For about half of the global population, it serves as the principal food source. Countries such as China, Indonesia, Pakistan, India, Vietnam, Thailand, Myanmar, Bangladesh, Philippines, and Japan are among the most important rice-producing nations, accounting for 92% of the total global production. In Bangladesh, it is the most important food crop, accounting for one-half of the country's agricultural gross domestic products and one-sixth of the country's total income. Bangladesh came in third place in terms of rice production worldwide (FAO, 2022). There are around 11.70 million hectares of land area dedicated to the cultivation of rice, of which approximately 27 percent is devoted to the cultivation of fine rice types (BBS, 2022). On a daily basis, there is a growing need for fine rice due to the fact that it possesses excellent qualities in terms of its nutrients, palatability, flavor, cooking quality, and fragrance (Kaul et al., 1982). Aromatic rice has a distinct flavor and is hence one of the most popular types of fine rice. Aromatic rice cultivation is attracting the attention of farmers due to its high price and export potential (Dutta et al., 2002). This type of rice accounts for about fifteen to eighteen percent of all rice traded internationally (Giraud, 2013).

Numerous varieties of aromatic rice are grown in many countries and are different from one another. It is important to note that the yield and quality of this rice

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are dependent on the genetic composition of the cultivars, the environment in which they are grown, and the management strategies that are utilized in the cultivation process. Therefore, the selection of the appropriate genotype(s) is of the utmost importance in order to increase its output. The process of selecting a rice genotype for use, future research and development requires a systematic approach to gathering, storing, categorizing and characterizing various rice genetic resources (Patwardhan et al., 2014). Choosing the correct type parent plants is a challenge when trying to get better variations. The use of molecular analysis to identify suitable types of aromatic rice parents outperformed the use of traditional methods based on morphological and physiological traits (Jonah et al., 2011). From the perspective of phylogenetics and the genetic distance between individuals, molecular markers are able to convey information about the similarities and differences between individuals (Thomas et al., 2006). They offer an opportunity to provide data on species-level variation both within and between countries, from a specific geographic area. For efficient gathering and utilization of genetic resources, they are an invaluable resource. When it comes to genetic diagnostics, population genetics, taxonomy, and genetic mapping, RAPD is seen as a neutral and unbiased marker that does not need prior knowledge of DNA sequencing among DNA markers (Michelmore et al., 1991; Chapco et al., 1992; Hadrys et al., 1992; Haig et al., 1994). It is extensively utilized to explore genetic variation and relatedness of many organisms (Yasmin et al., 2006; Rahman et al., 2007; Easmin et al., 2008; Alam et al., 2010; Khan et al., 2015; Khan et al., 2016; Saclain et al., 2016; Bala et al., 2017; Rifat et al., 2019; Tamanna et al., 2023). To properly identify and preserve natural resources, it is crucial to use RAPD markers to trace the genetic material of aromatic rice germplasm. Here, we investigated the genetic diversity of some prominent aromatic rice genotypes in Bangladesh using PCR-RAPD.

Materials and Methods

Collection of samples

Eleven rice genotypes viz. Atashail, Basmoti, Binadhan-9, Kalizira, Uknimodhu, BR5, BRRI dhan 34, BRRI dhan 37, BRRI dhan 38, BRRI dhan 50 and Zira katarti were used in the study. Seeds were collected from the Bangladesh Rice Research Institute (BRRI), Bangladesh Institute of Nuclear Agriculture (BINA), and different districts of Bangladesh (Table 1). After the seeds were soaked and allowed to germinate, they were then sown in the pot. For the purpose of conducting RAPD analysis, genomic DNA was derived from fresh and young leaf samples obtained from 11 different types of rice seedlings aged 18 days.

Table 1. Name of aromatic rice genotypes and their sources of collection

SL. No.	Rice genotypes	Sources of collection
1	Atashail	Local farmer, Chockpara, Kalihati, Tangail
2	Basmoti	Local Farmer, Chockpara, Kalihati, Tangail
3	BINAdhan-9	BINA, Mymensingh
4	Kalizira	Local farmer, Hamidpur, Sakhipur, Tangail
5	Uknimodhu	BINA, Mymensingh
6	BR 5	BRRI, Gajipur
7	BRRI dhan 34	BRRI, Gajipur
8	BRRI dhan 37	BRRI, Gajipur
9	BRRI dhan 38	BRRI, Gajipur
10	BRRI dhan 50	BRRI, Gajipur
11	Zira katarti	Local Farmer, Khairkati, Chirirbandar, Dinajpur

Genomic DNA isolation, confirmation and quantification

For the purpose of isolating genomic DNA, fresh leaf tissues that were both immature and actively growing were taken. Isolation of whole genomic DNA was performed on rice leaves following Phenol: Chloroform: Isoamyl alcohol purification and ethanol precipitation method (Rahman et al., 2007). Electrophoresis on a 0.8% agarose gel verified the DNA's purity and the nanodrop one UV-vis spectrophotometer from Thermo ScientificTM was used to quantify it.

Primer test and PCR amplification

Four primers (Table 2) were used and tested for optimizing annealing temperature on three sub-samples of DNA. PCR was performed in 10µl reaction volume containing 50 ng of template DNA, 1µM of primer each, 1µl of dNTPs, 1 unit *Taq* polymerase (TAKARA, Japan), 1µl $MgCl_2$ and the required amount of sterile deionized water. The program in the thermal cycler (Biometra, Germany) was set at 94ºC for 3 min initial denaturation, then 35 cycles consisting of 1 min denaturation at 94ºC, 1 min for annealing at 36 ºC and 2 min for extension at 72ºC. To conclude the program, a final extension of amplicons was carried out at a 72 ºC for 7 min.

Table 2. RAPD primers with corresponding polymorphic bands and overall estimation of genetic variation in eleven aromatic rice genotypes

Primer code	Sequence (5'-3')	TB	P	PP	PIC	H	I
S1027	ACGAGCATGG	11	5	45.50	0.287	0.165	0.246
OPAB02	GGAAACCCCT	8	5	62.50	0.456	0.202	0.308
S1155	GAAGGCTCCC	11	6	54.55	0.328	0.219	0.319
OPA02	TGCCGAGCTG	6	1	16.67	0.165	0.083	0.115
Overall	-	36	17	47.22	0.309	0.176	0.260

TB, Total bands; P, Polymorphic bands; PP, % polymorphic bands; PIC, Polymorphic information content; H, Nei's (1978) gene diversity; I, Shannon Information Index

Gel electrophoresis of amplified products

An electrophoretic separation was performed on a 1.4% agarose gel to obtain the amplifiable end products. Agarose gel electrophoresis was conducted in 1X TBE buffer at 120 V for 1.5 hr. A 100 bp and a 1 kb DNA ladder, both manufactured by BIONEER Corporation, were run in parallel in the gel. After electrophoresis, gel was gently removed from the electrophoresis chamber and placed in a pre-made ethidium bromide (10 mg/ml) staining solution.

Documentation of the DNA samples

After the staining process was complete, the gel was cautiously removed from the tray and set on the gel doc's high-performance ultraviolet light box (UVP BioDoc-It™ imaging equipment) to check out the DNA bands and capture pictures of the gel.

RAPD data analysis

Since RAPD markers are dominant, we went with the assumption that every band was a representation of the phenotype at a single allelic locus (Elo et al., 1997). In order to assess the size of the amplification products, molecular weight markers were utilized. This was accomplished by comparing the distance travelled by each fragment with the distance travelled by molecular weight markers of known sizes. In this way, all of the different bands or fragments, which are RAPD markers, were assigned identification numbers based on their position on the gel. These numbers were then visually assessed based on whether or not they were present (1) or absent (0), and this was done separately for each individual and each primer. Following the completion of the RAPD analysis, the scores that were acquired by employing all of the primers were combined to form a single data matrix. Polymorphic loci, Nei's (1978) gene diversity, genetic distance, and Shannon information index were estimated from the single data matrix using the computer program POPGENE (Version 3.5) (Yeh et al., 1999). The NTSYS-PC (numerical taxonomy system) version 2.11 (Rohlf, 2000) was used to construct unweighted pair group method of arithmetic means dendrogram. Estimation of polymorphic information content was made following Botstein et al. (1980). To determine how similar two genotype's RAPD profiles were on the same gel, we used the following formula to get the similarity indices (S) between their RAPD markers.

$$S = 2 Nxy / Nx + Ny,$$

Where, Nxy is the sum of all RAPD bands that both x and y individuals have, while Nx and Ny are the total number of bands in x and y genotypes, respectively (Lynch, 1990). The average degree of similarity between any two genotypes, denoted as Sij, was determined for each set of paired genotypes i and j (Lynch, 1991).

Results

To identify genetic variation in studied eleven aromatic rice genotypes, four RAPD primers (Table 2) were employed. These primers produced a variety of banding patterns (Figure 1).

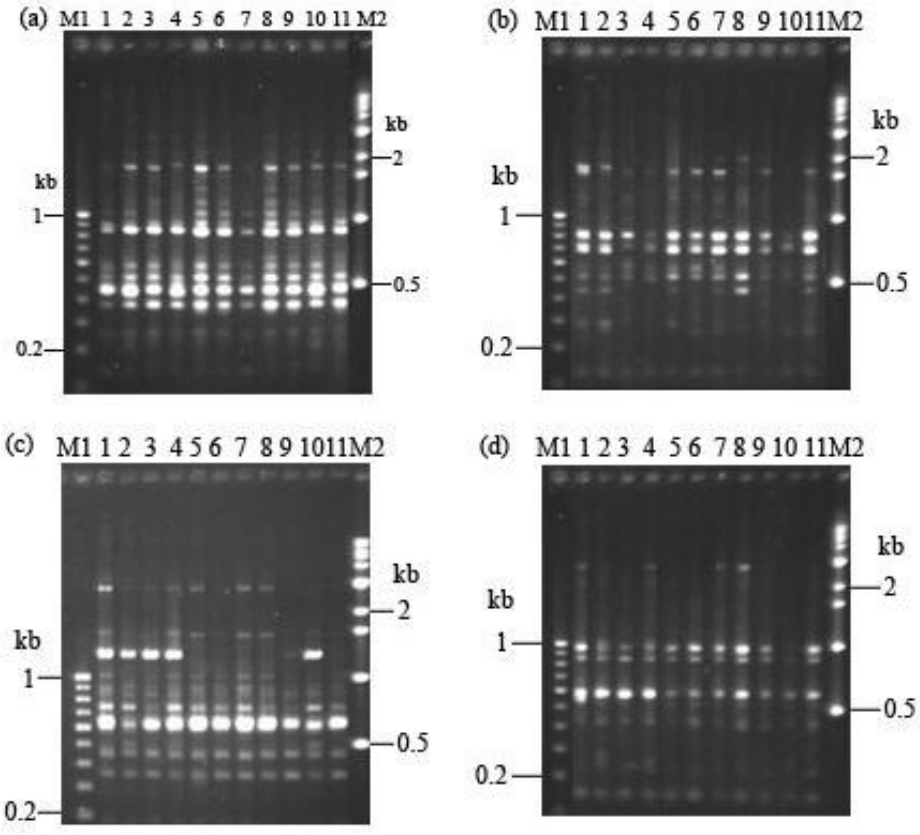

Figure 1. RAPD profiles of 11 aromatic rice genotypes using primer S1027 (a), OPAB02 (b), S1155(c) and OPA02 (d). Lane 1: Atashail, Lane 2: Basmati, Lane 3: BINAdhan-9, Lane 4: Kalizira, Lane 5: Uknimodhu, Lane 6: BR 5, Lane 7: BRRI dhan 34, Lane 8: BRRI dhan 37, Lane 9: BRRI dhan 38, Lane 10: BRRI dhan 50 and Lane 11: Zira Katari. M1: 100bp DNA ladder and M2: 1 kb DNA ladder.

Across 11 aromatic rice genotypes, the four primers produced a total of 36 bands. Of those 36 bands, 17 were determined to be polymorphic, and the overall polymorphism was found to be 47.22% (Table 2). The primers S1027 and S1155 produced the highest number of bands (11), while primer S1155 produced the highest number of polymorphic bands (6), demonstrating a polymorphism rate of 54.55% (Table 2). The 8 bands produced by primer OPA02 had an estimated polymorphism of 62.50%, with 5 of those bands exhibiting polymorphism. The lowest number of polymorphic bands (1) was found in primer OPA02 and it was 16.67 %. Table 2 also summarizes the results of the analyses performed on the polymorphic information content (PIC), Nei's gene diversity (H), and Shannon Information Index (I). All of the loci had PIC values that ranged from 0.165 to 0.456, with 0.309 being the average value. Both the overall Shannon Information Index and the Nei's (1978) gene diversity were determined to be 0.176 and 0.260, respectively, across all of the genotypes and RAPD markers.

The estimated values for band sharing based inter-genotype similarity indices and Nei's (1978) genetic distances are given in Table 3. The highest Nei's (1978) genetic distance (0.406) was found between BRRI dhan 34 and BRRI dhan 50 whereas the lowest genetic distance (0.057) was found between Kalizira and Binadhan-9. In addition, high level of band sharing based inter-genotype similarity indices (0.960) was estimated between Kalizira and Binadhan-9 and the lowest (0.778) between BRRI dhan 34 and BRRI dhan 50.

Table 3. Summary of band sharing based inter-cultivar similarity indices (above diagonal) and Nei's (1978) genetic distance (below diagonal) values between 11 aromatic rice genotypes

Genotypes	Atashail	Basmoti	Binadhan-9	Kalizira	Uknimodhu	BR5	BRRI dhan 34	BRRI dhan 37	BRRI dhan 38	BRRI dhan 50	Zirakatari
Atashail	---	0.938	0.864	0.903	0.883	0.850	0.913	0.906	0.814	0.825	0.869
Basmoti	0.118	---	0.871	0.908	0.860	0.850	0.886	0.882	0.850	0.887	0.875
Binadhan-9	0.216	0.216	---	0.960	0.913	0.930	0.894	0.845	0.783	0.857	0.856
Kalizira	0.150	0.150	0.057	---	0.874	0.886	0.898	0.851	0.812	0.846	0.811
Uknimodhu	0.216	0.288	0.182	0.251	---	0.899	0.864	0.936	0.867	0.849	0.946
BR5	0.251	0.251	0.149	o.216	0.150	---	0.873	0.853	0.918	0.862	0.925
BRRI dhan 34	0.150	0.216	0.251	0.182	0.182	0.216	---	0.925	0.794	0.778	0.849
BRRI dhan 37	0.182	0.251	0.288	0.288	0.087	0.251	0.150	---	0.844	0.827	0.923
BRRI dhan 38	0.325	0.251	0.288	0.365	0.216	0.118	0.365	0.251	---	0.893	0.919
BRRI dhan 50	0.288	0.150	0.182	0.251	0.251	0.216	0.406	0.288	0.150	---	0.861
Zirakatari	0.251	0.251	0.288	0.365	0.087	0.118	0.288	0.118	0.118	0.216	---

Nei's (1978) genetic distance was used to create a dendrogram that allowed 11 different aromatic rice genotypes to be separated into two primary clusters (Figure 2). Atashail, Kalizira, Basmoti, Binadhan-9 and BRRI dhan 34 were found in cluster I and Cluster II consisted of BR5, BRRI dhan 37, BRRI dhan 38, BRRI dhan 50 and Uknimodhu genotypes.

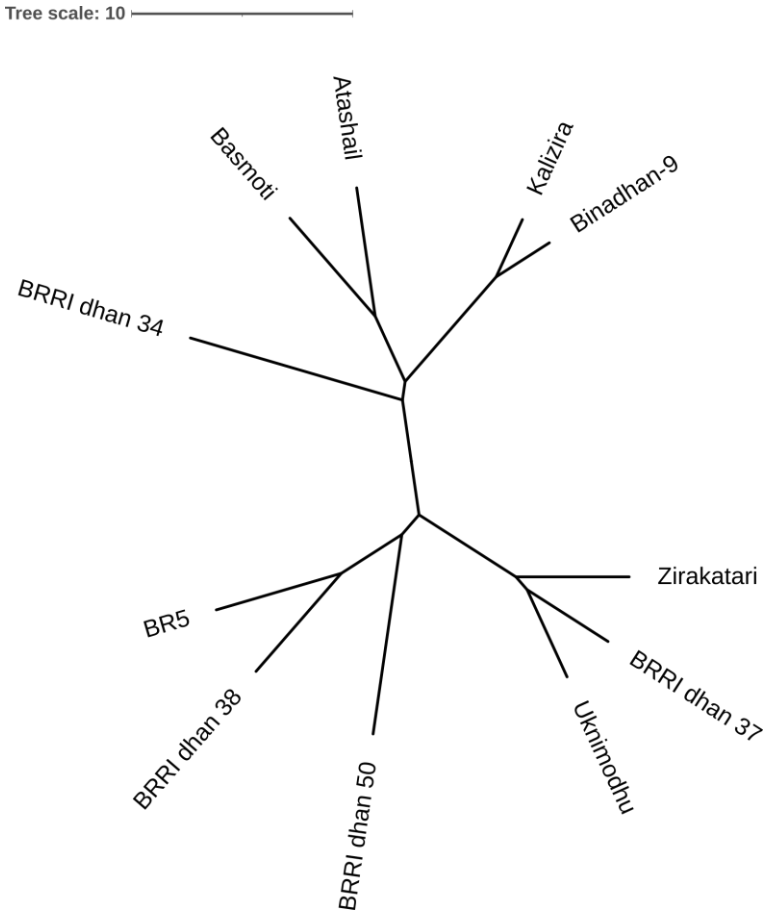

Figure 2. A UPGMA dendrogram based on Nei's (1978) genetic distance summarizing the data on differentiation among eleven aromatic rice genotypes according to RAPD analysis

Discussion

Despite the fact that rice is the most significant commercial crop in Bangladesh, there are only a few genotypes of aromatic rice that are available in the nation. Furthermore, there is a lack of knowledge regarding the utilization of molecular markers in the investigation of the genetic diversity of these rice varieties. To investigate the genetic diversity of 11 different aromatic rice genotypes, this study utilized 17 polymorphic bands that were amplified using four 10-mer RAPD primers. A modest level of genetic variety

was found in the genotypes that were revealed, according to the findings of the study, which took into account the number of total bands (36), polymorphic bands (17), polymorphism (47.22%), Shannon Information Index (0.260) and Nei's gene diversity (0.176). A RAPD analysis was performed on six local aromatic rice cultivars in Bangladesh by Rahman et al., (2007) and reported that the polymorphism was 86.36%. Similarly, various levels of polymorphism were reported; for instance, 82.5% in 35 rice cultivars by Tehrim et al., (2012), 78.79% in 30 aromatic rice cultivars by Hasan and Raihan, (2015), 92% in 45 aromatic rice by Singh et al. (2013), 35% in hybrid parental lines by Kiani and Katalani, (2018), 67.5% polymorphism among the tested varieties by Choudhury et al., (2001), and 80% among 9 upland and 4 lowland indica and japonica rice cultivars by Yu and Nguyen, (1994). In present study, the average polymorphic information content was 0.309 that varied between 0.165 to 0.456 (Table 2). In a study on genetic diversity in 21 aromatic rice varieties from Indonesia, Zakiyah et al., (2019) employed 38 decamer random primers to determine the PIC value of 0.263. However, the present investigation found greater average PIC value. Among the four primers tested, OPAB02 had the highest PIC value (0.456), suggesting that it could be the most useful marker for aromatic rice genotype identification and diversity assessment followed by S1027, S1155, and OPA02 primers. It is commonly understood that dominant markers, like the RAPD marker, produce heterozygosity and a PIC value between 0 and 0.5. This is because these markers can only identify two alleles per locus, and the frequency and amount of alleles affect both values (Zakiyah et al., 2019). In our study, Nei's (1978) gene diversity or anticipated heterozygosity was 0.219 and the maximum PIC value was 0.456, therefore none of these values was too low. The results of this study showed that aromatic rice genotypes could efficiently be differentiated using the RAPD primers utilized, with a polymorphism rate of 47.22%, an average PIC value of 0.309, and an average gene diversity of 0.176. However differences in the findings in different studies are likely due to the different aromatic rice genotypes, different loci amplified and different experimental set up used.

An inverse relationship exists between the genetic distance and the band-sharing based similarity index, two crucial parameters used to distinguish between genotypes; that is, a higher genetic distance is associated with a lower band-sharing based similarity index, and the reverse is also true. This study's genotypes were quite comparable, with genetic distance values ranging from 0.057 to 0.406 and band-sharing based similarity indices from 0.778 to 0.960. Rice genotypes were found to have genetic distance values ranging from 0.070 to 0.370, as shown by Rahman et al., (2007). According to the findings of our study, the genetic distance between BRRI dhan 34 and BRRI dhan 50 was the highest (0.406), and the band sharing based inter-genotype similarity indices were the lowest (0.778). On the other hand, the genetic distance between Kalizira and Binadhan-9 was the lowest (0.057), and the band sharing based inter-genotype similarity indices were the highest (0.960). In Bangladesh, aromatic rice genotype BRRI dhan 50 is based on the *boro* season, which runs from December to May, while BRRI dhan 34 is based on the *aman* season, which runs from July to December. Although the yield of BRRI dhan 34 is lower than that of BRRI dhan 50, the plant height of BRRI dhan 34 is greater. The other variant, known as Binadhan-9, was developed through the process of hybridization between the native Kalizira and an unusual mutant strain known as Y-1281 (Azad et al., 2016). The seeds of both genotypes are black in color. Therefore, based on morphological traits and RAPD-based genetic distance values, it was determined that BRRI dhan 34 and BRRI dhan 50 were rice genotypes that were distantly linked to one another, but Kalizira and Binadhan-9 were closely connected to one another. In the dendrogram that was constructed using the genetic distances, all of the aromatic rice genotypes were separated into two primary clusters. Cluster I consisted of five genotypes, which were BRRI dhan 34, Basmoti, Atashail, Kalizira, and Binadhan-9. Cluster II consisted of BR5, BRRI dhan 38, BRRI dhan 50, Uknimodhu, BRRI dhan 37, and Zirakatari genotypes. When compared to the genetic distances that exist between genotypes of different clusters, the genetic distances that are shared by genotypes within a cluster are significantly shorter. The outcomes of this study have a significant influence on the process of developing new types of rice. This study will help plant breeders to select parent genotypes in case of breeding purposes.

Conclusion

Aroma is one of the most significant quality features. In order to maintain the stability of aromatic rice genotypes and to develop better varieties with new genetic combinations, it is crucial to determine the genetic variation of rice. A few polymorphic DNA bands that may be useful for genotype identification and differentiation were revealed in the current study. It may be possible to preserve and enhance their potential by estimating the genetic diversity and relatedness among the eleven aromatic rice varieties. In addition, plant breeders may find this study's results helpful when deciding which crops to cultivate and how to choose them. The study's drawbacks include its small sample size and small number of primers used. More

primers, genotypes, and informative co-dominant SSR markers are required to obtain more accurate information about the genetic diversity of aromatic rice.

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