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EVALUATION OF AROMA IN MALAYSIAN RICE LANDRACES THROUGH SENSORY TEST AND MOLECULAR APPROACH

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ABSTRACT

Aroma is one of the primary rice grain quality traits that play a vital role in consumer preferences. In Malaysia, consumers have shown a marked preference for high-quality rice. Therefore, it is essential to identify rice landraces with considerable aroma for promotion as potential donors for the development of high-quality rice in future breeding programs to meet consumer demand and reduce dependency on imported high-quality rice types. Thirty-three rice genotypes comprising thirty Malaysian rice landraces and three modern rice cultivars were evaluated for aroma using sensory tests and molecular markers. The presence of aroma in cooked rice was determined through nose sensory evaluation by five panellists. Molecular analysis was performed using two aroma-specific SSR primers i.e., FMbadh2-E7 and Badex7-5. The sensory test revealed that only five rice landraces had a strong aroma; whereas eleven had a slight aroma. FMbadh2-E7 was found to have most of the alleles with a major allele frequency of 0.53. Whereas, Badex7-5, has a major allele frequency of 0.51, with five alleles at the locus. The dendrogram was generated by UPGMA cluster analysis using two aroma-specific primers, which characterized the rice genotypes into two distinct clusters; Cluster 1 consisted of fifteen rice landraces with non-aroma standards, while Cluster 2 consisted of fifteen rice landraces with aroma standards. Based on the sensory test and molecular approach, the identified rice landraces with aroma were i.e., Kenawit, Gertok, Pandan, Nangka, Tiga Bulan, Lumpur, Grik, Sanguo Pandan, Kurau, Bidor, and Wangi, which can be further promoted as potential donors through breeding.

Keywords: Rice landraces (*Oryza sativa* L.), aromatic rice, sensory test, 2-acetyl-1-pyrroline, molecular approach, SSR primers, cluster analysis

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INTRODUCTION

Rice is the most important food crop of the genus *Oryza* and has the second position after wheat worldwide (Kellogg, 2009). Based on 169 nuclear simple sequence repeats (SSRs), rice has been classified into five distinct groups known as *indica*, aus, aromatic, *temperate japonica*, and *tropical japonica* (Glaszmann, 1987). Aromatic or fragrant rice is a specialty type of rice that is sold at a premium price due to consumers' demand for its unique texture and pleasant scent (Bharti and Anand, 2020; Kaewmungkun *et al*., 2023). The two most popular aromatic rice cultivars on the global market are Basmati rice from India and Pakistan and Jasmine rice from Thailand (Sakthivel *et al*., 2009).

Aroma is one of the primary grain quality traits that play a considerable role in consumer preferences; the others are grain physical appearance, cooking and eating quality, grain physical appearance, and nutritional value (Fitzgerald *et al*., 2009; Nadaf *et al*., 2016). 2-acetyl-1-pyrroline (2-AP), a volatile compound, was discovered to be a major contributor to the pleasant scent of aromatic rice, primarily Basmati and Jasmine rice (Buttery *et al*., 1983). The accumulation of 2-AP was controlled by the gene osBadh2 (also known as fgr / badh2 / os2AP / osbadh2 / LOC_Os08g0424500) on chromosome 8, which had undergone multiple mutations and degradation that resulted in the emission of aroma in rice (Niu *et al*., 2008). Nonetheless, the genetic factor is not the only factor that contributes to rice's aroma; cultivation practices and environmental factors also play a role. Temperature, relative humidity, moisture content, pH, day length, sunlight intensity, and soil are environmental factors that influence the aroma of rice (Bharti and Anand, 2020; Kaewmungkun *et al*., 2023). Moreover, cultivation under stress conditions, such as in drought-

prone regions or areas with arid, and sandy soil, may increase the 2-AP concentration in rice (Prodhan and Shu, 2020).

Majority of the Malaysian people rely on rice as their primary source of nutrition and calorie intake. Recently, consumers have demonstrated a marked preference for nutritious and higher-quality rice types (Abubakar *et al*., 2015). Consumer purchasing decisions for aromatic rice such as Basmati rice, have shifted a greater emphasis on the cooked rice's quality and texture rather than its price (Abdullahi Farah *et al*., 2011). Consumer demand for imported cultivars of high-quality rice in Malaysia is estimated to be 30% annually (Jamal *et al*., 2014) or approximately 192,000 tons of total imported specialty rice. As a result of rising consumer demand, the Malaysian market is currently deluged with jasmine, basmati, glutinous rice, parboiled rice, and brown rice imported from Vietnam, Thailand, India, Pakistan, and the United States (Abubakar *et al*., 2015).

In order to solve the issue, an initiative has been made to reduce dependence on imported rice and meet consumer demand by introducing new rice cultivars that have the same characteristics as imported high-quality rice (Jamal *et al*., 2014). This initiative is implemented by the Malaysian Agricultural Research and Development Institute (MARDI), a statutory entity that has been conducting extensive research and development of rice cultivars for decades. Until now, MARDI has released 52 modern rice cultivars, including those with high quality and specialty traits, such as MRQ50, MRQ74, MRQ76, MARDI Wangi 88, and MRQ104 for white aromatic rice; and MRM16 and MARDI WARNA 98 for colored rice (Ab Razak *et al*., 2020; Nur Suraya *et al*., 2020). Khao Dawk Mali (Thailand), Kasturi (India), and Cuicak Wangi (local landrace) are examples of rice genetic resources conserved in the National Rice Genebank that have been used to develop modern rice cultivars with high quality and specialty traits in Malaysia (Ab Razak *et al*., 2020). Rice genetic resources are essential for breeding programs because they serve as sources of essential traits; however, a lack of information on potential traits among rice genetic resources has caused the most significant limitation in plant breeding programs.

Nowadays, the reliability and accuracy of the information used to measure the genetic diversity of rice have become increasingly important to assist breeders in selecting potential donors for breeding purposes. In this regard, the use of simple sequence repeats (SSRs) is preferred due to their high polymorphism, co-dominant inheritance, and ability to accurately determine the heterozygosity (Gour *et al*., 2017). Thus, the present study aimed to evaluate the aroma of thirty-three rice genotypes consists thirty Malaysian rice landraces and three modern rice cultivars through sensory tests and molecular analysis using aroma-specific SSR primers. The results are indispensable for comprehending the extent of genetic diversity among Malaysian rice landraces based on aroma, and the identified aromatic rice landraces can be promoted as potential donors for the development of high-quality or specialty rice.

MATERIALS AND METHODS

Materials used and sample preparation: A total of thirty-three rice genotypes were used in this study, comprising thirty Malaysian rice landraces and three modern rice cultivars. Malaysian rice landraces were chosen from the National Rice Genebank database and represent each of Malaysia's major provinces, namely Peninsular Malaysia, Sabah, and Sarawak (Table 1). The modern rice cultivars i.e., Malinja (MRGB00839), MR 219 (MRGB11633), and Sempadan 303 (MRGB13001) were used as controls in this study. The harvested grains were dehusked with a THU35B Testing Husker (Satake, Japan) and polished with a Testing Mill (Satake, Japan). The TRG05B Testing Rice Grader was then used to separate the broken rice from the unbroken milled rice.

Aroma evaluation by sensory test: The presence of aroma in rice was determined using a method proposed by Lestari *et al*., (2011) with minor modifications. Modifications were made to the method's rice quantity and boiling time. A 5.0 ml screw cap tube was filled with 3 g of newly harvested rice grain. The tube was filled with 3.0 ml of distilled water, and sealed with aluminium foil. The samples were immersed in a bath of boiling water for 30 minutes. After allowing the cooked samples to cool, the presence of aroma was determined by nose sensory evaluation for each sample. Five panellists rated each sample individually; the scores were stated as follows: no aroma (score 0), slight aroma (score 1), medium aroma (score 2), and strong aroma (score 3). The score of each sample was averaged and classified into three categories, namely aromatic (score >1.0), slightly aromatic (score 0.6 to 1.0), and not aromatic (score <0.5). Three rice cultivars, namely MRQ 74 (with a strong aroma), MRQ 76 (with a slight aroma), and MR 219 (with no aroma) were used as standards.

Molecular analysis for aroma

Genomic DNA extraction: Two leaf samples from each rice landrace including the control cultivars and the standards, were collected, dried with silica gel, and then frozen at -20° C. A tiny piece of leaf samples was placed on a plate with a 5 mm stainless steel bead and immediately frozen at -80° C for at least 24 hours for DNA extraction. The DNA was extracted following the protocol by Mace *et al*., (2003) with few modifications; the modification involved using semi-robotic equipment for high-throughput DNA extraction. The frozen tissue was ground using a Tissue Lyser (Qiagen, Germany), and

the extraction buffer was added. The integrity and concentration of the DNA were determined using 0.8% agarose gels and Fluoroskan Ascent (Thermo Fisher Scientific, United States).

PCR amplification, gel electrophoresis, and scoring: The PCR reaction was carried out following the procedure developed by Schuelke (2000). The procedure was performed using two aroma-specific SSR primers namely FMbadh2-E7 and Badex7-5, and the primers were ligated with a non-fluorescent M13 sequence tail (TGT AAA ACG GCC AGT). The final volume of the PCR reaction was 10 µL, and it contained 5.4 µL of dH_2O, 0.5 µL of $MgCl_2$, 1.0 µL of 1x buffer, 1.0 µL of dNTP, 0.1 µL of bovine serum albumin (BSA), 0.4 µL of diluted forward and reverse primers, 0.5 µL of fluorescence-labelled M13 primer, 0.1 µL of Taq polymerase, and 1.0 µL of DNA of rice landraces. GeneAmp® PCR System 9700 (Applied Biosystems, United States) was utilized to amplify the target sequence. Initial denaturation at 94° C for 5 minutes was followed by 34 cycles of 94° C for 30 seconds, 41 to 65° C for 45 seconds based on the annealing temperature of each primer, and 72° C for 45 seconds, with a final extension at 72° C for 10 minutes. After amplification, the PCR products were mixed with 80 µL of HiDi and 20 µL of GeneScan™ 500 LIZ™ as a standard ladder. Then, the PCR products were then differentiated using ABI 3730xl DNA Analyzer.

Statistical analysis: The experiment was laid out in a completely randomized design (CRD), with three replications. The SAS software version 9.4 (SAS Institute Inc, Cary, NC, USA) was used to analyze variance (ANOVA) of aroma score using one-way ANOVA at 1% (p<0.01) and 5% (p<0.05) levels of significance. The least significant difference (LSD) test was used to compare and separate the rice landraces using a significance level of 5% (p<0.05). The allele size was determined using GeneMapper™ software version 5. The major allele frequency, number of genotypes, number of alleles, availability, gene diversity, heterozygosity, and polymorphism information content (PIC) were estimated using the Power Marker. The Power Marker was also employed for the cluster analysis, which performed the UPGMA (unweighted pair group method with arithmetic mean) based on the distance matrix and also the pairwise genetic distance of each genotype.

RESULTS

Sensory test for aroma determination: Thirty-three rice genotypes comprising thirty Malaysian rice landraces and three modern cultivars were evaluated for aroma using sensory test with cooked rice. The analysis of variance revealed that rice genotypes owned significant (p<0.01) differences of aroma (Table 2). Five rice landraces, including the standard cultivar MRQ 74, were found to have a strong aroma; eleven rice landraces including the aroma standard, MRQ 76 had a slight aroma; and the remaining genotypes had no aroma (Table 3). Among the identified aromatic rice genotypes, Kenawit received the maximum score of 1.80 and demonstrated a significant difference based on the mean comparison. Conversely, the remaining rice genotypes exhibited similarity based on mean comparisons with the mean values of 1.53 in Gertok, 1.47 in Nangka, 1.33 in Tiga Bulan, and 1.20 in Pandan. In contrast, Bidor received the highest score of 1.00 among the slightly aromatic rice landraces, followed by Kurau with a score of 0.93 and Grik with the lowest score of 0.60. The sensory test discovered a wide range of panellist ratings; however, all panellists rated Kenawit and Tiga Bulan similarly for aroma presence. It was also found that one of the panellists reported a score of no aroma in the slightly aromatic rice landraces: Silou, Bidor, Wangi, Lumpur; and in aromatic rice landraces: Pandan, Nangka, Gertok.

Aroma detection using simple sequence repeats (SSRs): In this study, a total of 10 alleles were amplified with two SSR primers (Table 4). Primer FMbadh2-E7 was found to have the most alleles (6 alleles) with a major allele frequency of 0.53. Badex7-5 has a major allele frequency of 0.51, with five alleles detected at the locus. The PIC value represents the degree of DNA marker polymorphism. Primers with the highest PIC value provide the most informative DNA band patterns and are hence the most useful molecular markers. According to Dalimunthe *et al*. (2020), the PIC were classified into three categories: highly informative (PIC > 0.5), moderate (0.25 > PIC > 0.5), and low informative (PIC < 0.25). The PIC values of FMbadh2-E7 and Badex7-5 was 0.44 and 0.46, respectively. Thus, these primers are categorized as moderately informative but are still effective in determining the genetic divergence of aroma between rice landraces, control cultivars, and aroma standards. This finding was also proved by Li *et al*. (2020) and Sakthivel *et al*. (2009), who discovered that the primers had a significant tendency to discriminate between fragrant and non-fragrant genotypes.

Genetic diversity of aroma in Malaysian rice landraces: For aroma analysis, the dendrogram was generated by UPGMA cluster analysis using two aroma-specific SSR primers i.e., FMbadh2-E7 and Badex7-5 (Figure 1). The primers effectively separated the aroma standards from the non-aroma standards into two distinct clusters. Cluster 1 consists of fifteen rice landraces with non-aroma standards, while Cluster 2 consists of fifteen rice landraces with aroma standards. In Cluster 2, eleven rice landraces were scored as aromatic based on the sensory test: Tiga Bulan, Gertok, Pandan, Kenawit, and Nangka were characterized as aromatic, while Lumpur, Grik, Sanguo Pandan, Kurau, Bidor, and Wangi were

characterized as slightly aromatic. The pairwise genetic distance, as determined by the shared alleles, is presented in Table 5. The values ranged from 0.00 to 1.00, with 0.00 indicating identical alleles and 1.00 indicating dissimilarity, respectively (Ab Razak *et al*., 2020). The following eight rice landraces exhibited the highest similarities to aroma standards (MRQ 74 and MRQ 76) i.e., Apit, Sanguo Pandan, Pandan, Kenawit, Kurau, Bidor, Wangi, and Nangka. There are several discrepancies between these two analyses: the sensory test scored Pulut Bukit, Silou, Jangrai, Kantan Merah, and Mepawan as slightly aromatic, but they were grouped with non-aroma standards in Cluster 1. Meanwhile, four rice landraces namely Tutumoh, Beruang, Telinga, and Apit, were scored as non-aromatic by the sensory test but were grouped in Cluster 2 along with aroma standards. In fact, based on the individual ratings, one to two panellists rated these rice landraces as having an aroma.

Table 1. Malaysian rice landraces were selected from three major provinces in Malaysia i.e., Peninsular Malaysia (Pahang, Perak, Kelantan), Sarawak, and Sabah.

No.	Accession Number	Variety Name	Origin	No.	Accession Number	Variety Name	Origin
1	MRGB12635	Grik	Pahang	16	MRGB13079	Pandan	Sarawak
2	MRGB12639	Apit	Pahang	17	MRGB13084	Brio Pendek	Sarawak
3	MRGB12640	Lumpur	Pahang	18	MRGB13089	Keramat Hitam	Sarawak
4	MRGB12647	Kantan Merah	Pahang	19	MRGB13080	Kenawit	Sarawak
5	MRGB12482	Jangrai	Perak	20	MRGB13098	Miyah	Sarawak
6	MRGB12483	Nangka	Perak	21	MRGB09855	Bokilong	Sabah
7	MRGB12488	Gertok	Perak	22	MRGB09869	Pulut Bukit	Sabah
8	MRGB12397	Bidor	Kelantan	23	MRGB09872	Kolomintuhon	Sabah
9	MRGB12387	Kurau	Kelantan	24	MRGB09909	Kadim	Sabah
10	MRGB12435	Wangi	Kelantan	25	MRGB09925	Lakatan	Sabah
11	MRGB12938	Muduh	Sarawak	26	MRGB09933	Silou	Sabah
12	MRGB12939	Mepawan	Sarawak	27	MRGB09938	Tutumoh	Sabah
13	MRGB13063	Sanguo Pandan	Sarawak	28	MRGB09951	Beruang	Sabah
14	MRGB13065	Topoi	Sarawak	29	MRGB09955	Tiga Bulan	Sabah
15	MRGB13077	Pulut Belacan	Sarawak	30	MRGB09961	Telinga	Sabah

Table 2. Analysis of variance (ANOVA) of aroma for Malaysian rice landraces, control cultivars, and aroma standards.

Source of Variation	Rice Germplasm	Error
Degrees of freedom	34	70
Aroma	1.24**	0.008

Values represent the Mean Square of three replicates
** Significant at $p < 0.01$; * Significant at $p < 0.05$, ns: not significant

Table 3. The score and mean of the aroma of Malaysian rice landraces, control cultivars, and aroma standards using a sensory test of cooked rice.

Variety Name	Panelist 1	Panelist 2	Panelist 3	Panelist 4	Panelist 5	Mean and Standard Error (±)	Category of Aroma
MRQ76	±	±	±	±	±	1.00 ± 0.00^{f}	slightly aromatic
MRQ74	++	++	++	++	++	3.00 ± 0.00^{a}	Aromatic
Malinja	±	-	-	-	±	0.40 ± 0.00^{lm}	not aromatic
MR219	-	-	-	-	-	0.00 ± 0.00^{p}	not aromatic
Sempadan303	-	-	-	-	-	0.00 ± 0.00^{p}	not aromatic
Bokilong	-	±	±	±	-	0.53 ± 0.07^{kl}	not aromatic
Pulut Bukit	+	+	-	-	±	0.87 ± 0.07^{fgh}	slightly aromatic
Kolomintuhon	-	-	-	-	-	0.00 ± 0.00^{p}	not aromatic
Kadim	-	-	-	-	-	0.00 ± 0.00^{p}	not aromatic
Lakatan	-	-	-	-	±	0.27 ± 0.07^{mno}	not aromatic

Silou	±	±	±	-	±	0.67 ± 0.07^{ijk}	slightly aromatic
Tutumoh	-	-	±	-	-	0.33 ± 0.07^{mn}	not aromatic
Beruang	-	-	-	±	±	0.27 ± 0.07^{mno}	not aromatic
Tiga Bulan	±	±	±	++	±	1.33 ± 0.07^{de}	aromatic
Telinga	-	-	-	±	±	0.33 ± 0.07^{mn}	not aromatic
Kurau	+	-	-	++	-	0.93 ± 0.07^{fg}	slightly aromatic
Bidor	±	-	±	+	±	1.00 ± 0.00^{f}	slightly aromatic
Wangi	±	+	±	±	-	0.87 ± 0.07^{fgh}	slightly aromatic
Jangrai	±	+	-	-	-	0.67 ± 0.07^{ijk}	slightly aromatic
Nangka	±	+	-	++	±	1.47 ± 0.07^{cd}	aromatic
Gertok	+	+	-	+	±	1.53 ± 0.07^{c}	aromatic
Grik	±	±	-	±	-	0.60 ± 0.00^{jk}	slightly aromatic
Apit	-	-	-	±	±	0.40 ± 0.00^{lm}	not aromatic
Lumpur	±	±	±	-	±	0.80 ± 0.12^{ghi}	slightly aromatic
Kantan Merah	+	±	-	-	±	0.73 ± 0.07^{hij}	slightly aromatic
Muduh	-	-	-	-	-	0.00 ± 0.00^{p}	not aromatic
Mepawan	±	±	±	-	-	0.67 ± 0.07^{ijk}	slightly aromatic
Sanguo Pandan	-	-	±	±	+	0.73 ± 0.07^{hij}	slightly aromatic
Topoi	-	-	-	-	±	0.20 ± 0.00^{no}	not aromatic
Pulut Belacan	-	-	-	-	-	0.00 ± 0.00^{p}	not aromatic
Pandan	-	±	±	++	±	1.20 ± 0.00^{e}	aromatic
Kenawit	+	+	±	±	++	1.80 ± 0.00^{b}	aromatic
Brio Pendek	-	-	±	-	-	0.13 ± 0.07^{p}	not aromatic
Keramat Hitam	-	-	-	-	-	0.00 ± 0.00^{p}	not aromatic
Miyah	-	-	-	-	-	0.00 ± 0.00^{p}	not aromatic
Grand Mean						**0.65**	
Maximum						**3.00**	
Minimum						**0**	
CV (%)						**98.47**	
LSD (α = 0.05)						**0.14**	

LSD test significant difference at 5% (p<0.05) level of significance. Mean followed by the same letter is not significantly different. (++): strong aroma, score 3; (+): medium aroma, score 2; (±): slight aroma, score 1; (-): no aroma, score 0

Table 4. Genetic diversity parameters using two SSR primers of aroma.

Marker	Major Allele Frequency	Genotype No.	Sample Size	No. of observation	Allele No.	Availability
FMbadh2-E7	0.53	8	210	203	6	0.97
Badex7-5	0.51	7	210	209	4	1.00
Mean	0.52	7.5	210	206	5	0.98

Marker	Gene Diversity	Heterozygosity	PIC
FMbadh2-E7	0.54	0.03	0.44
Badex7_5	0.55	0.05	0.46
Mean	0.55	0.04	0.45

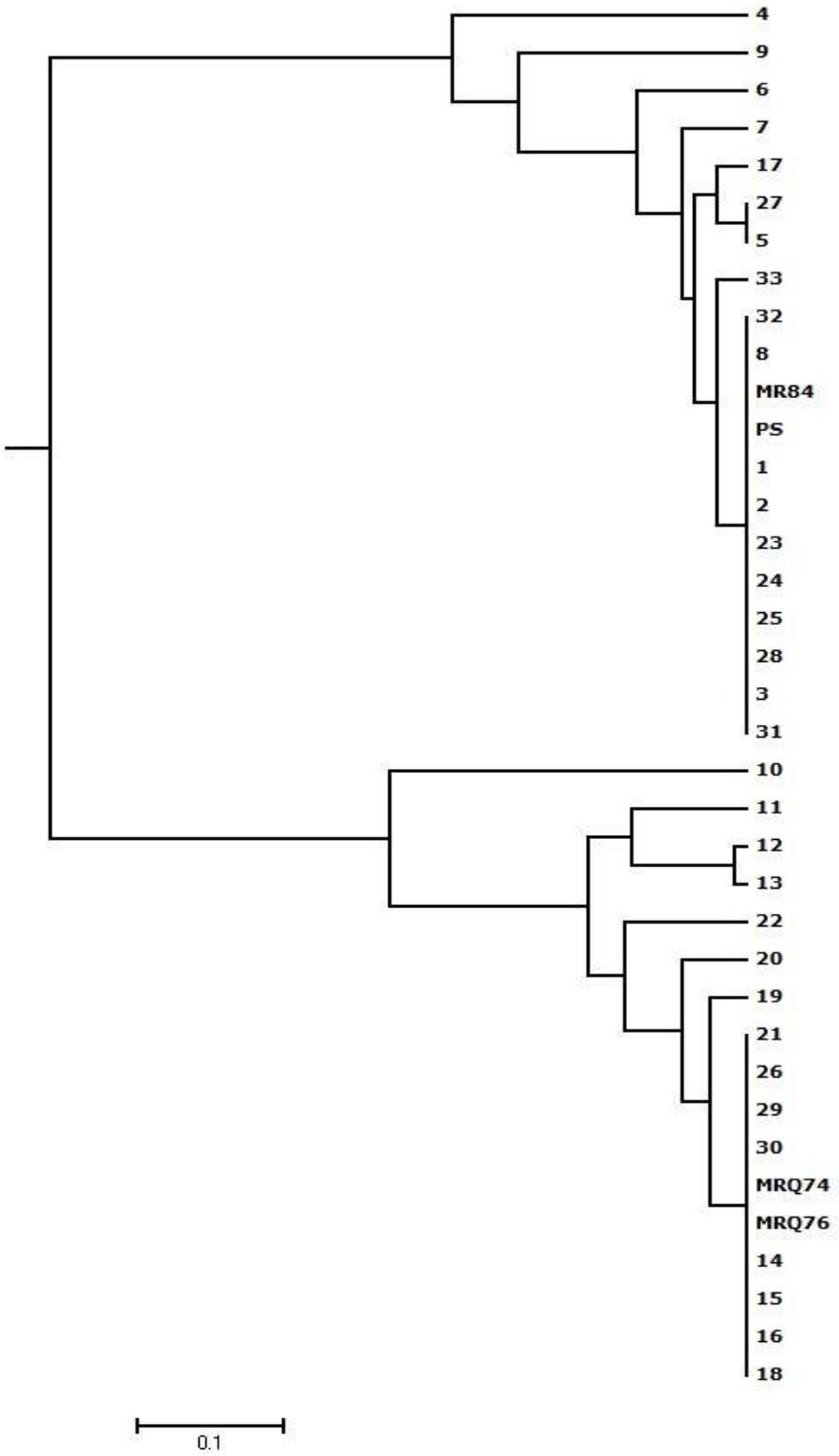

Figure 1. The dendrogram resulting from UPGMA cluster analysis generated by two aroma-specific SSR primers, FMbadh2-E7 and Badex7-5.

Table 5. The pairwise genetic distance between each genotype based on shared alleles.

	Malinja	Tutumoh	Beruang	Tiga Bulan	Telinga	Kurau	Bidor	Wangi	Jangrai	Nangka	Gertok	MR 219	Grik	Apit	Lumpur	Kantan Merah	Muduh	Mepawan	Sanguo Pandan	Topoi	Pulut Belacan	Pandan	Sempadan 303	Kenawit	Brio Pendek	Keramat Hitam	Miyah	Bokilong	Pulut Bukit	Kolomintuhon	Kadim	Lakatan	Silou
Malinja	0.00																																
Tutumoh	0.83	0.00																															
Beruang	1.00	0.50	0.00																														
Tiga Bulan	1.00	0.50	0.15	0.00																													
Telinga	1.00	0.50	0.17	0.02	0.00																												
Kurau	1.00	0.50	0.33	0.18	0.17	0.00																											
Bidor	1.00	0.50	0.33	0.18	0.17	0.00	0.00																										
Wangi	1.00	0.50	0.33	0.18	0.17	0.00	0.00	0.00																									
Jangrai	0.04	0.79	1.00	1.00	1.00	1.00	1.00	1.00	0.00																								
Nangka	1.00	0.50	0.33	0.18	0.17	0.00	0.00	0.00	1.00	0.00																							
Gertok	1.00	0.50	0.28	0.13	0.12	0.05	0.05	0.05	1.00	0.05	0.00																						
MR 219	0.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00	0.04	1.00	1.00	0.00																					
Grik	1.00	0.50	0.25	0.10	0.08	0.08	0.08	0.08	1.00	0.08	0.13	1.00	0.00																				
Apit	1.00	0.50	0.33	0.18	0.17	0.00	0.00	0.00	1.00	0.00	0.05	1.00	0.08	0.00																			
Lumpur	0.83	0.33	0.33	0.18	0.17	0.17	0.17	0.17	0.83	0.17	0.17	0.83	0.17	0.17	0.00																		
Kantan Merah	0.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00	0.04	1.00	1.00	0.00	1.00	1.00	0.83	0.00																	
Muduh	0.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00	0.04	1.00	1.00	0.00	1.00	1.00	0.83	0.00	0.00																
Mepawan	0.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00	0.04	1.00	1.00	0.00	1.00	1.00	0.83	0.00	0.00	0.00															
Sanguo Pandan	1.00	0.50	0.33	0.18	0.17	0.00	0.00	0.00	1.00	0.00	0.05	1.00	0.08	0.00	0.17	1.00	1.00	1.00	0.00														
Topoi	0.08	0.75	1.00	1.00	1.00	1.00	1.00	1.00	0.04	1.00	1.00	0.08	1.00	1.00	0.83	0.08	0.08	0.08	1.00	0.00													
Pulut Belacan	0.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00	0.04	1.00	1.00	0.00	1.00	1.00	0.83	0.00	0.00	0.00	1.00	0.08	0.00												
Pandan	1.00	0.50	0.33	0.18	0.17	0.00	0.00	0.00	1.00	0.00	0.05	1.00	0.08	0.00	0.17	1.00	1.00	1.00	0.00	1.00	1.00	0.00											
Sempadan 303	0.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00	0.04	1.00	1.00	0.00	1.00	1.00	0.83	0.00	0.00	0.00	1.00	0.08	0.00	1.00	0.00										
Kenawit	1.00	0.50	0.33	0.18	0.17	0.00	0.00	0.00	1.00	0.00	0.05	1.00	0.08	0.00	0.17	1.00	1.00	1.00	0.00	1.00	1.00	0.00	1.00	0.00									
Brio Pendek	0.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00	0.04	1.00	1.00	0.00	1.00	1.00	0.83	0.00	0.00	0.00	1.00	0.08	0.00	1.00	0.00	1.00	0.00								
Keramat Hitam	0.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00	0.04	1.00	1.00	0.00	1.00	1.00	0.83	0.00	0.00	0.00	1.00	0.08	0.00	1.00	0.00	1.00	0.00	0.00							
Miyah	0.04	0.83	1.00	1.00	1.00	1.00	1.00	1.00	0.08	1.00	1.00	0.04	1.00	1.00	0.83	0.04	0.04	0.04	1.00	0.13	0.04	1.00	0.04	1.00	0.04	0.04	0.00						
Bokilong	0.41	0.48	0.64	0.64	0.64	0.64	0.64	0.64	0.41	0.64	0.64	0.41	0.64	0.64	0.48	0.41	0.41	0.41	0.64	0.41	0.41	0.64	0.41	0.64	0.41	0.41	0.41	0.00					
Pulut Bukit	0.08	0.75	1.00	1.00	1.00	1.00	1.00	1.00	0.04	1.00	1.00	0.08	1.00	1.00	0.83	0.08	0.08	0.08	1.00	0.00	0.08	1.00	0.08	1.00	0.08	0.08	0.13	0.41	0.00				
Kolomintuhon	0.17	0.71	1.00	1.00	1.00	1.00	1.00	1.00	0.13	1.00	1.00	0.17	1.00	1.00	0.83	0.17	0.17	0.17	1.00	0.08	0.17	1.00	0.17	1.00	0.17	0.17	0.13	0.41	0.08	0.00			
Kadim	0.08	0.75	0.92	0.92	0.92	0.92	0.92	0.92	0.08	0.92	0.92	0.08	0.92	0.92	0.75	0.08	0.08	0.08	0.92	0.13	0.08	0.92	0.08	0.92	0.08	0.08	0.08	0.33	0.13	0.17	0.00		
Lakatan	0.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00	0.04	1.00	1.00	0.00	1.00	1.00	0.83	0.00	0.00	0.00	1.00	0.08	0.00	1.00	0.00	1.00	0.00	0.00	0.04	0.41	0.08	0.17	0.08	0.00	
Silou	0.33	0.50	1.00	1.00	1.00	1.00	1.00	1.00	0.29	1.00	1.00	0.33	1.00	1.00	0.83	0.33	0.33	0.33	1.00	0.25	0.33	1.00	0.33	1.00	0.33	0.33	0.33	0.41	0.25	0.21	0.33	0.33	0.00
MR 84	0.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00	0.04	1.00	1.00	0.00	1.00	1.00	0.83	0.00	0.00	0.00	1.00	0.08	0.00	1.00	0.00	1.00	0.00	0.00	0.04	0.41	0.08	0.17	0.08	0.00	0.33
MRQ74	1.00	0.50	0.33	0.18	0.17	0.00	0.00	0.00	1.00	0.00	0.05	1.00	0.08	0.00	0.17	1.00	1.00	1.00	0.00	1.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	0.64	1.00	1.00	0.92	1.00	1.00
MRQ76	1.00	0.50	0.33	0.18	0.17	0.00	0.00	0.00	1.00	0.00	0.05	1.00	0.08	0.00	0.17	1.00	1.00	1.00	0.00	1.00	1.00	0.00	1.00	0.00	1.00	1.00	1.00	0.64	1.00	1.00	0.92	1.00	1.00
Pulut Siding	0.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00	0.04	1.00	1.00	0.00	1.00	1.00	0.83	0.00	0.00	0.00	1.00	0.08	0.00	1.00	0.00	1.00	0.00	0.00	0.04	0.41	0.08	0.17	0.08	0.00	0.33

A value of 0.00 indicates identical alleles; and a value of 1.00 indicates genotype dissimilarity

DISCUSSION

The study revealed that twenty-one rice landraces had comparable sensory test and molecular findings, with eleven genotypes identified as having aroma and ten genotypes identified as having no aroma. The presence of aroma was identified in Kenawit, Gertok, Pandan, Nangka, Tiga Bulan, Lumpur, Grik, Sanguo Pandan, Kurau, Bidor, and Wangi. However, this study revealed several discrepancies in the aroma results between the two methods. Five rice landraces, namely Pulut Bukit, Silou, Jangrai, Kantan Merah, and Mepawan, were shown to have a slight aroma in a sensory test but were not detected using molecular markers. Carsono *et al*. (2020) and Yeap *et al*. (2013) also discovered undetectable aromas using molecular markers in their respective studies. Buttery *et al*. (1983), reported that 2-acetyl-1-pyrroline, or 2-AP, a volatile compound with a popcorn-like aroma, is the only component that has been identified as a significant contributor to the pleasant scent of aromatic rice and the only compound shown to quantify rice's aroma (Hu *et al*., 2020). However, a recent study discovered approximately 500 volatile compounds in aromatic and non-aromatic rice varieties (Lina and Min, 2022). Various advanced methods recently developed, such as gas chromatography-mass spectrophotometry and high-density molecular marker mapping, and genome sequencing, a higher number of aromatic compounds can potentially be detected. According to Yang *et al*. (2008) and Marawal *et al*. (2008), in addition to 2-AP, other active compounds in cooked rice that have been successfully quantified include guaiacol, indole, p-xylene, oct-1-en-3-one, propanol, 2-butanone, acetaldehyde, hexanol, and pentanal. Thus, the presence of distinct groups of aromatic compounds may result in undetectable molecular markers due to chemical compound involvement in varying proportions (Pachauri *et al*., 2010) or the involvement of other fgr genes controlling aroma (Fitzgerald *et al*., 2009).

Moreover, the primers FMbadh2-E7 and Badex7-5 were designed to target the flanking sequences of the 8-bp deletion in exon 7 of the badh2 gene located on Chromosome 8 (Li *et al*., 2020; Sakthivel *et al*., 2009). Nonetheless, several studies have reported that the aroma of rice is controlled by one, two, or three dominant or recessive genes or QTLs (quantitative trait loci). As discovered by Lorieux *et al*. (1996) and Amarawathi *et al*. (2008), several QTLs were discovered on chromosomes 8, 4, and 12 via RLFP and STS; whereas other QTLs were discovered on chromosomes 3, 4, and 8 via SSR markers, respectively. Consequently, this may be another factor that contributes to the undetectable aroma by molecular markers.

On the other hand, Tutumoh, Beruang, Telinga, and Apit were scored as non-aromatic based on the sensory test but were identified as aromatic based on molecular markers. According to Carsono *et al*. (2020), the extremely low concentration of 2-AP in the genotypes may result in aromas being undetected by panellists in sensory testing. Meanwhile, Alrufaye *et al*. (2018) suggested that it could be because of the allegations of minor genes that regulate rice aroma. Even though, the sensory test was declared unreliable by Bharti and Anand (2020) and Hien *et al*. (2006), because of significant variations in the analyst's ability to recognize aromas and personal bias resulting from varying levels of nasal sense saturation. Moreover, rather than being influenced by subjective factors such as emotion, physical condition, and environment, sensory evaluation was considered to be highly subjective and not quantitative (Hu *et al*., 2020). Nevertheless, this method remains recognized as a practical and acceptable technique for detecting the aroma of rice, despite the fact that the presence of this 2-AP compound is often detected through sensory tests (Carsono *et al*., 2020; Hien *et al*., 2006). In addition, previous research has shown that the aromatic character of rice as measured by sensory tests correlates significantly with the 2-AP concentration (Ishitani and Fushimi, 1994). In addition, this study employs qualified panellists from the Rice Quality Laboratory, so the potential for error that could compromise the accuracy of the evaluation is reduced. In accordance with Hu *et al*. (2020), trained panellists are one of the prerequisites for sensory evaluation, along with a unified description lexicon and reference standards for rice aroma.

According to Alrufaye *et al*. (2018), the determination of aroma cannot rely solely on sensory testing but must be complemented by other techniques. In addition to Carsono *et al*. (2020), the results from the two methods are more reliable and accurate compared to a single method because the aroma of rice is highly influenced by genetic composition and environmental conditions. Combining several approaches to detect aroma in rice has been a common practice for decades, such as gas chromatography with molecular marker mapping (Lorieux *et al*., 1996); Allele Specific Amplification (External Antisense Primer, External Sense Primer, Internal Nonfragrant Sense Primer, and Internal Fragrant Antisense Primer) with sensory evaluation on leaves and grains (Yeap *et al*., 2013); molecular markers using ESP (External Antisense Primer), IFAP (Internal Fragrant Antisense Primer), INSP (Internal Non fragrant Sense Primer) and EAP (External Antisense Primer) with Sensory test using KOH 1.7% solution (Carsono *et al*., 2020). In this study, the identification of aroma was performed using molecular markers in conjunction with a sensory test. These procedures are not merely important for the detection of aromas, but also for the validation of trait expression, which is necessary for the identification of possible promising rice landraces. Kenawit, Gertok, Pandan, Nangka, Tiga Bulan, Lumpur, Grik, Sanguo

Pandan, Kurau, Bidor, and Wangi have been identified as aromatic rice landraces through both methods. Among them, Sanguo Pandan, Kenawit, Pandan, Kurau, Bidor, and Wangi exhibited the highest similarity to the aroma standards when considering their pairwise genetic distance. As a result, the above-mentioned rice landrace can be considered as possible potential donors in rice breeding programs.

Conclusion: Sensory test for aroma in rice landraces is more accurate and reliable when combined with molecular analysis. The aromatic rice landraces that have been identified through sensory test and molecular markers are valuable for future breeding programs, primarily for the development of high-quality or specialty rice to meet the consumer demands. The SSR markers employed in this study can be recommended for large-scale and routine aroma genotyping because they can differentiate aromatic rice from non-aromatic rice; and are also simple, inexpensive, and repeatable. Moreover, detecting the aroma of rice may be made more efficient and accurate by incorporating other advanced techniques.

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