P-ISSN 2586-9000
E-ISSN 2586-9027
Homepage : https://tci-thaijo.org/index.php/SciTechAsia
Science & Technology Asia
Vol.28 No.4 October - December 2023
Page: [273-288]
*Original research article*

# Analysis of Fragrant Characteristics in Myanmar Rice Accessions Through Biochemical and Molecular Approaches

San Thandar[1], April Nwet Yee Soe[1], Moe Moe Myint[1], Thwe Thwe Lin[1], Kyaw Min Thu[2], Tin Mar Lynn[1,*]

[1]*Molecular Genetics Laboratory, Department of Biotechnology Research, Kyaukse 05151, Myanmar*
[2]*Plant and Agricultural Biotechnology Research Department, Department of Biotechnology Research, Kyaukse 05151, Myanmar*

Received 27 June 2023; Received in revised form 9 October 2023
Accepted 31 October 2023; Available online 27 December 2023

**ABSTRACT**

Aroma is a significant quality trait of rice, and cultivars with aroma command a premium on the marketplace. Fifteen rice cultivars were characterized for their aroma by three methods; sensory test, determination of proline content and molecular marker analysis. Sensory analysis revealed that eight rice cultivars were fragrant whereas the remaining ones were not. The proline content of rice leaves was analyzed at the flowering stage, and higher contents of proline were detected in fragrant cultivars than non-fragrant ones. A total of 35 simple sequence repeat (SSR) markers distributed over the whole rice genome, including 17 markers on chromosome 8 which were closely associated to the quantitative trait loci (QTLs) and/or genes for aroma, were employed to analyze genetic diversity of rice cultivars. Moreover, four functional markers were used to diagnose the aroma trait allele of the *badh2* gene. At 29 SSR loci, a total of 85 alleles were found, with the number of alleles per marker ranging from 2 to 5. Values for polymorphism information content were between 0.2 and 0.67. Eight SSR markers on chromosome 8 could discriminate the presence of the fragrant gene in 8 rice accessions, while three functional markers could confirm two mutation alleles within the *badh2* gene. Using the unweighted pair group method with arithmetic mean (UPGMA) approach, cluster analysis divided 15 rice accessions into two major clusters, effectively differentiating the short and medium bold cultivars from the slender cultivars. The three methods used in this study were strong enough to distinguish aromatic rice varieties.

**Keywords:** Aroma; Molecular marker analysis; Proline content; Rice; Sensory test

***Corresponding author:** tinmarlynn.biotec@gmail.com

## 1. Introduction

Rice (*Oryza sativa* L.) is a mainstay crop for more than fifty percent of the global population, with Asian nations producing almost 90% of the world's rice [1]. The rise in socioeconomic status in Asia and developing nations has recently highlighted an increasing demand for high-quality rice [2]. Aroma stands out as one of rice's most crucial attributes, and aromatic varieties command a higher price in both domestic and global markets, as it is related with its local, cultural, and national identity [3]. Aromatic varieties, such as jasmine and basmati rice, are widely favored by consumers throughout the world [4].

Paw San Hmwe, one of the Paw San rice varieties in Myanmar is reputed as Pearl Paw San in the worldwide market because of its potent aroma, excellent cooking qualities and significant grain elongation while cooking [5]. There are different Paw San varieties and their names vary with location. The widely grown aromatic quality rice varieties in Myanmar are Paw San Hmwe, Lone Thwe Hmwe and Basmati [6]. Another aromatic rice variety known as Namathalay (NMTL) has long been cultivated in Myanmar. It has small grains and is easy to digest which is suitable for the elderly. NMTL rice is an environmentally friendly rice variety which possesses abiotic stress tolerance and requires less nitrogen fertilizer (https://docplayer.net/90312829-Minn-san-thein-and-khin-mar-oo.html). Although NMTL possess several advantages, this variety is neglected and underutilized, and information on this plant is still rare. Furthermore, although the aroma of Paw San rice varieties has been studied [7], there are only a few reports that compare the aromatic and genetic characteristics of Paw San, Lone Thwe Hmwe, and Namathalay rice varieties.

Aroma is a complicated characteristic and more than one hundred volatile aromatic metabolites are responsible for its expression in rice [8]. Among them, 2-acetyl-1-pyrroline (2AP) is widely regarded as the primary component associated with a popcorn-like aroma in rice [9]. 2AP is present in every part of the rice plant except for its root system. Its concentration in fragrant rice is 10 to 15 times greater than non-fragrant rice [10]. Proline was discovered to be a precursor of 2AP and a rise in the levels of free proline is associated with higher concentration of 2AP in many cultivars of scented rice [11]. Although 2AP has been measured with GC-MS in several reports, this method is expensive and not widely available in developing countries [12]. Therefore, determination of proline content could be an alternative approach to estimate the fragrance strength of aromatic rice.

Genetically, at least four chromosomes (3, 4, 8 and 12) have been involved in the mapping of aroma traits, with chromosomes 4, 8 and 12 identified by Lorieux et al. [9] and 3, 4 and 8 identified by Amarawathi et al. [13]. It has been found that rice aroma is linked to the recessive Os2AP gene on chromosome 8 [14]. Bradbury et al. [15] stated that aroma of rice is caused by the inactivation of the BADH2 enzyme, which is encoded by the betaine aldehyde dehydrogenase 2 (*badh2*) gene on chromosome 8. Mutations within *badh2*, which consists of 15 exons and 14 introns, have been associated with the build-up of 2AP, which affects the aroma of rice.

According to genetic analysis, rice aroma is an inheritable characteristic that is primarily governed by recessive monogenes and not by cytoplasmic genes [16]. However, aroma has also been regarded as a quantitative trait, and many genes were found to be involved in its expression [16] which indicated the complexity of genes controlling the rice aroma [12]. The amount of 2AP in rice can be significantly influenced not only by genetics but also by ecological factors and cultivation methods [17]. Smelling leaf tissues and grains heated with water or reacted with KOH solution aid in selecting fragrant rice [18], but there are

major limitations to testing fragrance in this way that include potential harm to nasal passages, olfactory fatigue, and variation between individual testers [19].

Genetic diversity is essential for rice adaptation on varied agroecological environments. The genetic diversity of a crop population arises from various mechanisms such as mutation, selection, and recombination [20], and is primarily evaluated based on morphological variations of quantitatively important traits [21]. However, this method is limited by time, space, and labor costs and cannot accurately quantify the genetic diversity of germplasms due to additive gene action on the expression of economically important traits, thus making environmental factors conceal their true phenotypic impact [22]. Microsatellite markers have become a popular method for screening, characterizing, and assessing genetic diversity in many crop varieties in recent years [23]. The development of PCR-based fragrance markers has several benefits including being affordable, easy to use, fast, and having a minimal tissue requirement [24].

Functional markers derived from sequence variations within the *fgr* gene [13] offer superior genotype selection compared to DNA markers located nearby but at functionally unrelated sites [25]. It was found that most aromatic rice varieties exhibit an 8 bp deletion and 3 single nucleotide polymorphism sites within the 7$^{th}$ exon of the *badh2* gene, though variation sites were also found in 1$^{st}$ , 10$^{th}$ , 13$^{th}$ and 14$^{th}$ exons [15]. Therefore, researchers have employed functional markers to assess whether the rice has aroma, in addition to the sensory evaluation method using KOH and DNA markers linked to the aroma trait.

In this study, simple sequence repeat (SSR) markers scattered throughout 12 different chromosomes of the rice genome were employed to analyze the genetic diversity and relationships among aromatic and non-aromatic rice accessions. In addition, the objective of this study was to distinguish between fragrant and non-fragrant rice accessions using functional markers and SSR markers linked to aroma traits, sensory test and measurement of proline content at the flowering stage.

## 2. Materials and Methods

### 2.1 Plant materials

A total of 15 rice accessions including three non-aromatic check varieties (MNTK, AYPD, and IR64) were used in our study. These rice accessions were collected from the Seed Bank at the Department of Agricultural Research, Nay Pyi Taw in Myanmar and information of these accessions is described in Table 1.

**Table 1.** List of rice accessions and their information.

| Sr. No. | Accession No. | Cultivar name | Code | Township | State /Region |
|---|---|---|---|---|---|
| 1 | 000804 | Paw San Bay Kyar | PSBK1 | Pathein | Ayeyarwady Region |
| 2 | 002174 | Paw San Bay Kyar | PSBK2 | Sittwe | Rakhine State |
| 3 | - | Paw San Bay Kyar | PSBK3 | Kyaukse | Mandalay Region |
| 4 | 002500 | Paw San Hmwe | PSH1 | Minhla | Bago Region |
| 5 | 002522 | Paw San Hmwe | PSH2 | Myaungmya | Ayeyarwady Region |
| 6 | 009765 | Paw San Hmwe | PSH3 | Minbya | Rakhine State |
| 7 | - | Lone Thwe Hmwe | LTM1 | Kyaukse | Mandalay Region |
| 8 | 002453 | Lone Thwe Hmwe | LTM2 | - | Nay Pyi Taw |
| 9 | 007310 | Lone Thwe Hmwe | LTM3 | Myeik | Tanintharyi Region |
| 10 | 001635 | Na Ma Tha Lay | NMTL1 | Kungyangon | Yangon Region |
| 11 | 007797 | Na Ma Tha Lay | NMTL2 | Kalay | Sagaing Region |
| 12 | 000022 | Na Ma Tha Lay | NMTL3 | - | Mon State |
| 13 | 009926 | Aye Yar Padaethar | AYPD | Kyaukse | Mandalay Region |
| 14 | 003470 | Manawthukha | MNTK | - | Nay Pyi Taw |
| 15 | 001191 | IR-64 | IR64 | - | Nay Pyi Taw |

**2.2 Grain characteristics of rice genotypes**

Grain characteristics of the selected rice varieties were determined to identify desirable traits in rice and develop new varieties with improved quality along with aroma.

***2.2.1 Grain dimension***

Ten grains per sample were randomly selected and measured for length and width using a portable vernier caliper (SOLEX). Grains were classified into four length categories: short (<5.5 mm), medium (5.5-6.6 mm), long (6.6-7.5 mm), and extra-long (>7.5 mm). Grain shape was determined by the ratio of length to width, with four categories: slender (>3.0), medium (2.1-3.0), bold (1.1-2.0), and round (<1.1) [26].

***2.2.2 Measurement of elongation ratio***

To measure the elongation ratio, ten rice grains were soaked in 5 mL of tap water in a 20 mL test tube for 20 min, then boiled for around 30 min. Length and width of the grains were measured before and after cooking, and the ratio was calculated by dividing the average length of ten cooked rice grains by the average length of ten uncooked grains [27].

**2.3 Biochemical analysis of aroma**

The rice cultivation process was conducted at the research field of Plant and Agricultural Research Department, Department of Biotechnology Research (DBR), Kyaukse.

***2.3.1 Leaf aroma test***

Fragrance was evaluated according to the method described by Sood and Siddiq [18] with slight modifications.

The aroma of leaf tissues at three developmental stages (tillering, flowering, and milking) was evaluated by incubating 100 mg of green rice leaves in a 50 mm petri dish with 5 mL of 0.5% KOH at room temperature for 15 min, followed by olfactory examination.

***2.3.2 Grain aroma test***

Twenty white grains of each accession were soaked in a 50 mm petri plate containing 5 mL of 0.5% KOH solution at room temperature for 1 hour [18]. In addition, five brown rice seeds from each accession were incubated in 1.5 mL centrifuge tubes containing 200 µL distilled water at 65 ºC for 30 min, then cooled [28].

Samples were evaluated for aroma intensity on a 0-3 scale by opening each lid individually after incubation. Scores were assigned as follows: 0 = absence of aroma, 1 = slight aroma, 2 = moderate aroma, and 3 = strong aroma. To minimize desensitization, evaluations were conducted randomly and in duplicate. Eight panelists from DBR tested and scored the aroma of rice grains and leaf samples from each rice accession.

**2.4 Proline determination**

Proline content was assessed using the approach described by Ábrahám et al. [29]. Three replicates of leaf samples (100 mg) at the flowering stage were homogenized in 500 µL of 3% sulfosalicylic acid and centrifuged at 12000 rpm for 5 min. A reaction mixture consisting of 100 µL of 3% sulfosalicylic acid, 200 µL of glacial acetic acid, and 200 µL of acid ninhydrin solution (1.25 g of ninhydrin dissolved in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid) was prepared. After centrifugation, 100 µL of the supernatant was added to the reaction mixture for the estimation of proline, and the tube was incubated at 96°C for 60 min. After termination on ice, 1 mL of toluene was added to the contents, vortexed for 20 sec, and left for 5 min to allow phase separation. Finally, absorbance at 520 nm was measured using toluene as a blank, and proline content was determined by comparing the absorbance value with the standard curve ($y$ = 0.00948$x$-0.0066; $R^2$ = 0.9592).

### 2.5 DNA extraction

Genomic DNA was extracted from young leaves of 4-week-old seedlings using a modified CTAB method with 1 M KCL instead of CTAB and mercaptoethanol [30]. DNA concentration and purity were determined using a nanodrop spectrophotometer (ND 1000, Thermo Scientific, Madison, USA), and quality was checked by 1% agarose gel electrophoresis. The gel was visualized under a UV trans-illuminator (Spectroline, USA).

### 2.6 Selection of SSR markers, PCR amplification and electrophoresis

To assess genetic diversity among the 15 rice varieties, 35 SSR markers dispersed across the 12 rice chromosomes were selected, including 17 markers on chromosome 8 that were tightly linked to QTLs and genes for aroma. All marker information, except for the annealing temperature and allele size range, was obtained from the Gramene database (http://www.gramene.org) and is listed in Table 2. Additionally, four functional markers were used to diagnose the aroma trait allele (Table 3). Prior to the assay, an empirical optimization survey was undertaken for each primer to identify optimal annealing temperatures and primer concentrations.

PCR reactions for SSR primers were conducted in a Proflex Thermal Cycler (Applied Biosystems, USA) using a total reaction volume of 10 µL, including 5 µL of 2*x* Taq DNA Polymerase Master Mix Kit (VWR, Denmark), 0.5 µL of each 10 µM forward and reverse primers, 3.5 µL dd$H_2O$, and 0.5 µL of each template DNA (200 ng/µL). PCR conditions involved initial denaturation at 95°C for 4 min, proceeded by 30 cycles of denaturation at 95°C for 30 sec, annealing at the suitable temperature (55°C-67°C depending on the primer) for 30 sec, extension at 72°C for 1 min, and concluded with final extension at 72°C for 5 min.

**Table 2.** Information of selected SSR markers spread over 12 chromosomes.

| No. | Primer | Chromosome | Repeat Motif | Expected Size (bp) | Ta (°C) | Allele Size Range (bp) |
|---|---|---|---|---|---|---|
| 1 | RM1 | 1 | (GA)26 | 113 | 55 | 80-124 |
| 2 | RM485 | 2 | (TA)18 | 291 | 59 | 300-315 |
| 3 | RM154 | 2 | (GA)21 | 183 | 64 | 120-220 |
| 4 | RM232 | 3 | (CT)24 | 158 | 55 | 142-166 |
| 5 | RM273 | 4 | (GA)11 | 207 | 56 | 175-275 |
| 6 | RM252 | 4 | (CT)19 | 216 | 55 | 184-267 |
| 7 | RM122 | 5 | (GA)7A(GA)2A(GA)11 | 227 | 57 | 184-267 |
| 8 | RM169 | 5 | (GA)12 | 167 | 67 | 130-200 |
| 9 | RM190 | 6 | (CT)11 | 124 | 55 | 90-130 |
| 10 | RM253 | 6 | (GA)25 | 141 | 55 | 97-143 |
| 11 | RM204 | 6 | (CT)44 | 169 | 55 | 104-194 |
| 12 | RM510 | 6 | (GA)15 | 122 | 55 | 120-125 |
| 13 | RM11 | 7 | (GA)17 | 140 | 55 | 121-147 |
| 14 | RM85 | 8 | (TGG)5(TCT)12 | 107 | 55 | 70-110 |
| 15 | RM223 | 8 | (CT)25 | 165 | 55 | 141-165 |
| 16 | RM339 | 8 | (CTT)8CCT(CTT)5 | 148 | 55 | 130-170 |
| 17 | RM42 | 8 | (AG)6-(AG)2T(GA)5 | 166 | 55 | 166-176 |
| 18 | RM342 | 8 | (CAT)12 | 141 | 55 | 100-200 |
| 19 | RM152 | 8 | (GGC)10 | 151 | 55 | 150-155 |
| 20 | RM210 | 8 | (CT)23 | 140 | 55 | 121-163 |
| 21 | RM44 | 8 | (GA)16 | 99 | 55 | 80-124 |
| 22 | RM256 | 8 | (CT)21 | 127 | 56 | 89-147 |
| 23 | RM344 | 8 | (TTC)2-5-(CTT)3-(CTT)4 | 163 | 55 | 160-172 |
| 24 | RM126 | 8 | (GA)7 | 171 | 55 | 165-190 |
| 25 | RM72 | 8 | (TAT5)(ATT)15 | 166 | 55 | 80-190 |
| 26 | RM337 | 8 | (CTT)4-19-(CTT)8 | 142 | 55 | 60-480 |
| 27 | RM38 | 8 | (GA)16 | 250 | 55 | 235-278 |
| 28 | RM447 | 8 | (CTT)8 | 111 | 55 | 100-115 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 29 | RM310 | 8 | (GT)19 | 105 | 55 | 80-120 |
| 30 | RM331 | 8 | [(CTT)4GTT]2(CTT)4 | 176 | 55 | 145-180 |
| 31 | RM215 | 9 | (CT)16 | 148 | 57 | 138-152 |
| 32 | RM434 | 9 | (TC)12 | 152 | 57 | 170 |
| 33 | RM590 | 10 | (TCT)10 | 137 | 55 | 145-155 |
| 34 | RM287 | 11 | (GA)21 | 118 | 55 | 80-140 |
| 35 | RM277 | 12 | (GA)11 | 124 | 55 | 100-125 |

**Table 3.** Functional markers used for analysis of fragrance in rice.

| No. | Gene marker | *badh2* alleles | Annealing temperature (°C) | Approximate allele size range (bp) | Reference |
|---|---|---|---|---|---|
| 1 | External Sense Primer (ESP)<br>Internal Fragrant Antisense Primer (IFAP)<br>Internal Non-fragrant Sense Primer (INSP)<br>External Antisense Primer (EAP) | *badh2-E7* | 58 | ESP-EAP 585<br>ESP-IFAP 257<br>INSP-EAP 355 | Bradbury et al. (2005b) |
| 2 | *fgr1* | *badh2-E7* | 57 | 267-275 | Jin et al. (2010) |
| 3 | 8bpDEL | *badh2-E7* | 55 | 392-400 | Myint et al. (2012a) |
| 4 | 3bpIN | *badh2-E13* | 55 | 194-197 | Myint et al. (2012a) |

The allele-specific amplification (ASA) together with four primers (ESP, EAP, IFAP and INSP) were amplified following the protocol from Bradbury et al. [31], while the *fgr1* marker was amplified according to Jin et al. [32], and the PCR amplification protocol of the 3bp insertion (3bpIn) and 8bp deletion (8bpDel) markers were followed by the methodology of Myint et al. [14].

PCR amplified products were electrophoresed on 3% agarose gel stained with ethidium bromide and 8% polyacrylamide gel stained with silver nitrate (0.1%) in 0.5*x* TBE buffer at 120 V, with running time based on PCR product size. The size of the PCR products was verified against a 100 bp DNA ladder molecular size standard.

### 2.7 Data analysis

Grain characteristics and proline content of tested rice cultivars were computed using SPSS.16 software (SPSS Inc., Chicago, IL, USA), with significance defined as $P < 0.05$, and the difference among the means was determined using Duncan's Multiple Range Test. The genotypes were scored with a binary coding method, where '1' represented the presence of a band and '0' denoted its absence for each SSR primer and analyzed with NTSYS-pc version 2.1 [33]. The SIMQUAL program was used to compute Jaccard's similarity coefficient, and the resultant similarity matrix was utilized for constructing an unweighted pair group method with arithmetic mean (UPGMA)-based dendrogram. PowerMarker version 3.25 [34] was employed for calculating genetic diversity parameters including major allele frequency, alleles per locus, polymorphism information content (PIC) values and heterozygosity.

## 3. Result and Discussion

### 3.1 Morphological traits of studied rice varieties

Some parameters of grain characteristics were examined based on the standard protocols [26]. The count for the four types of grain length were as follows: 4 long types, 4 medium types, 5 short types, and 2 extra-short types (Table 4). LTM1 exhibited the longest length (7.30±0.14 mm) among long-type grains, whereas IR64 displayed the shortest length (6.66±0.12 mm). For medium-type grains, the longest length was observed in PSBK2 (6.31±0.13 mm), and the shortest length was found in

NMTL2 (5.64±0.12 mm). The length of short-type grains ranged from PSBK1 (5.43±0.05 mm) to PSH2 (5.21±0.07 mm), while NMTL1 (4.07±0.04 mm) and NMTL3 (3.91±0.06 mm) exhibited the longest and shortest length for extra short-type grains, respectively. Long-grain rice is generally preferred in the Indian subcontinent, while medium to medium-long grain rice is the preferred choice in Southeast Asia [35]. The length and width of the grain determine the shape, which is another characteristic of preference among consumers along with aroma [36].

**Table 4.** Grain characteristics, sensory analysis and proline content of selected rice accessions.

| Rice accession | Grain characteristics | | | | | Sensory analysis | Proline (μg/g fresh leave) |
|---|---|---|---|---|---|---|---|
| | Elongation ratio | White rice Length (mm) | Grain size | Length-Width ratio | Shape | | |
| PSBK1 | 1.86±0.08$^{bc}$ | 5.43±0.05$^{fg}$ | Short | 1.95±0.04$^{f}$ | Bold | Strong | 88.54±0.16$^{a}$ |
| PSBK2 | 1.62±0.06$^{de}$ | 6.31±0.13$^{c}$ | Medium | 2.15±0.05$^{e}$ | Medium | None | - |
| PSBK3 | 2.03±0.07$^{a}$ | 5.37±0.11$^{g}$ | Short | 2.00±0.05$^{f}$ | Bold | Strong | 46.48±0.27$^{e}$ |
| PSH1 | 1.92±0.04$^{ab}$ | 5.25±0.07$^{g}$ | Short | 1.95±0.05$^{f}$ | Bold | None | 39.97±0.16$^{h}$ |
| PSH2 | 1.79±0.04$^{bc}$ | 5.21±0.07$^{g}$ | Short | 1.86±0.03$^{f}$ | Bold | Strong | 42.51±0.48$^{f}$ |
| PSH3 | 1.82±0.02$^{bc}$ | 5.26±0.07$^{g}$ | Short | 1.90±0.04$^{f}$ | Bold | Moderate | 80.13±0.57$^{b}$ |
| LTM1 | 1.59±0.05$^{e}$ | 7.30±0.14$^{a}$ | Long | 3.50±0.08$^{a}$ | Slender | Moderate | 56.48±0.27$^{d}$ |
| LTM2 | 1.54±0.02$^{ef}$ | 7.20±0.08$^{a}$ | Long | 3.62±0.05$^{a}$ | Slender | Strong | 60.29±0.27$^{c}$ |
| LTM3 | 1.48±0.04$^{ef}$ | 7.11±0.08$^{a}$ | Long | 3.49±0.04$^{a}$ | Slender | Moderate | 59.81±0.48$^{c}$ |
| NMTL1 | 1.49±0.05$^{ef}$ | 4.07±0.04$^{h}$ | Extra Short | 2.00±0.02$^{f}$ | Bold | None | 33.62±0.48$^{j}$ |
| NMTL2 | 1.49±0.05$^{ef}$ | 5.64±0.12$^{ef}$ | Medium | 2.71±0.04$^{c}$ | Medium | None | 18.86±0.55$^{k}$ |
| NMTL3 | 1.74±0.05$^{cd}$ | 3.91±0.06$^{h}$ | Extra Short | 1.86±0.02$^{f}$ | Bold | Slight | 41.08±0.16$^{g}$ |
| AYPD | 1.42±0.04$^{f}$ | 5.95±0.03$^{d}$ | Medium | 2.56±0.06$^{d}$ | Medium | None | 29.17±0.42$^{k}$ |
| MNTK | 1.57±0.03$^{e}$ | 5.86±0.06$^{de}$ | Medium | 2.79±0.03$^{c}$ | Medium | None | 38.38±0.16$^{i}$ |
| IR64 | 1.41±0.05$^{f}$ | 6.66±0.12$^{b}$ | Long | 3.05±0.07$^{b}$ | Slender | None | 28.38±0.27$^{k}$ |

**Note:** Data represent the mean ± SE. Small letters indicate significant difference among fifteen rice varieties ($P < 0.05$).

According to the length-breadth ratio, 7 varieties were bold-type, 4 varieties were medium-type, and 4 varieties were slender-type grains. LTM2 (3.62±0.05) had the highest length-to-breadth ratio and the lowest for PSH2 (1.86±0.03) and NMTL3 (1.86±0.02). The largest elongation ratio was observed in PSBK3 (2.03±0.03) and the smallest elongation ratio was recorded in IR64 (1.41±0.05) followed by AYPD (1.42±0.04) and LTM3 (1.48±0.04).

These findings are consistent with previous research that has also shown that the shape and elongation ratio of rice grains can vary significantly among rice cultivars. Consumers have a preference for aromatic rice that exhibits a higher elongation ratio, which in turn commands a higher price in the market [37].

### 3.2 Sensory evaluation

The aroma characteristics of rice varieties were evaluated with sensory methods such as reacting leaf tissues and rice grains with 0.5% KOH solution and heating rice grains with water. The accessions were classified into four groups based on their fragrance intensity (strongly fragrant, moderately fragrant, slightly fragrant, and non-fragrant) at four stages: tillering, flowering, milking, and grain stage (Table 4). It was discovered that rice grains smelled more distinctively after reacting with KOH than they did after being heated with water. Among the tested accessions, four (PSBK1, PSBK3, PSH2 and LTM2) were categorized as strongly fragrant, three (PSH3, LTM1 and LTM3) as moderately fragrant, one (NMTL3) as slightly fragrant and six (PSBK2, PSH1, NMTL1, NMTL2, AYPD, MNTK and IR64) as non-fragrant accessions. Even though PSBK2 and PSH1 belong to the group of aromatic PS rice varieties, this study has found that they are non-aromatic.

According to Aye et al. [38], the variation in aroma score among PS rice varieties could be attributed to dissimilarities

in environmental factors, including temperature fluctuations during grain maturation, as well as soil conditions such as soil texture and pH levels at the site of cultivation.

### 3.3 Determination of proline at the flowering stage

Several studies have proven that proline plays a crucial role in reproductive development particularly during the flowering stage and serves as a readily available source of energy in pollen [39]. In scented rice cultivars, proline content remained stable during vegetative development, increased dramatically during flowering, and reduced during grain filling and maturity. Hinge et al. [40] reported that highest proline content was found in scented rice cultivars during the flowering stage. Therefore, in our study, the proline content was determined for leaf tissues of fragrant and non-fragrant rice cultivars at the flowering stage (Table 4).

In PS group, the highest proline content was detected in fragrant accession PSBK1 followed by PSH3, PSBK3, PSH2 and the lowest was detected in the non-fragrant accession PSH1. The proline content exhibited slight variations among all LTM fragrant varieties. However, in NMTL group, the highest proline content was detected in the aromatic variety NMTL3 and the lowest was in the non-aromatic variety NMTL2.

According to the data, the proline content at the flowering stage was higher in fragrant varieties than in non-fragrant varieties, suggesting that the build-up of proline may influence the aroma of rice. The Pearson's correlation coefficient between proline content in leaf tissues at the flowering stage and the sensory test (0.5% KOH) of the leaf tissues and rice grains is shown in Table 5.

The results of correlation analysis between proline and sensory assessment (0.5% KOH) of leaf tissues, brown grains, and white grains of rice among 14 varieties revealed significant correlations between proline and aroma of leaf tissues (r = 0.603*), proline and aroma of brown grains (r = 0.576*), and proline and aroma of white grains (r = 0.657**). In our research, proline analysis was conducted on the leaf tissues of only 14 rice varieties during the flowering stage, as the proline content of PSBK2 could not be measured due to the perishing of rice plants of this variety during planting.

**Table 5.** Pearson's correlation between proline content at flowering stage and aroma of rice grains and leaf tissues.

| | Proline | Aroma of brown rice | Aroma of white rice | Aroma of leaves at flowering stage |
|---|---|---|---|---|
| Proline | 1 | | | |
| Aroma of brown rice | 0.576* | 1 | | |
| Aroma of white rice | 0.657** | 0.932** | 1 | |
| Aroma of leaves at flowering stage | 0.603* | 0.958** | 0.943** | 1 |

* Correlation is significant at the 0.05 level 2-tailed, ** Correlation is significant at the 0.01 level 2-tailed.

**Table 6.** Characteristics of SSR markers in studied genotypes (PowerMarker).

| Marker | Major Allele Frequency | Genotype No. | Sample Size | Allele No. | Gene Diversity | Heterozygosity | PIC |
|---|---|---|---|---|---|---|---|
| RM223 | 0.73 | 5 | 15 | 5 | 0.44 | 0.00 | 0.43 |
| RM277 | 0.87 | 2 | 15 | 2 | 0.23 | 0.00 | 0.20 |
| RM590 | 0.87 | 3 | 15 | 3 | 0.24 | 0.00 | 0.23 |
| RM510 | 0.67 | 2 | 15 | 2 | 0.44 | 0.00 | 0.35 |
| RM252 | 0.53 | 4 | 15 | 4 | 0.63 | 0.00 | 0.58 |
| RM190 | 0.67 | 2 | 15 | 2 | 0.44 | 0.00 | 0.35 |
| RM339 | 0.53 | 2 | 15 | 2 | 0.50 | 0.00 | 0.37 |
| RM122 | 0.87 | 2 | 15 | 2 | 0.23 | 0.00 | 0.20 |
| RM169 | 0.80 | 2 | 15 | 2 | 0.32 | 0.00 | 0.27 |
| RM485 | 0.50 | 5 | 15 | 4 | 0.61 | 0.07 | 0.53 |
| RM342 | 0.50 | 3 | 15 | 2 | 0.50 | 0.07 | 0.38 |
| RM204 | 0.57 | 5 | 15 | 4 | 0.60 | 0.07 | 0.55 |
| RM85 | 0.67 | 3 | 15 | 3 | 0.48 | 0.00 | 0.41 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| RM1 | 0.53 | 3 | 15 | 3 | 0.55 | 0.00 | 0.46 |
| RM152 | 0.73 | 3 | 15 | 3 | 0.42 | 0.00 | 0.37 |
| RM11 | 0.53 | 3 | 15 | 3 | 0.55 | 0.00 | 0.46 |
| RM154 | 0.60 | 4 | 15 | 4 | 0.58 | 0.00 | 0.53 |
| RM287 | 0.60 | 3 | 15 | 3 | 0.55 | 0.00 | 0.48 |
| RM210 | 0.57 | 5 | 15 | 4 | 0.59 | 0.47 | 0.53 |
| RM44 | 0.53 | 3 | 15 | 3 | 0.53 | 0.07 | 0.42 |
| RM253 | 0.80 | 3 | 15 | 3 | 0.34 | 0.00 | 0.31 |
| RM232 | 0.60 | 3 | 15 | 3 | 0.56 | 0.00 | 0.50 |
| RM42 | 0.87 | 2 | 15 | 2 | 0.23 | 0.00 | 0.20 |
| RM337 | 0.57 | 4 | 15 | 3 | 0.54 | 0.07 | 0.45 |
| RM72 | 0.57 | 3 | 15 | 2 | 0.49 | 0.07 | 0.37 |
| RM38 | 0.80 | 2 | 15 | 2 | 0.32 | 0.00 | 0.27 |
| RM447 | 0.33 | 4 | 15 | 4 | 0.72 | 0.00 | 0.67 |
| RM310 | 0.47 | 4 | 15 | 4 | 0.65 | 0.00 | 0.59 |
| RM331 | 0.53 | 2 | 15 | 2 | 0.50 | 0.00 | 0.37 |
| Mean | 0.63 | 3.14 | 15 | 2.93 | 0.48 | 0.03 | 0.41 |

### 3.4 Allelic diversity with SSR markers

SSR markers covering 12 chromosomes were utilized to analyze the genetic diversity and discriminate aromatic from non-aromatic varieties from different locations in Myanmar. Out of the thirty-five SSR markers screened, twenty-nine were found to be polymorphic, while the remaining six markers were monomorphic and therefore discarded. A total of 85 alleles were recorded, with the number of alleles per locus varying from 2 to 5 and averaging at 2.93 (Table 6).

The average number of alleles found in the current study was higher than the average number of alleles reported by Shah et al. [20]. In contrast, the average number of alleles observed during this study was lower compared to the findings of Jayamani et al., Thomson et al. and Pervaiz et al. [41-43] who reported an average of 7.7, 13.0 and 4.5 alleles per locus, respectively. The inconsistency observed in the number of alleles per locus among reports could be a result of the genotypes utilized and the selection of microsatellite primers with identifiable alleles [20].

PIC is a measure indicating the degree of polymorphism or genetic variation within a population [12]. PIC values ranged from 0.2 to 0.67 with an average of 0.41 and the highest PIC value was obtained with RM447 (Fig. 1). Seven out of the twenty-nine markers had PIC values above 0.5. Prasad et al. [12] stated that genetic markers with a PIC value of 0.5 or more are particularly informative for genetic research, and they can effectively differentiate the polymerphism rate of a specific locus. Major allele frequency varied from 0.33 to 0.87. The capacity of each of the 29 microsatellite markers to identify genetic diversity among varieties ranged from 0.23 to 0.72, with an average of 0.48. Heterozygous alleles for certain markers were observed in some accessions. The majority of the SSR markers had zero observed heterozygosity, implying that the majority of the rice cultivars used in this investigation was pure and completely homozygous.

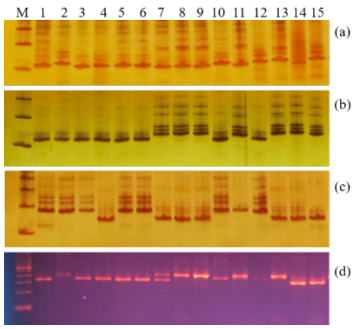


**Fig. 1.** Microsatellite profiles of rice varieties. M = Molecular weight marker (100 bp DNA ladder). Numbers on the top of the lanes correspond to genotypes given in Table 1. (a) RM447, (b) RM11 and (c) RM339 in polyacrylamide gel, (d) RM485 amplification on agarose gel showing polymorphisms among 15 rice varieties.

In our study, 14 SSR markers (RM223, RM590, RM510, RM252, RM169, RM485, RM204, RM85, RM1, RM11, RM154, RM287, RM232 and RM38) were detected as informative markers for genetic diversity studies based on the banding pattern. Despite producing two alleles, some of them were strong enough to discriminate diverse genotypes or different accessions of the same group. Therefore, the information about the genetic diversity obtained by using SSR markers from this study will prove highly valuable for proper identification and selection of suitable parents in breeding programs. The examination of genetic diversity within a breeding population is crucial as it forms the foundation of any breeding and improvement initiative. By introducing foreign genes, it aids in the development of crop that is appropriate for and adaptive to rapid climate change [44]. Assessing genetic diversity using SSR markers is important as it provides unbiased and accurate evaluations and reveals in-depth information about the genetic differences among germplasm materials [45].

The PS group exhibited a clear distinction between non-aromatic and aromatic accessions through the utilization of five SSR markers situated on chromosome 8, comprising RM339 for PSH1 and four additional SSR markers (RM342, RM38, RM447 and RM310) for PSBK2 (Fig. 1). RM590 is a marker of choice for distinguishing aromatic cultivars PSBK1 from PSBK3, PSH2 and PSH3. This variety specific allele (RM590) can be utilized in identifying varieties and creating DNA fingerprints, even for closely related PS varieties. This suggests that the markers mentioned above could be employed for genetic analysis of PS aromatic rice cultivars from diverse regions.

Three SSR markers (RM223, RM152 and RM447) on chromosome 8 could discriminate all aromatic LTM varieties from non-aromatic check varieties, while two SSR markers RM223 and RM42 could also differentiate aromatic NMTL3 from non-aromatic NMTL accessions (Table 4). RM252 and RM287 are ideal for distinguishing between two rice varieties (NMTL1 and NMTL3) of different colours but similar size and shape. These two markers have the potential to distinguish and differentiate closely related NMTL varieties for variety identification and DNA fingerprinting. Five SSR markers (RM342, RM152, RM38, RM447 and RM310) on chromosome 8 could discriminate NMTL2 as non-fragrant accession from NMTL group.

Seven primers (RM510, RM485, RM1, RM11, RM44, RM337 and RM331) were able to separate a clear grouping of the accessions according to their grain shape (Fig. 1). SSR markers located on chromosome 8, namely RM85, RM152, RM44, RM42, RM337, RM72, and RM331, amplified identical alleles across all six PS varieties. On the other hand, markers located on different chromosomes such as RM223, RM277, RM122, RM204, RM152, RM154, RM253, and RM42 amplified similar alleles in both PS and LTM groups but not in the NMTL group and non-aromatic check varieties.

Two non-aromatic varieties PSBK2 from PS varieties and NMTL2 from NMTL group give different alleles from their relative varieties with RM485, RM342, RM287, RM232, RM38, RM447 and RM310 (Fig. 1). The current study showed no significant genetic variation within the PS group or the LTM group, although there was variation in NMTL group and check varieties.

### 3.5 Cluster analysis and genetic relationship

A dendrogram based on UPGMA using the binary data from 29 SSR markers classified the 15 rice varieties into two major clusters (Fig. 2). Cluster I contained all the short and medium bold type PS varieties and the two extra short bold type NMTL varieties

and was subsequently divided into two sub-clusters: i and ii. In sub-cluster i, PSBK3, PSH2 and PSH3 (fragrant rice accessions) were grouped with PSBK1 and PSH1 (non-fragrant rice accessions), while in sub-cluster ii, PSBK2 (non-fragrant rice accession) was separated distantly from the rest of PS group and clustered together with NMTL1 (non-fragrant rice accession) and NMTL3 (fragrant rice accession). Although the present study was based on the markers located on 12 chromosomes, it was found that three rice accessions (PSBK3, PSH2 and PSH3) are clustered together with 100% genetic similarity (Fig. 2).

Therefore, a higher number of markers will be needed to distinguish these three accessions. The PS group has a close relationship with NMTL1 and NMTL3 from NMTL group as compared to other varieties. Except for PSBK2 in sub-cluster ii, a group of five PS varieties from different regions were clustered together, indicating a narrow genetic base.

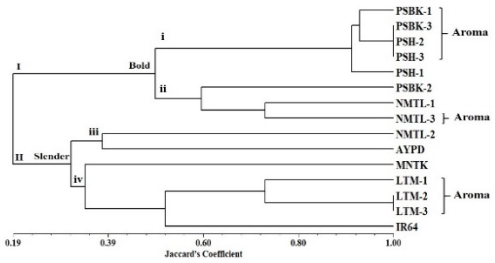


**Fig. 2.** Dendrogram derived from UPGMA cluster analysis based on Jaccard's similarity coefficient showing genetic diversity and relatedness among the 15 rice varieties.

Cluster II exhibited greater diversity, consisting of seven rice varieties (all fragrant LTM varieties and four non-fragrant varieties, being NMTL2, AYPD, MNTK and IR64) which were further subdivided into two sub-clusters, iii and iv. Sub-cluster iii consisted of two non-aromatic varieties (NMTL2 and AYPD), while sub-cluster iv consisted of LTM group that was clustered together with non-fragrant rice accessions (IR64 and MNTK). MNTK was quite divergent from the rest of IR64 and LTM varieties in sub-cluster iv. Interestingly, our results revealed that all the bold-grained types were completely separated from the rest of medium and slender grained rice varieties. Our findings are consistent with previous research, as Joshi and Behera [46] reported that 38 aromatic rice varieties could be classified into three major groups based on grain shape using cluster analysis. In addition, Sofi et al. [47] also observed that the rice varieties they studied were grouped together based on grain shape.

Jaccard's similarity matrix was utilized to determine the level of genetic relationship among studied accessions (Table 7). The pairwise genetic similarity indices revealed that the highest genetic similarity was among PSBK3, PSH2, and PSH3 (100%) as well as between LTM2 and LTM3 (100%). The lowest genetic similarity among rice varieties was between PSBK1 and AYPD (12%), NMTL1 and MNTK (12%), NMTL3 and MNTK (12%), and finally NMTL3 and IR64 (12%). Unsurprisingly, it was observed that genetic similarity between the aromatic and non-aromatic rice cultivars was comparatively low. In line with our findings, the study conducted by Pervaiz et al. [48] also demonstrated a clear differentiation between aromatic and non-aromatic rice varieties in the cluster analysis.

### 3.6 Molecular analysis with functional markers

Four functional markers, namely the ASA primer, *fgr1*, 3bpIn, and 8bpDel, were used to assess variations within the *badh2* gene, which is responsible for aroma in the aromatic rice accessions (Table 3).

**Table 7.** Jaccard's similarities among 15 rice accessions using 29 SSR markers.

| | PSBK1 | PSBK2 | PSBK3 | PSH1 | PSH2 | PSH3 | LTM1 | LTM2 | LTM3 | NMTL1 | NMTL2 | NMTL3 | AYPD | MNTK | IR64 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PSBK1 | 1.00 | | | | | | | | | | | | | | |
| PSBK2 | 0.49 | 1.00 | | | | | | | | | | | | | |
| PSBK3 | 0.93 | 0.53 | 1.00 | | | | | | | | | | | | |
| PSH1 | 0.86 | 0.49 | 0.93 | 1.00 | | | | | | | | | | | |
| PSH2 | 0.93 | 0.53 | 1.00 | 0.93 | 1.00 | | | | | | | | | | |
| PSH3 | 0.93 | 0.53 | 1.00 | 0.93 | 1.00 | 1.00 | | | | | | | | | |
| LTM1 | 0.21 | 0.29 | 0.24 | 0.27 | 0.24 | 0.24 | 1.00 | | | | | | | | |
| LTM2 | 0.20 | 0.25 | 0.23 | 0.26 | 0.23 | 0.23 | 0.73 | 1.00 | | | | | | | |
| LTM3 | 0.20 | 0.25 | 0.23 | 0.26 | 0.23 | 0.23 | 0.73 | 1.00 | 1.00 | | | | | | |
| NMTL1 | 0.45 | 0.60 | 0.49 | 0.45 | 0.49 | 0.49 | 0.23 | 0.20 | 0.20 | 1.00 | | | | | |
| NMTL2 | 0.22 | 0.19 | 0.22 | 0.19 | 0.22 | 0.22 | 0.28 | 0.24 | 0.24 | 0.14 | 1.00 | | | | |
| NMTL3 | 0.47 | 0.58 | 0.51 | 0.47 | 0.51 | 0.51 | 0.18 | 0.14 | 0.14 | 0.73 | 0.14 | 1.00 | | | |
| AYPD | 0.12 | 0.16 | 0.14 | 0.17 | 0.14 | 0.14 | 0.34 | 0.30 | 0.30 | 0.21 | 0.38 | 0.21 | 1.00 | | |
| MNTK | 0.15 | 0.14 | 0.17 | 0.20 | 0.17 | 0.17 | 0.38 | 0.28 | 0.28 | 0.12 | 0.36 | 0.12 | 0.33 | 1.00 | |
| IR64 | 0.15 | 0.17 | 0.17 | 0.20 | 0.17 | 0.17 | 0.49 | 0.53 | 0.53 | 0.14 | 0.36 | 0.12 | 0.39 | 0.44 | 1.00 |

ASA primer combination amplified the fragrant specific allele at 257 bp in three varieties (LTM2, LTM3 and NMTL3) and non-fragrant allele at 355 bp in 11 varieties (six PS varieties, two NMTL varieties and three check varieties). In one variety (LTM1), both the fragrant and non-fragrant loci were amplified when analyzed using ASA marker, indicating that it was heterozygous (Fig. 3a). Despite exhibiting a heterozygous allele, the LTM1 variety was found to possess aroma upon chemical analysis. In contrast to our results, Bradbury et al. [31] described that the presence of heterozygous alleles in the analysis using the ASA marker indicated the absence of fragrance in the rice variety.

When screening with the *fgr1* functional marker, we found that three LTM varieties and NMTL3 were aromatic with a 267 bp allele, while the other 11 accessions including three check varieties were non-aromatic with an allele size of 275 bp (Fig. 3b). Monomorphic or unambiguous bands were observed in all tested varieties using 8bpDel marker. The presence of the *badh2-E7* allele in four varieties (LTM1, LTM2, LTM3 and NMTL3) was demonstrated by the detection of the fragrant allele in ASA marker and *fgr1* analysis. The *badh2-E7* allele is highly prevalent among aromatic rice accessions, as nearly all of them carry this allele [31].

Although these three functional markers (ASA, *fgr1* and 8bpDel) were also not able to identify PS group as fragrant rice accessions, the 3bpIn marker could identify fragrant PS varieties from non-fragrant ones (Table 8). The 3bpIn marker could identify PSBK1, PSBK3, PSH2 and PSH3 as fragrant rice accessions at 197 bp (Fig. 3c), and also phenotypic analysis reconfirmed them as fragrant rice accessions. PSBK2 and PSH1 produce neither the *fgr* genotype nor the fragrant phenotype even though they were classified as PS accessions. In agreement with our findings, Myint et al. [14] reported that a 3-bp insertion in exon 13 of Os2AP was identified as a prominent allele in aromatic rice cultivars from Myanmar. Akwero et al. [49] noted that functional markers within the *badh2* gene offer the advantage of direct selection of accessions containing the gene responsible for the aromatic phenotype. This overcomes the restrictions of sensory analysis. The development of a fragrant line typically requires almost ten years due to extensive screening and field trials, though using the functional marker assessment system could aid in the development of fragrant rice within a more brief time frame [19].

**Table 8.** Functional marker-based assessment of rice aroma.

| Markers | PSBK1 | PSBK2 | PSBK3 | PSH 1 | PSH 2 | PSH 3 | LTM 1 | LTM 2 | LTM 3 | NMTL 1 | NMTL 2 | NMTL3 | AYPD | MNTK | IR64 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ASA | NA | NA | NA | NA | NA | NA | A | A | A | NA | NA | A | NA | NA | NA |
| 3bpIn | A | NA | A | NA | A | A | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| *fgr1* | NA | NA | NA | NA | NA | NA | A | A | A | NA | NA | A | NA | NA | NA |

## 4. Conclusion

SSR markers are an efficient tool for evaluating genetic diversity and determining rice accessions from various regions. Eight SSR markers, RM339, RM342, RM38, RM447, RM310, RM223, RM152 and RM42 on chromosome 8, could clearly differentiate non-aromatic from aromatic rice accessions, and these trait-specific markers could be used in marker-assisted selection. Microsatellite analysis has revealed certain cultivar-specific alleles that can be used in cultivar identification and the creation of DNA fingerprints. PSBK3, PSH2 and PSH3 in PS group and LTM2 and LTM3 in LTM group had 100% genetic similarity which indicates the potential existence of a moderately comparable genetic profile, requiring additional markers to distinguish them. Functional marker analysis indicated the presence of the *badh2-E7* allele in four rice accessions (LTM1, LTM2, LTM3 and NMTL3) and *badh2-E13* allele in four rice accessions (PSBK1, PSBK3, PSH2 and PSH3) within this collection. The proline content in leaf tissues at the flowering stage was significantly and positively correlated with the aroma of rice grains and leaf tissues. To obtain more conclusive data, proline content should be analyzed at different developmental stages. This study demonstrated that a combination of sensory analysis, proline determination and molecular marker analysis can provide a simple, cost-effective, and fast approach to distinguish fragrant from non-fragrant accessions. The integration of these three approaches would be valuable in a breeding attempt for the improvement of highly aromatic rice varieties.

## Acknowledgements

Department of Biotechnology Research (DBR), Ministry of Science and Technology, Kyaukse City, Myanmar provided valuable support for this study. The authors extend their heartfelt gratitude to Professor Dr. Aye Aye Khai, Director General, DBR for her valuable guidance. The authors also appreciate Seed Bank, Department of Agricultural Research, Ministry of Agriculture and Livestock, Nay Pyi Taw, Myanmar for providing rice seeds. The authors would like to express their gratitude to Dr. Myat Minn and his team at DBR for their assistance. The authors are also grateful to panelists from DBR for performing the sensory analysis to determine the aroma.

## References

[1] Bazrkar-Khatibani L, Fakheri B-A, Hosseini-Chaleshtori M, Mahender A, Mahdinejad N, Ali J: Genetic Mapping and Validation of Quantitative Trait Loci (QTL) for the Grain Appearance and Quality Traits in Rice (*Oryza sativa* L.) by Using Recombinant Inbred Line (RIL) Population. International Journal of Genomics 2019, 2019:3160275.

[2] Mané I, Bassama J, Ndong M, Mestres C, Diedhiou PM, Fliedel G: Deciphering urban consumer requirements for rice quality gives insights for driving the future acceptability of local rice in Africa: Case study in the city of Saint-Louis in senegal. Food Science Nutrition, 2021;9(3):1614-24.

[3] Kovach MJ, Calingacion MN, Fitzgerald MA, McCouch SR: The origin and evolution of fragrance in rice (Oryza sativa L.). Proceedings of the National Academy of Sciences, 2009;106(34):14444-9.

[4] Jaiswal HK, Waza SA, Rai VP: Validation of Functional Markers Associated with Genes for Fragrance in Rice (*Oryza sativa* L.). Indian Journal of Plant Genetic Resources, 2019;32(2):217-22.

[5] Thein M: Genetic Diversity of Meedon Rice (*Oryza sativa* L.) Germplasm in Myanmar. Yezin, Myanmar: PhD Thesis. Yezin Agricultural University; 2011.

[6] Nwe KT, Myint TT: Aspects of quality rice improvement in Myanmar. Rice Research Activities in Myanmar Commemoration of International Year of Rice Publication, Myanmar, 2004:59-76.

[7] Myint KM, Courtois B, Risterucci A-M, Frouin J, Soe K, Thet KM, Vanavichit A, Glaszmann J-C: Specific patterns of genetic diversity among aromatic rice varieties in Myanmar. Rice, 2012;5(1):1-13.

[8] Mathure SV, Jawali N, Thengane RJ, Nadaf AB: Comparative quantitative analysis of headspace volatiles and their association with BADH2 marker in non-basmati scented, basmati and non-scented rice (*Oryza sativa* L.) cultivars of India. Food chemistry, 2014;142:383-91.

[9] Lorieux M, Petrov M, Huang N, Guiderdoni E, Ghesquière A: Aroma in rice: genetic analysis of a quantitative trait. Theoretical Applied Genetics, 1996;93:1145-51.

[10] Lestari AP, Abdullah B, Junaedi A, Aswidinnoor H: Performance of grain quality and aroma of aromatic new plant type promising rice lines. Indonesian Journal of Agricultural Science, 2011;12(2):83-93.

[11] Mo Z, Li W, Pan S, Fitzgerald TL, Xiao F, Tang Y, Wang Y, Duan M, Tian H, Tang X: Shading during the grain filling period increases 2-acetyl-1-pyrroline content in fragrant rice. Rice, 2015;8:1-10.

[12] Prasad G, Padmavathi G, Suneetha K, Madhav M, Muralidharan K: Assessment of diversity of Indian aromatic rice germplasm collections for morphological, agronomical, quality traits and molecular characters to identify a core set for crop improvement. CABI Agriculture and Bioscience, 2020;1(13):1-24.

[13] Amarawathi Y, Singh R, Singh AK, Singh VP, Mohapatra T, Sharma TR, Singh NK: Mapping of quantitative trait loci for basmati quality traits in rice (*Oryza sativa* L.). Molecular Breeding, 2008;21:49-65.

[14] Myint KM, Arikit S, Wanchana S, Yoshihashi T, Choowongkomon K, Vanavichit A: A PCR-based marker for a locus conferring the aroma in Myanmar rice (*Oryza sativa* L.). Theoretical Applied Genetics, 2012;125:887-96.

[15] Bradbury LM, Fitzgerald TL, Henry RJ, Jin Q, Waters DL: The gene for fragrance in rice. Plant biotechnology journal, 2005;3(3):363-70.

[16] Hashemi FG, Rafii M, Ismail M, Mahmud T, Rahim H, Asfaliza R, Malek M, Latif M: Biochemical, genetic and molecular advances of fragrance characteristics in rice. Critical Reviews in Plant Sciences, 2013;32(6):445-57.

[17] Kongchum M, Harrell DL, Linscombe SD: Comparison of 2-Acetyl-1-Pyrroline (2AP) in Rice Leaf at Different Growth Stages Using Gas Chromatography. Agricultural Sciences, 2022;13(2):165-76.

[18] Sood B, Siddiq E: A rapid technique for scent determination in rice [India]. Indian Journal of Genetics and Plant Breeding, 1978;38:268-71.

[19] Kottearachchi N, Priyangani E, Attanayaka D: Identification of fragrant gene, fgr, in traditional rice varieties of Sri Lanka. J Natl Sci Foundation of Sri Lanka, 2010;38(2):139-43.

[20] Shah SM, Naveed SA, Arif M: Genetic diversity in basmati and non-basmati rice varieties based on microsatellite markers. Pak J Bot, 2013;45(S1):423-31.

[21] Jasim Aljumaili S, Rafii M, Latif M, Sakimin SZ, Arolu IW, Miah G: Genetic diversity of aromatic rice germplasm revealed by SSR markers. BioMed research international, 2018;2018(7658032):1-11.

[22] Schulman AH: Molecular markers to assess genetic diversity. Euphytica, 2007;158(3):313-21.

[23] Miah G, Rafii MY, Ismail MR, Puteh AB, Rahim HA, Islam KN, Latif MA: A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. International journal of molecular sciences, 2013;14(11):22499-528.

[24] Rai VP, Singh AK, Jaiswal HK, Singh SP, Singh RP, Waza SA: Evaluation of molecular markers linked to fragrance and genetic diversity in Indian aromatic rice. Turkish Journal of Botany, 2015;39(2):209-17.

[25] Andersen JR, Lübberstedt T: Functional markers in plants. Trends in plant science, 2003;8(11):554-60.

[26] Bocevska M, Aldabas I, Andreevska D, Ilieva V: Gelatinization behavior of grains and flour in relation to physico-chemical properties of milled rice (*Oryza sativa* L.). Journal of food quality, 2009;32(1):108-24.

[27] Myint M M, Yee Soe A N, Thandar S, Lin T T, Aung W, Lynn T M. Evaluation of physico-chemical characteristics and genetic di-versity of widely consumed rice varieties in Kyaukse area, Myanmar. Plant Science Today, 2023;10(2): 415-25.

[28] Yi M, Nwe KT, Vanavichit A, Chai-arree W, Toojinda T: Marker assisted backcross breeding to improve cooking quality traits in Myanmar rice cultivar Manawthukha. Field Crops Research, 2009;113(2):178-86.

[29] Ábrahám E, Hourton-Cabassa C, Erdei L, Szabados L: Methods for determination of proline in plants. Plant Stress Tolerance*:* Methods and Protocols, 2010, pp. 317-31.

[30] Doyle JJ, Doyle JL: A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 1987;19(1).

[31] Bradbury LM, Henry RJ, Jin Q, Reinke RF, Waters DL: A perfect marker for fragrance genotyping in rice. Molecular Breeding, 2005;16:279-83.

[32] Jin L, Lu Y, Shao Y, Zhang G, Xiao P, Shen S, Corke H, Bao J: Molecular marker assisted selection for improvement of the eating, cooking and sensory quality of rice (*Oryza sativa* L.). Journal of Cereal Science, 2010;51(1):159-64.

[33] Rohlf FJ: NTSYSpc version 2.0: user guide. Applied Biostatistics Inc, 1998:1-38.

[34] Liu K, Muse SV: PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics, 2005;21(9):2128-29.

[35] Cruz ND, Khush G: Rice grain quality evaluation procedures. In: *Aromatic rices.* Edited by Singh RK, Singh, U.S. and Khush, G.S., vol. 3. New Delhi: Oxford and IBH Publishing Co Pvt. Ltd.; 2000: 15-28.

[36] Ahmad Sarhadi W, Ookawa T, Yoshihashi T, Khalid Madadi A, Yosofzai W, Oikawa1 Y, Hirata Y: Characterization of aroma and agronomic traits in Afghan native rice cultivars. Plant production science, 2009;12(1):63-9.

[37] Golam F, Prodhan ZH: Kernel elongation in rice. Journal of the Science of Food and Agriculture, 2013;93(3):449-56.

[38] Aye HN, Khaing AA, Tun PT, Htun TM, Win KK: Grain Quality of Paw San Varieties from Pathein, Pyapon and Shwebo Townships. Journal of Agricultural Research, 2019;6:2.

[39] Lehmann S, Funck D, Szabados L, Rentsch D: Proline metabolism and transport in plant development. Amino acids, 2010;39:949-62.

[40] Hinge VR, Patil HB, Nadaf AB: Aroma volatile analyses and 2AP characterization at various developmental stages in Basmati and Non-Basmati scented rice (*Oryza sativa* L.) cultivars. Rice, 2016;9(1):1-22.

[41] Jayamani P, Negrao S, Martins M, Macas B, Oliveira M: Genetic relatedness of Portuguese rice accessions from diverse origins as assessed by microsatellite markers. Crop Science, 2007;47(2):879-84.

[42] Thomson MJ, Septiningsih EM, Suwardjo F, Santoso TJ, Silitonga TS, McCouch SR: Genetic diversity analysis of traditional and improved Indonesian rice (*Oryza sativa* L.) germplasm using microsatellite markers. Theoretical and Applied Genetics, 2007;114:559-68.

[43] Pervaiz ZH, Rabbani MA, Khaliq I, Pearce SR, Malik SA: Genetic diversity associated with agronomic traits using microsatellite markers in Pakistani rice landraces. Electronic Journal of Biotechnology, 2010;13(3):4-5.

[44] Alam MA, Juraimi AS, Rafii M, Hamid AA, Arolu IW, Latif M: Application of EST-SSR marker in detection of genetic variation among purslane (*Portulaca oleracea* L.) accessions. Brazilian Journal of Botany, 2015;38:119-29.

[45] Ahmad F, Hanafi MM, Hakim MA, Rafii MY, Arolu IW, Akmar Abdullah SN: Genetic divergence and heritability of 42 coloured upland rice genotypes (*Oryza sativa*) as revealed by microsatellites marker and agro-morphological traits. PLoS One, 2015;10(9):e0138246.

[46] Joshi RK, Behera L: Identification and differentiation of indigenous non-Basmati aromatic rice genotypes of India using microsatellite markers. African journal of Biotechnology, 2007:6(4).

[47] Sofi NR, Mushtaq RS, Yetoo N-U-N, Rafiqee S, Khan RS, Mir S, Dar MS, Shikari A, Mir RR, Khan G et al: Identification and characterization of rice (*Oryza sativa* L.) advanced breeding lines to uncover novel genes for engineering new genotypes in response to agro-morphological traits and blast disease. In.: Research Square; 2022.

[48] Pervaiz ZH, Rabbani MA, Pearce SR, Malik SA: Determination of genetic variability of Asian rice (*Oryza sativa* L.) varieties using microsatellite markers. African Journal of Biotechnology, 2009;8(21):5641-51.

[49] Akwero A, Ocan D, Akech W, Lamo J, Ochwo-Ssemakula M, Rubaihayo P: Allelic variations in aroma gene in cultivated rice varieties. African Crop Science Journal, 2020;28(2):241-54.