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RESEARCH ARTICLE

Evaluation of physicochemical characteristics and genetic diversity of widely consumed rice varieties in Kyaukse area, Myanmar

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Abstract

Consumer preferences are greatly influenced by eating and cooking quality of rice grains, along with the economic value of a specific rice variety. This study was to evaluate ten rice varieties including the check variety IR64 on their physicochemical, cooking and eating quality as well as to identify their genetic diversity using SSR markers. Most rice varieties are medium-grain types based on length-breadth ratio, whereas the famous Myanmar rice variety, *Paw San Bay Kyar*, (PSBK) is bold. PSBK showed the best cooking and eating quality traits with intermediate amylose content (AC), intermediate gelatinization temperature (GT), soft gel consistency (GC), and the highest elongation ratio among the studied rice varieties. Seventeen SSR markers linked with cooking and eating traits were used to assess the extent of polymorphism and genetic variation among ten rice varieties. There were 49 alleles in total, with an average of 2.88 alleles per locus. RM592 had the maximum number of alleles. The average PIC value ranged from 0.22 (RM540) to 0.77 (RM592). Cluster analysis with UPGMA method based on Jaccard's similarity coefficient divided ten rice varieties into two main groups and four sub-clusters. In multiple regression analysis, RM190 and Wx primers were discovered to be significantly associated with AC, GC and GT of cooking and eating quality traits. This study could contribute to the choice of rice varieties with superior cooking and eating quality for rice breeding programmes by implementing physicochemical characteristics and molecular analysis .

Keywords

rice; cooking and eating quality; SSR markers; genetic diversity; multiple regression analysis

Introduction

Rice (*Oryza sativa* L.) is mainly cultivated in Asia because the climate there is ideal for rice cultivation. In Asia and some parts of the Pacific, rice is the staple food. The Asia-Pacific region is where more than 90% of the world's rice is cultivated and consumed (1). Myanmar is the world's seventh-largest rice producer, with rice accounting for approximately 43% of total agricultural production. Out of 67.6 million hectares of land in Myanmar, 12.8 million are used in cultivation (https://www.trade.gov/country-commercial-guides/burma-agriculture). According to a survey conducted by Myanmar Rice Federation in 2016, the average annual per capita consumption of the nation is projected to be 115 kilograms.

Consumer choice of rice is generally governed by taste, price, convenience, variety and quality. Rice grain quality, cooking and eating quality are critical in determining its economic value in the export market as well as consumer acceptance (2). Physical properties of the grains including size, shape, colour, uniformity, general appearance, chalkiness, milling and head rice recovery, are the most important traits determining the overall quality (3). The preference of grain size and shape varies among rice consuming countries. Long grain rice marketed for a high price on the international market. However, the most famous aromatic indigenous rice varieties of Myanmar, known as the Paw San varieties, are short-bold types. Paw San rice varieties are premium quality rice of Myanmar and awarded the World's best rice in 2011, and have a high potential for export revenue (4).

The three primary criteria directly related to the quality of the rice for cooking and eating are amylose content (AC), gelatinization temperature (GT), and gel consistency (GC). AC is the major criterion for milled rice's cooking and eating quality (5). Majority of Myanmar people prefer intermediate amylose rice (23-24%) contributing to the quality of hardness (6). AC is primarily controlled by waxy gene (Wx) located on chromosome 6. Wx encodes the enzyme granule-bound starch synthase, which is essential for rice amylose synthesis (7). GT is defined by the alkali spreading value (ASV), which is related to the chain length distribution of amylopectin, and it represents how easy or difficult it is to boil the rice (8). There is a negative correlation between GT and the resistance of seeds to alkali digestion; low GT varieties are significantly easier to digest when consumed, and vice versa (9). The GC is a measure of firmness of the rice after cooking and is performed to classify rice with the same amylose content, particularly those with high AC into hard, medium or soft texture (10). In general, soft GC and low GT are usually considered to be of fine cooking and eating quality (11). Some research works on loci controlling rice physicochemical properties have reported that these three parameters are under the control of the waxy locus or a genomic region closely related to this locus (7). Moreover, elongation, volume expansion, and water absorption are all important factors in determining the quality of cooked rice grains (12, 13). Therefore, it can be said that the quality of rice grains for cooking and eating is a crucial basic quality component that must be prioritized in future rice breeding programmes to serve market demands, on both a local and global scales.

Developing rice varieties with high cooking and eating quality has been challenging because of the genetic complexity of these quality and the difficulties in accurately evaluating them in early breeding generations (14). The development of DNA markers has provided new opportunities for the genetic improvement of rice grain quality and the use of DNA markers can complement physicochemical analyses, contributing for studies of rice quality assessment. Molecular markers have been shown to be useful in several types of genetic research, such as construction of molecular maps and gene mapping, genetic purity test, construction of fingerprints, analysis of germplasm diversity, and in molecular assisted breeding (15, 16). Methods that use SSR markers, are fast and cost-effective when it comes to assessing the quality of rice. SSR markers have been used for comparative analysis of specific regions of rice genome and quality evaluation of rice varieties (17, 18).

In the present study, we used 17 SSR markers linked to eating and cooking quality to evaluate the genetic variation and also investigated physicochemical properties of nine rice varieties that are widely consumed in Kyaukse area of Myanmar, and one check variety, IR64. The objective of this research was to determine the potential of these markers for genetic variation among rice varieties and to suggest the most informative markers for marker assisted selection.

Materials and Methods

Sample Collection and Preparation

Nine rice varieties that were mostly preferred in Kyaukse area, Mandalay Region and the check variety IR64 were kindly provided by Department of Agricultural Research (DAR), Kyaukse, Myanmar (Table 1). Fifteen paddy seeds of each rice variety were pre-germinated in petri and dishes. After three days, these sprouted seeds were hydroponically cultivated in trays containing Peters' nutrient solution. The pH was adjusted to 5.0, and the volume of the Peters' solution was adjusted to the level of touching the seedling float at two-day intervals. Fresh young leaves of 21-day old rice seedlings were used for DNA extraction. The rice grains were stored under dry condition with similar moisture content to facilitate further determination of physicochemical traits. Dehusking and polishing activities were done using a sheller and a rice polisher (Satake Corp., Japan) in Plant and Agricultural Biotechnology Research Department, Department of Biotechnology Research (DBR), Kyaukse.

Table 1. List of rice varieties used in this study

No.	Rice	Accession	Origin
1	Paw San Bay Kyar	PSBK	Myanmar
2	Ayarmin	AYM	Malaysia
3	Sin Thu Kha	STK	Myanmar
4	Ma Naw Thu Kha	MNTK	India
5	A Ka Ri Mhwe	AKRM	IRRI
6	Lone Thwe Mhwe	LTM	Thailand
7	Shwe Thwe Yin	STY	Philippines
8	Shwe Ma Naw	SMN	India
9	Y90	Y90	Thailand
10	IR64	IR64	Philippines

Physicochemical and Cooking Characteristics of Rice Varieties

All rice varieties were examined for physicochemical characteristics and cooking quality. The length-width ratio, grain size, grain shape, and alkali spreading value of whole grain were investigated. The raw rice was powdered in a blender (Panasonic, Japan) and analyzed for gel consistency and amylose content. Further, the rice was cooked and analyzed for its water absorption ratio, volume expansion ratio, elongation ratio and cooked length-breadth ratio.

Grain dimension

A total of ten intact grains were randomly selected from each sample for the measurement of length and width. The measurements were carried out with a portable vernier caliper (SOLEX). The measurement of length classifies grains into the following categories: short (<5.5mm), medium (5.5-6.6mm), long (6.6-7.5mm) and extra-long (>7.5mm). The ratio of length to width determines the shape of grains as follows: slender (>3.0mm), medium (2.1-3.0mm), bold (1.1-2.0mm) and round (<1.1mm) (19).

Determination of AC

AC of rice varieties was determined by using a spectrophotometric technique (20). Milled rice samples were ground to pass through a 100-mesh sieve. Finely ground 100 mg rice powder was transferred in triplicate to 100 ml volumetric flasks and 1 ml of 95% ethanol and 9 ml of 1 N NaOH were carefully added and heated for 10 min, then cooled for 5 min and the volumes were made up to 100 ml. Five millilitres of starch solution were pipetted into a 100 ml volumetric flask and then 1 ml of 1 N acetic acid and 2 ml of iodine solution were added. The solution was made up to volume with distilled water, shaken and allowed to stand for 20 min. Absorbance was measured at 620 nm with a UV-Vis spectrophotometer (UV2950 Labomed, USA).

Determination of GT

GT was indexed by the alkali spreading test (21). Alkali spreading value involved incubating six grains of intact milled rice, which were placed on a petri dish to which 5 mL of 1.7% KOH was added and incubated at 30 °C for 23 h. It measures the degree of spreading using a seven point scale based on kernels (1 = not affected, 2 = swollen, 3 = swollen collar complete or narrow, 4 = swollen, collar complete and wide, 5 = split or segregated; collar complete and wide, 6 = dispersed; merging with collar, 7 = completely dispersed and intermingled) and corresponds to GT as follows: 1-3, high (75-79 ˚C); 4-5, intermediate (70-74 ˚C); and 6-7, low (55-69 ˚C) (IRRI). A low ASV corresponds to a high GT; conversely, a high ASV indicates a low GT (22).

Determination of GC

Finely ground 100 mg rice powder was placed into 13x100 mm tubes and wetted with 0.2 ml of thymol blue (95% ethanol containing 0.025% thymol blue). The tube was shaken to suspend the starch by using a vortex at 6 speed, and then 2 ml of 0.2 N KOH was immediately added and the mixture was dispersed using a vortex. The tubes were covered with glass marbles and put into a vigorously boiling water bath to reflux for 8 min. The samples were then removed from the water bath to set at room temperature for 5 min and then cooled in an ice water bath for 20 min. The tubes were then laid horizontally on a table lined with millimetre graph paper, and the length of the gel was measured from the bottom of the test tube to the top of the gel after 60 min (23).

Volume expansion ratio and water absorption ratio

Volume expansion ratio is calculated as volume of cooked rice to volume of raw rice, while water absorption ratio is determined by dividing the weight of cooked rice with that of raw rice (20).

The two ratios are calculated by the formulae given below:

$$\text{Volume expansion ratio} = \frac{\text{Cooked rice volume}}{\text{Raw rice volume}}$$

$$\text{Water absorption ratio} = \frac{\text{Cooked rice weight}}{\text{Raw rice weight}}$$

Elongation ratio

The elongation ratio is given by the average length of ten cooked rice divided by the average length of ten uncooked rice (22). The elongation ratio is expressed as:

$$\text{Elongation ratio} = \frac{\text{Average length of cooked rice (mm)}}{\text{Average length of uncooked rice (mm)}}$$

Cooked length-breadth ratio (CLBR)

The cooked rice length-breadth ratio is expressed using this equation (22).

$$\text{Cooked length-breadth ratio} = \frac{\text{Length of cooked rice (mm)}}{\text{Breadth of cooked rice (mm)}}$$

DNA Extraction

DNA was extracted from fresh young leaves of 21-day old rice seedlings according to the CTAB method with some modifications (24) in which CTAB and mercaptoethanol were substituted with 1 M KCL in the extraction buffer. DNA quality was checked by running in 1% agarose gel electrophoresis. DNA concentration and purity were determined by a Nanophotometer (IMPLEN P330, UK).

Microsatellite Markers Selection and PCR Amplification

Thirty-one microsatellite SSR markers linked to loci controlling rice eating and cooking characteristics were screened in our research. Out of 31 markers, 17 SSR markers that showed polymorphic banding patterns were selected for molecular analysis in 10 rice varieties (Table 2). Information about all the markers used in this study was obtained from Gramene database (http://www.gramene.org).

PCR reaction was conducted in a reaction solution of 10 µl containing 5 µl of 2x Taq DNA Polymerase Master Mix Kit (VWR, Denmark), 0.5 µL of each 10 µM forward and reverse primers, 3.5 µl ddH_2O and 0.5 µl of each genomic DNA (200 ng/µl). The PCR amplification was performed

Table 2. SSR markers used for the determination of molecular analysis

No	Primer	Chromosome No	Repeat Motif	Species	Expected Size (bp)	Annealing Temperature (˚C)
1	RM232	3	(CT)24	Indica	158	55
2	RM251	3	(CT)29	Indica	147	58
3	RM252	4	(CT)19	Indica	216	57
4	RM164	5	(GT)16TT(GT)4	Japonica	216	60
5	RM159	5	(GA)19	Japonica	248	67
6	RM592	5	(ATT)20	Japonica	270	55
7	RM3	6	(GA)2GG(GA)25	Indica	145	57
8	RM190	6	(CT)11	Indica	124	55
9	RM204	6	(CT)44	Indica	169	60
10	RM225	6	(CT)18	Indica	140	55
11	RM253	6	(GA)25	Indica	141	56
12	RM276	6	(AG)8A3(GA)33	Indica	149	60
13	RM314	6	(GT)8(CG)3 (GT)5	Indica	118	59
14	RM540	6	(AG)16	Japonica	172	55
15	RM541	6	(TC)16	Japonica	158	58
16	RM180	7	(ATT)10	Japonica	110	55
17	Wx	6	(CT)n	-	121	55

using a Proflex Thermal Cycler (Applied Biosystems, USA) according to the cycle profile: initial denaturation at 95˚C for 4 min and then 30 cycles of 30 sec denaturation at 95˚C, 30 sec annealing at the temperature depending on the marker used (55˚C to 67˚C), 30 sec extension at 72˚C for 1 min and 5 min at 72˚C for final product extension. The resultant PCR products were analyzed by electrophoresis in 8 % polyacrylamide gel in 1x TBE buffer at 120 V for

a period of 1:15 h. The gel was stained in silver nitrate solution (0.1%), and 0.4 M NaOH and 4% formaldehyde were used as a developer solution. The size of each band was determined by comparing it with the molecular weight marker 100 bp DNA ladder (TaKaRa, Japan).

Data Analysis

Analysis of variance (ANOVA) technique and Duncan's Multiple Range Test were applied by using the software programmes SPSS version 16 software (SPSS Inc., Chicago, IL, USA) with significance defined as $P<0.05$. Amplified bands were scored on the basis of their presence (1) or absence (0), separately for each variety of rice and each SSR primer. Using the PowerMarker version 3.25 software (25), the resulting binary matrix was analysed to determine polymorphic information content (PIC). Genetic similarities were estimated from the matrix of binary data using Jaccard's similarity coefficient. A phylogenetic tree was constructed by the Unweighted Pair Group Method Arithmetic Average (UPGMA) cluster analysis by using NTSYSpc Version 2.0 software (26). Correlation and relationship between chemical characteristics for eating quality and SSR markers were recorded using multiple regression analysis by SPSS software. Quality characteristics as dependent variables and scoring of amplified DNA as independent variables were considered (27).

Results & Discussion

Physical Characteristics of Rice Varieties

The physical and chemical characteristics of different rice varieties were compiled in Table 3. Consumers base their concept of cooking and eating quality on the appearance, size and shape of the grain. Aside from PSBK, the width of the rice grains showed slight variation among the tested rice varieties. According to our data, PSBK, AYM, STK, STY, SMN, and MNTK were short rice grains, while AKRM, LTM, Y90, and IR64 were medium-sized rice grains (Fig. 1). The grain size directly affects rice yield and it is an important determinant of rice quality (28). Of all different types of rice, Y90 was recorded the highest length-width ratio whereas PSBK was the lowest in length-width ratio followed by MNTK, SMN, STY, STK, AYM, IR64, LTM and AKRM. The rice length-width ratio is used to determine the shape of varieties, and increased in length of rice is mostly preferred (29). Based on the length-width ratio, PSBK was classified as a bold shaped rice variety. Thi *et al.* described that Paw San rice grains were short and broad, but became long and slender after cooking (30). This characteristic is highly desirable since short and broad grains reduce grain breaking during the milling process, while many customers like the slender shape of cooked grains. Six varieties such as AYM, STK, MNTK, STY, SMN, and IR64 exhibited medium type grain shape while the remaining three varieties; AKRM, LTM, and Y90 showed in slender shape. Grain size and grain shape are crucial factors for evaluating the quality of rice in breeding programmes designed to generate improved varieties for commercial production (31).

Chemical Characteristics of Rice Varieties

Generally, the rice grain is opted by the consumers from cooking and eating characteristics view point. AC and GT affect the texture and are the basic for determination of cooking and eating values. Amylose plays a vital role in gelatinization of starch and its consequent influence on cooking and eating quality of milled rice. Cooked rice becomes moist and sticky due to low AC, whereas rice containing high AC is less tender, and becomes hard upon cooling (32).

Shahidullah *et al.* reported that AC in all good grades of rice ranged between 20.7-21.4% (33). The amylose content of the studied rice varieties ranged from 17.84% to 29.8%. Amylose content was found as its maximum in STY and minimum in IR64 followed by LTM. A higher percentage of amylose content was shown in rice varieties: AYM, Y90, AKRM, MNTK, and SMN. PSBK and STK were indicated as having an intermediate percentage of AC (Table 3). This result indicated that both PSBK and STK varieties of local origin had intermediate AC which were mostly preferred by local people. Similarly, Singh *et al.* stated that rice with intermediate AC of 20-25% were the most preferred by consumers, as they were reported to

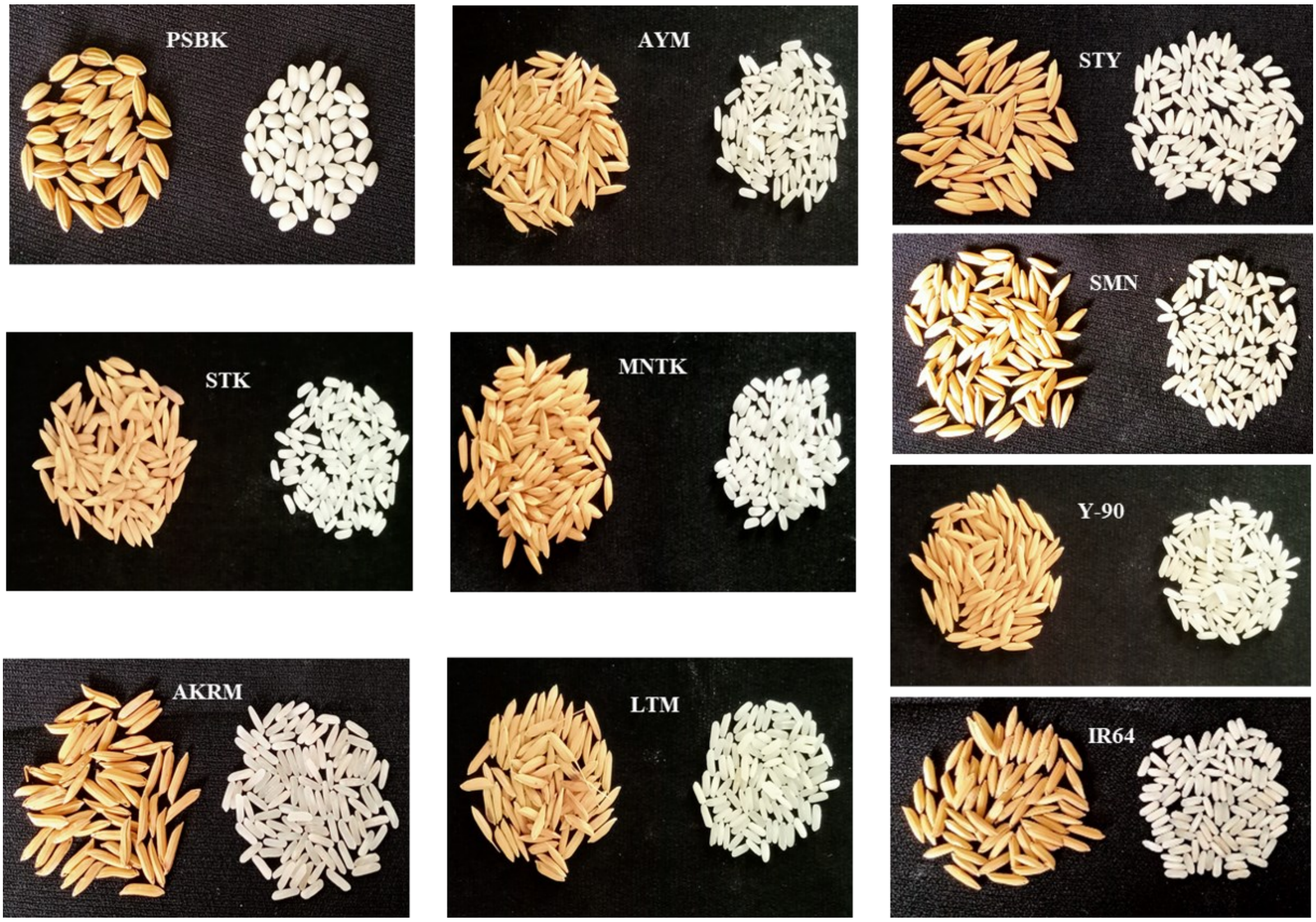

Fig. 1. Morphology of paddy and milled rice grains of ten rice varieties used in present study.

cook moist and remained soft even after cooling (13). Thomas *et al.* reported that amylose content played a significant role in determining the overall cooking and eating quality of a rice variety, even though the quality was also affected by proteins, lipids, or amylopectin (34).

It is assumed that chemical characteristics determine cooking quality and are positively or negatively connected with cooking characteristics (35). The alkali spreading value, which is an indirect method of determining the GT, of fine rice varieties is affected considerably due to various temperature treatments and storage intervals (36). In this study, the highest ASV and lowest GT were recorded in AKRM (Fig. 2). Consumer preference for low-GT rice is minimal due to the negative outcomes of linear kernel elongation, water absorption, and volume expansion of rice varieties (37). Rodríguez-Torres *et al.* reported that the rice variety with higher AC had higher GT (38). This observation was in line with the findings of our study regarding the AYM, MNTK, STY, and SMN rice varieties; nevertheless, it was in contradiction with the finding concerning the AKRM variety. Myanmar local variety PSBK and check variety IR64 were noted in intermediate ASV and GT which conferring good quality trait (Table 3).

In this study, rice varieties were classified into soft, medium, and hard GC. Such a classification could be possible as a result of the different expression levels of the waxy gene and the variation in the Wx gene locus (39). GC differs depending on the source of starch and variety of grains. Cooked rice with hard GC hardens faster than those with a soft one. Rice with a soft gel consistency cooks tenderly and remains its softness after cooling; hence, it is preferred by most consumer (22). The soft GC was found in PSBK, LTM and IR64 whereas AYM, STY, SMN, and Y90 were recorded as hard GC rice varieties (Table 3).

Cooking Characteristics of Rice Varieties

Cooking characteristics of rice are linked to consumer preferences and are very important as rice is consumed almost immediately after cooking (34). During cooking, rice kernels absorb water and increase in volume through an increase in length or breadth. Water uptake capacity of rice is related to tenderness, stickiness and palatability of cooked rice. In the present study, the variety AKRM exhibited a significantly higher water absorption value (3.09±0.04) than other varieties including the check variety IR64, although it had high ASV and low GT (Table 4). PSBK, AYM, STK and IR64 rice varieties also had the higher values of water absorption ratio. It was noted that the minimum water absorption ratio was obtained in SMN (2.2±0.05). The amount of water absorbed by rice during cooking is regarded as an economic quality as it gives some estimate of the volume increase during cooking (12). Keawpeng *et al.* found that water absorption of rice increased when the rice was hard and absorbed more water during cooking (40).

The volume expansion ratio of rice varieties was affected considerably by treatments and storage. The results showed a significant difference among the studied

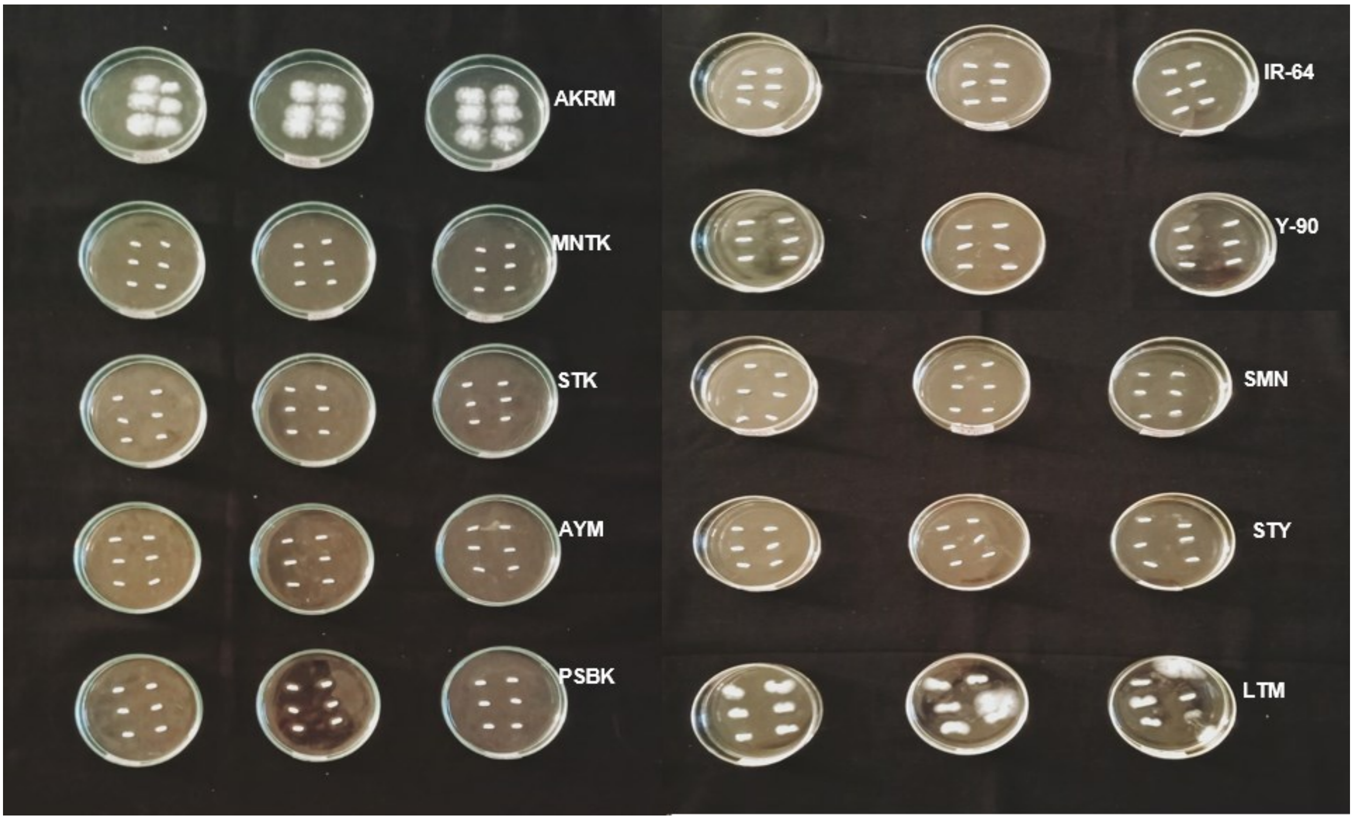

Fig. 2. Variation in gelatinization temperature (GT) among ten rice varieties.

Table 3. Physical and chemical characteristics of ten rice varieties

Rice	Grain Dimension					Amylose Content (%)	Alkali spreading value	Gelatinization Temperature	Gel Consistency
	Length (mm)	Width (mm)	L/W ratio	Grain Size	Grain Shape				
PSBK	4.75	2.47	1.93 ± 0.03^{g}	Short	Bold	21.66 ± 2.10^{de}	4.00 ± 0.0^{c}	Intermediate	Soft
AYM	5.42	1.98	2.74 ± 0.06^{d}	Short	Medium	28.68 ± 0.11^{ab}	3.72 ± 0.06^{d}	High	Hard
STK	5.4	2.00	2.70 ± 0.05^{d}	Short	Medium	24.23 ± 0.42^{cd}	3.22 ± 0.06^{e}	High-Intermediate	Medium
MNTK	5.05	2.01	2.51 ± 0.04^{f}	Short	Medium	26.14 ± 0.55^{abc}	2.94 ± 0.06^{f}	High	Medium
AKRM	6.14	1.95	3.15 ± 0.05^{a}	Medium	Slender	26.17 ± 2.25^{abc}	5.94 ± 0.06^{a}	Low	Medium
LTM	6.16	2.04	3.02 ± 0.02^{b}	Medium	Slender	18.47 ± 1.12^{e}	4.44 ± 0.06^{b}	Low-Intermediate	Soft
STY	5.33	1.98	2.69 ± 0.05^{de}	Short	Medium	29.80 ± 1.28^{a}	3.06 ± 0.06^{ef}	High	Hard
SMN	5.00	1.95	2.57 ± 0.02^{ef}	Short	Medium	25.46 ± 0.34^{bcd}	3.06 ± 0.06^{ef}	High	Hard
Y90	6.32	2.00	3.16 ± 0.05^{a}	Medium	Slender	26.83 ± 1.96^{abc}	3.61 ± 0.06^{d}	High-Intermediate	Hard
IR64	5.65	2.00	2.83 ± 0.07^{cd}	Medium	Medium	17.84 ± 0.33^{e}	3.67 ± 0.10^{d}	Intermediate	Soft

rice varieties (Table 4). The average volume expansion ratio ranged from Y90 (1.23±0.00) to AKRM (1.38±0.00). There was no significant difference among rice varieties; PSBK, AYM, STY, SMN, and IR64. Juliano revealed that rice with high AC showed high volume expansion ratio during cooking and cooked dry, becoming less tender and harder upon cooling (20, 21). That finding was not compatible with our results since volume expansion ratio of the studied rice varieties fluctuated regardless of AC.

Elongation ratio is an important criterion in determining the cooking and eating quality of rice. The grain elongation on cooking is dependent on genetic factors as well as the degree of milling (41). PSBK was recorded as having the highest elongation ratio (2.08±0.06), while IR64 was found to have the lowest (1.27±0.06) (Table 4). Cooked rice with a higher elongation ratio was preferred over rice with a lower elongation ratio, as observed by Shahidullah *et al.* although consumers preferred rice with a lower volume expansion ratio (33). Some rice varieties elongate more than others following hydration and starch gelatinization without increasing in girth, this is a desired cooking quality trait in most high

Table 4. Cooking characteristics of ten rice varieties

Rice	Water Absorption Ratio	Volume Expansion Ratio	Elongation Ratio	Cooked Length-Breadth Ratio
PSBK	2.62±0.06^{c}	1.25±0.04^{bc}	2.08 ± 0.06^{a}	3.13 ± 0.06^{ab}
AYM	2.63±0.05^{c}	1.3±0.00^{bc}	1.62 ± 0.07^{b}	2.93 ± 0.08^{bc}
STK	2.84±0.02^{b}	1.31±0.02^{b}	1.54 ± 0.04^{b}	2.75 ± 0.05^{cd}
MNTK	2.55±0.01^{c}	1.3±0.01^{b}	1.59 ± 0.04^{b}	2.65 ± 0.03^{d}
AKRM	3.09±0.04^{a}	1.38±0.00^{a}	1.59 ± 0.04^{b}	3.31 ± 0.09^{a}
LTM	2.4±0.06^{d}	1.32±0.03^{ab}	1.63 ± 0.02^{b}	3.26 ± 0.05^{a}
STY	2.58±0.03^{c}	1.26±0.02^{bc}	1.62 ± 0.05^{b}	2.92 ± 0.06^{bc}
SMN	2.2±0.05^{e}	1.25±0.02^{bc}	1.50 ± 0.06^{b}	3.06 ± 0.19^{ab}
Y90	2.27±0.03^{de}	1.23±0.00^{c}	1.55 ± 0.04^{b}	3.26 ± 0.11^{a}
IR64	2.86±0.07^{b}	1.28±0.03^{bc}	1.27 ± 0.06^{c}	1.99 ± 0.08^{e}

quality rice around the world. Basmati rice of India and Pakistan, Bahra of Afghanistan, Domsiah of Iran, Bashful of Bangladesh, and D25-4 from Myanmar were reported to elongate 100% upon cooking, and were considered high quality rice internationally (42).

The average CLBR was significantly different among rice varieties ($P<0.05$) with the highest in AKRM (3.31±0.09) and the lowest in IR64 (1.99±0.08). High expansion breadth wise is not a desirable quality attribute in high quality rice, although high-quality rice varieties are characterized and favoured based on the rise in length (12).

Polymorphism of Microsatellite Markers

DNA markers can differentiate plant varieties depending on their target genomic regions, which are defined by the type of primers used in each DNA marker system. Ten rice varieties from Kyaukse area, Mandalay Region were analysed on their genetic variation using 17 SSR microsatellite markers. These markers on chromosomes 3 to 7 were also tightly linked to cooking and eating quality. The number of observed alleles in each locus is a good indicator of genetic variation (27). A total of 49 alleles were identified across rice varieties using SSR primers for analyzing the genetic diversity of rice varieties (Table 5). Among 17 SSR primers, RM592 exhibited the highest number of alleles (Fig.3). The number of alleles ranged from 2 (RM252, RM159, RM190, RM253, RM276, RM540, RM180, and Wx marker) to 6 (RM592) with an average of 2.88 alleles per locus. The average value of alleles per locus obtained in this study was comparable to 2 to 7

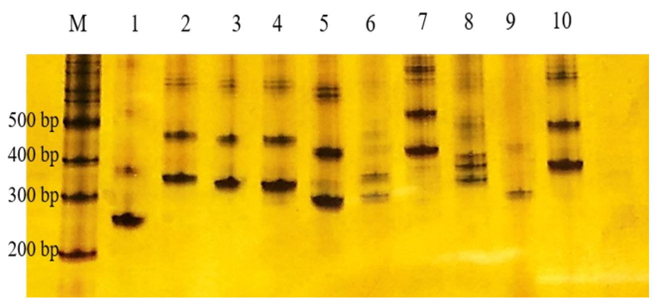

Fig. 3. SSR banding pattern of ten rice varieties generated by primer RM592. The lanes represent (M) 100 bp molecular size marker; (1) PSBK, (2) AYM, (3) STK, (4) MNTK, (5) AKRM, (6) LTM, (7) STY, (8) SMN, (9) Y90, (10) IR64

Table 5. Parameters for genetic analysis of 17 SSR loci across ten rice varieties using PowerMarker software

Marker	Major Allele Frequency	Geno-type No.	Allele No.	Gene Diversity	Hetero-zygosity	PIC
RM232	0.40	3.00	3.00	0.66	0.00	0.59
RM251	0.40	3.00	3.00	0.66	0.00	0.59
RM252	0.70	2.00	2.00	0.42	0.00	0.33
RM164	0.70	3.00	3.00	0.46	0.00	0.41
RM159	0.50	2.00	2.00	0.50	0.00	0.38
RM592	0.30	6.00	6.00	0.80	0.00	0.77
RM3	0.45	5.00	4.00	0.66	0.10	0.59
RM190	0.60	2.00	2.00	0.48	0.00	0.36
RM204	0.60	6.00	4.00	0.59	0.20	0.54
RM225	0.60	3.00	3.00	0.54	0.00	0.47
RM253	0.80	2.00	2.00	0.32	0.00	0.27
RM276	0.60	2.00	2.00	0.48	0.00	0.36
RM314	0.50	4.00	4.00	0.65	0.10	0.59
RM540	0.85	3.00	2.00	0.26	0.10	0.22
RM541	0.50	3.00	3.00	0.58	0.00	0.49
RM180	0.55	3.00	2.00	0.50	0.10	0.37
Wx	0.60	2.00	2.00	0.48	0.00	0.36
Mean	0.57	3.18	2.88	0.53	0.04	0.45

alleles per SSR locus for various classes of microsatellites as reported by Chakravarthi and Naravaneni (43). Therefore, these markers can be used with different parental lines for an international molecular breeding programmes. The loci on chromosome 5 were found to have the most alleles with an average of 3.33, followed by chromosome 3 with an average of 3, while chromosome 4 and 7 were detected to have the least allele 2, next to chromosome 6 (2.8).

The level of polymorphism among 10 rice varieties was evaluated by calculating PIC values for each of the 17 SSR loci. PIC values fluctuated from 0.22 (RM540) to 0.77 (RM592), with an average of 0.45 (Table 5). Similar to our finding, Verma et al. discovered that RM592 had the highest number of alleles and the highest Nei's Gene diversity, with a PIC value of 0.72. PIC value is a reflection of allele diversity and frequency among the varieties (45). The higher the PIC value of a locus, the higher the number of alleles detected. This observed pattern was consistent with the finding of Lapitan *et al.* (17). In this study, the highest PIC value was observed at the primer RM592, which indicated that this marker was the most polymorphic and informative. Five out of seventeen markers (RM232, RM251, RM3, RM204, and RM314) had PIC values above 0.5. (44)

Clustering analysis

Similarity coefficient and cluster analysis method were suitable for using the information derived from SSR markers to group the rice varieties. Genetic similarity based on Jaccard's coefficient of similarity was used to assess the level of relatedness among the studied rice varieties (Table 6). The pair-wise genetic similarity coefficients ranged from 0.06 to 0.89. Maximum similarity was found between MNTK and STK, showing similar AC, GC and GT.

Table 6. Jaccard's genetic similarity index among ten rice varieties using 17 microsatellite markers

	PSBK	AYM	STK	MNTK	AKRM	LTM	STY	SMN	Y90	IR64
PSBK	1.00									
AYM	0.21	1.00								
STK	0.06	0.62	1.00							
MNTK	0.06	0.55	0.89	1.00						
AKRM	0.42	0.10	0.06	0.10	1.00					
LTM	0.25	0.21	0.09	0.13	0.25	1.00				
STY	0.10	0.26	0.31	0.36	0.31	0.25	1.00			
SMN	0.15	0.30	0.30	0.34	0.26	0.21	0.56	1.00		
Y90	0.17	0.26	0.31	0.36	0.48	0.25	0.55	0.39	1.00	
IR64	0.31	0.06	0.10	0.13	0.62	0.21	0.36	0.34	0.48	1.00

Cluster analysis based on Jaccard's similarity coefficients, conspicuously placed the ten rice varieties into two major groups (Fig. 4). Group I was comprised of seven rice varieties and was further subdivided into three sub-clusters (i, ii, and iii). Sub-cluster (i) with a similarity coefficient of about 25% consisted of two rice varieties, PSBK and LTM, which were similar in their GT values.

Sub-cluster (ii) consisted of STY and SMN, which were clustered together at a coefficient of 56%. These two varieties had high AC and high GT. Sub-cluster (iii) consisted of check varieties IR64, AKRM and Y90 although these three varieties possessed different AC, GT and GC. Inclusion of these varieties into one sub-cluster could be due to their other key quality attributes and not due to chemical characteristics. According to the reports of National Seed Committee, Department of Agriculture, Ministry of Agriculture, Livestock and Irrigation, Myanmar (http://www.myanmarseedportal.gov.mm), AKRM and Y90 also had good eating trait, which may be the reason for being in the same group as the check variety IR64. In 2009, Fitzgerald *et al.* reported that the check variety, IR64, a cultivar released in Philippines, showed intermediate AC, soft GC, intermediate GT, and good cooking and eating quality (46). However, this variety showed a low amylose content in our research, which might be due to the changes in its cultivated area, although the values of GT and GC were consistent with the finding of Fitzgerald *et al.* (46).

Group II consisted of three rice varieties: AYM, STK and MNTK, which exhibited intermediate to high AC, and high-intermediate to high GT. A Similarity coefficient of about 89% showed the closest genetic relationship between MNTK and STK since STK was obtained by the crossing of MNTK and IR BB 21 (47). This fact confirmed

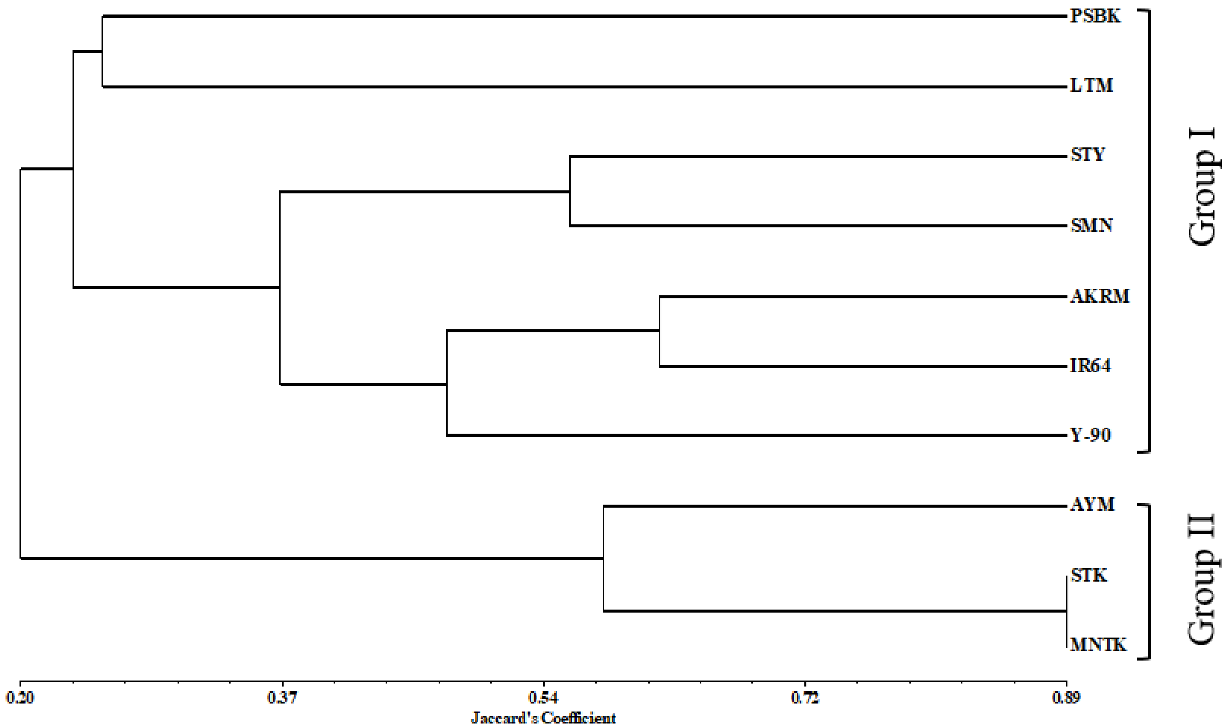

Fig. 4. A UPGMA cluster dendrogram showing the genetic relationship among ten rice varieties based on the alleles detected by 17 SSR markers (Jaccard's Similarity).

the high level of genetic relatedness and close percentage among these two varieties. The results of this research showed that the clusters did not appear to be associated only with eating quality, despite the fact that 17 markers were linked to cooking and eating quality traits. This might be due to cross recombination and mutation during selection.

Multiple regression analysis revealed that RM190 and Wx primer were significantly correlated with AC and GC and explained more than 50% of variation in chemical traits for eating quality. Moreover, RM159, RM190, RM204, RM276 and Wx had a significantly correlation with GT (Table 7). Similar to our result, Wan *et al.* discovered that rice genotypes unveiled by RM190 were significantly correlated with AC, and 59.3% of AC variance was attributed to genetic polymorphism in Wx gene (48). And also, RM190 was found in the waxy gene and studied for AC, GC and GT traits, while RM204 was used to study the GT trait (49). According to the research of Tan *et al.* the three quality responsible for the rice's cooking and eating quality (AC, GC, and GT) are controlled by the waxy gene or a genomic area closely connected to it (7). Several reports described that GC is associated with Wx gene (7, 46). In addition, QTL analysis revealed that a major QTL specifying GT was located at the interval RM276-RM121 and at the same locus as the alkali degeneration gene (50). Lapitan *et al.* reported that SSR markers could differentiate quality rice varieties with comparable cooking and eating quality (17).

Improving cooking and eating quality traits is complex because of their polygenic inheritance and environmental interactions (17). In addition to genetic complexity, environmental factors, cultural practices, and postharvest processes, such as air temperature during ripening, the amount of fertilizer, irrigation management, grain drying after harvest, and cooking methods, have a significant impact on the cooking and eating quality of rice (18).

Conclusion

In conclusion, our investigation revealed that physicochemical characteristics, cooking features, and genetic variation of rice exhibited varietal differences. The intermediate AC, the intermediate GT and the soft GC scored excellently on PSBK indicating a good cooked rice texture and consumer preference. Moreover, PSBK had the highest elongation ratio and a lower volume expansion ratio during cooking. According to these physicochemical studies, the PSBK showed the best cooking and eating quality characteristics, followed by IR64 and STK rice varieties. Using 17 SSR markers for cluster analysis, ten rice varieties formed two major groups and four sub-clusters. RM592 had the highest allele numbers and PIC value, making it the most reproducible and diverse marker suitable for differentiating most rice varieties. In multiple regression analysis, RM190, RM159, RM204, RM276 and Wx

Table 7. Correlation between SSR markers and cooking and eating quality traits

Primer	Amylose content			Gelatinization Temperature			Gel Consistency		
	R	R^2	Sig	R	R^2	Sig	R	R^2	Sig
RM232	0.349	0.122	0.634	0.527	0.278	0.32	0.407	0.165	0.531
RM251	0.349	0.122	0.634	0.527	0.278	0.32	0.407	0.165	0.531
RM252	0.272	0.074	0.447	0.281	0.049	0.54	0.309	0.096	0.385
RM164	0.329	0.108	0.67	0.185	0.034	0.885	0.584	0.341	0.232
RM159	0.61	0.373	0.061	0.665	0.442	0.036	0.588	0.346	0.074
RM592	0.855	0.732	0.235	0.746	0.557	0.512	0.724	0.524	0.565
RM3	0.626	0.392	0.57	0.531	0.282	0.744	0.739	0.546	0.328
RM190	0.746	0.556	0.013	0.716	0.513	0.02	0.832	0.692	0.003
RM204	0.819	0.67	0.167	0.947	0.898	0.011	0.884	0.782	0.066
RM225	0.601	0.362	0.208	0.44	0.194	0.47	0.531	0.282	0.313
RM253	0.086	0.007	0.813	0.403	0.162	0.248	0.027	0.001	0.941
RM276	0.167	0.028	0.645	0.728	0.53	0.017	0.289	0.084	0.418
RM314	0.186	0.035	0.973	0.547	0.30	0.513	0.374	0.14	0.807
RM540	0.529	0.28	0.317	0.363	0.131	0.611	0.469	0.22	0.42
RM541	0.528	0.279	0.318	0.344	0.118	0.644	0.37	0.137	0.597
RM180	0.148	0.022	0.926	0.424	0.18	0.499	0.569	0.324	0.254
Wx	0.746	0.556	0.013	0.716	0.513	0.02	0.832	0.692	0.003

primers were very informative on the cooking and eating parameters of rice varieties. The overall results showed that PSBK and STK, apart from the check variety IR64 were capable of assessing rice's palatability around Kyaukse area. Additional research will be required to identify and use more functional markers linked to cooking and eating properties in order to assist plant breeders in selecting good eating quality traits for future improvement programmes.

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Authors contributions

STD, ANYS, MMM, TTL and WWA conducted the experiments. ANYS and TML participated in the design of the study. TML and MMM performed the statistical analysis, and MMM prepared the manuscript. TML, STD and ANYS revised and improved the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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