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Analysis of Morphological and Biochemical Characters of Different Aromatic Rice (*Oryza sativa* L.) Varieties for Grain Quality Assessment

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ABSTRACT: Rice (*Oryza sativa* L.) is the main source of energy, nutrients, fibers, for the largest people in the world, and requirement of rice is increasing day by day. Rice grain quality is more complex character compare to other cereals crops. The appearance, eating and cooking quality in rice composed of numerous morphological and biochemical traits such as grain length, grain breadth, aroma, gel consistency, amylose content and texture of cooked rice. Nine aromatic rice genotypes were studied. Results revealed six genotypes includes, Pusa Basmati-1, Harayan Basmati-2, Pusa Basmati-1612, Vallabh Basmati-22, Pusa Basmati-1609 and Panjab Basmati-5 were extra-long slender grain. Pusa Basmati-1612 had the highest length of raw rice kernel 8.55±0.13 mm and the Kalanamak showed high breadth of raw rice kernel 2.05±0.14 mm. Vallabh Basmati-22 rice variety had high L/B ratio of 5.76 while Kalanamak had least L/B ratio i. e. 2.34. PB-1612 had the highest Test weight (25.03±0.20) among the varieties studied. Based on GC test, the mean value of gel consistency ranged from 33.66±0.57 mm in Panjab Basmati-4 to 122.80±1.70mm in PusaBasmati-1. Most of varieties had high ASV except Pusa Basmati-1612 had low ASV. Mostly aromatic Basmati genotypes had intermediate amylose content. The amylose content ranged from 15.55 % in Kalanamak to 23.79 % in Pusa Basmati-1609. Maximum elongation ratio of rice grain was 1.75 in Haryana Basmati-2 and Kalanamak recorded minimum (1.18) elongation ratio. The kernel length after cooking ranged from 17.48±0.32 mm to 5.68±0.17 mm. All genotypes had strong aroma.

Keywords: Grain quality, Aroma, Amylose, ASV, GC.

INTRODUCTION

Rice (*Oryza sativa* L.) is major crops which is consumed as a food crop by around 2.5 billion people of the world. Rice provides 21% of human calorie requirements and accounting for 76% of south-east Asia people calorie intake. Rice is important crop for nutritional and food security source for Asian people, also the only cereal crops which consumed and cooked whole grains, thus quality is most important (Hossain *et al.*, 2009; Wu *et al.*, 2014). The term aroma in rice is generally used for the varieties that have aroma and flavor, aroma of rice is due to being chemicals 2-acetyl-1-pyrroline (2AP) and other volatile compounds (Singh *et al.*, 2000) present in endosperm. Aromatic rice in Indian popularly known as Basmati rice, because of its superior quality it is most popular and preferred by the consumers and fetches a premium price in the international and local markets. Aromatic particularly basmati rice has satisfactory quality characters such as excellent cooking properties, slender grain and lengthwise elongation during cooking (Kaur *et al.*, 2011; Calingacion *et al.*, 2014; Verma *et al.*, 2015).

Grain quality of rice is the entirely of the rice characters features that meets the customer demands. Rice grain quality is divided into four categories: milling quality, appearance quality, cooking, eating qualities and nutritional quality (Tsukaguchi *et al.*, 2016; Pandey *et al.*, 2012; Bhullar *et al.*, 2013; Peng *et al.*, 2014). Grain appearance quality *viz.*, shape, size, and colour are the valuable aspect for consumers. The rice kernel with desirable fragrance, long slender and soft texture take high place than other rice varieties in market. Consumer acceptance on rice quality is different in a country and among countries. Endosperm is the main eating part of rice grain which have about 90% starch. Rice eating and cooking qualities are influenced by starch properties (Sun *et al.*, 2011). Thus, Rice grain quality is primary factor based on biochemical composition (such as aroma, gel consistency, alkali spreading value and amylose content), morphological properties (grain size and shape) and nutritional properties (Sharma and Khanna 2019). Consumer preference and market demand towards rice are dependent on various quality characteristics of grain, such as chemical, morphological cooking, and eating quality properties, flavor or nutritional parameters (Thilakarathna *et al.*, 2017), for rice producers, good quality has emerged as a key selection criteria (Li *et al.*, 2022). Therefore, the present study conducted to determine the grain quality of genetically diverse nine aromatic rice based on morphological, biochemical and cooked characteristics.

MATERIAL AND METHODS

Plant Material: Aromatic rice genotypes namely; Haryana Basmati 2 (HB-2), Vallabh Basmati 22 (VB-22), Kalanamak, Pusa Basmati-1 (PB-1), Malviya Basmati, Pusa Basmati-1609 (PB-1609), Pusa Basmati-1612 (PB-1612), Palam Basmati and Panjab Basmati-4were grown inSardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Technology Park fieldduring Kharif season 2022 and stored under dry conditions with similar moisture content for Bio-chemical studies.

Morphological traits: The morphological parameters such as grain length (L), grain breadth (B) of milled rice before cooking and after cooking were measured using a digital vernier caliper having the least count of 0.01mm. The measurement was done by using 10 milled rice grain in each varieties; therefore, the means of grains were recorded. The length and breadth (L/B) ratio was determined for milled rice by following equation (McCabe *et al.*, 2005).

L/B ratio = Length (mm)/Width (mm)

Test weight was determined by weighing100 randomly selected grain of each genotypes using electronic balance and the results multiplied to 10, the average value was taken from three replicate for each genotype. Kernel elongation after cooking and L/B ratio: twenty whole milled grain were soaked in 20 ml distilled water for 30 min. the samples were cooked in a hot water bath and temperature was maintain in 100ºC for 10 min. the cooked grain was then placed on blotting paper. The length of the ten whole was measured by placing on a graph paper. The elongation ratio was determined the average length of cooked rice kernel to the average length of raw rice kernel.

Bio-chemical traits:

Gel consistency (GC). Gel consistency was determined according to the procedure of Cagampang *et al.* (1973), Milled rice grain were ground with mortar & pestle to prepare fine flour, 100 ± 2 mg of rice flour was weighed with electric balance and put into test tube (13×100mm). Added 0.2 ml of 95 % ethanol containing 0.25% thymol blue followed by added 2.0 ml of 0.2 N KOH. The samples were mixed with shaker and cooked for 10 min in boiling water bath then allowed at room temperature for 5 min. the tubes were cooled in ice bath for twenty minute and lain horizontally on a millimeter graph paper. The length of the gel was measured from the bottom of the tube to gel front based on gel consistency test and categorized into hard gel less than 40 mm, medium gel 41-61 mm and soft gel more than 61 mm (Fig. 1).

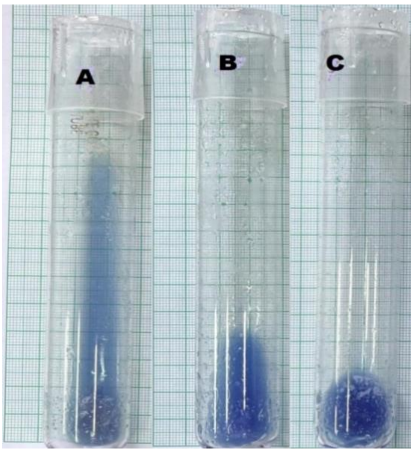

Fig. 1. Gel consistency of rice genotypes under study.

Amylose content (AC). Amylose content was carried out by using method Juliano (1971), 100 mg of rice flour taken from each sample in a volumetric flask to which 1ml of 95% ethyl alcohol and then immediately 9 ml of 1N NaOH was added. Sample were shaken with electric shaker for better dissolving. Then heated in boiling water bath for 12 min to gelatinize starch. Diluted each sample in 90 ml distilled water. 5ml of diluted sample was taken in a new volumetric flask. Then added 1ml of acetic acid (57.7 ml Glacial Acetic Acid in 1000ml distilled water) and 2ml iodide solution (2% Sodium Iodide with 0.2 % Iodine) to the starch extract and the volume was made up to 100ml. samples are incubated at room temperature for 20 min. then by using spectrophotometer (GENESYS 180) the absorbance has been measured at 620 nm. The amylose content (AC) of each varieties were calculated in comparison with standard graph (Perez and Juliano 1978). Based on amylase %, rice varieties can be classified as follows: Waxy (0-2%), very low (3-9%), low (10-19%), intermediate (20-25%) and high (>25%).

Aroma Test. Aroma test was done according to aroma sensory method IRRI, (1971), one gram milled rice from each variety is placed into flask (50 ml round bottom flask). 20 ml of distilled water added. The flask covered with cotton plug. The samples are put in boiling water bath for 10 min, and then allowed to cool and the presence of aroma is sensory by four panel members determined for each sample. To prevent overwhelming sense of panel the 10 samples were scored at a time; 1= slightly aroma, 2= moderate aroma and 3= strong aroma. Scored were averaged across panel members.

Alkali Spreading Value (ASV). Alkali spreading value was carried out using the method of Little *et al.* (1958), six whole grain milled rice of each samples was scaled and placed in petri dish. Added 10ml of Potassium Hydroxide (1.7% KOH solution) to each petri dish and arranged the rice kernel to enough spaces between each other to allow for spreading. The petri dish were covered and incubate at room temperature for 23 hour each grain visual examined based on a 7-point numerical spreading scale (Bhattacharya and Sowbhagya 1972).

Score	Kernel ASV
1	Not affected
2	Swollen
3	Swollen with incomplete collar
4	Swollen with complete collar
5	Split kernel with wide collar
6	Dispersed kernel with merging kernel
7	Completely dispersed kernel

Statistical analysis. In this study, the results are analyzed, presented as mean and standard deviation (S.D.) and correlations among selected quality characters using excel and SPSS software (IBM SPSS statistic 20).

RESULT AND DISCUSSION

The characters associated with nutrition, texture and quality of grain of nine rice germplasm are indicated in (Table 1). The length of raw kernel ranged from 4.08±0.20 mm for Kalanamak to 8.55±0.13 mm for Pusa Basmati-1609 varieties, the mean value is 7.46. The breadth of raw kernel ranged from 1.39±0.16 mm for variety Pusa Basmati-1to 2.05±0.14 mm for variety Kalanamak with the mean value of these variety is 1.58. The first criteria of grain quality are grain size and shape which consider in varieties development and releasing rice varieties for commercialization purposes (Adair *et al.,* 1966). Aromatic rice genotypes with kernel length 6.00 mm and above are broadly acceptable size (Kaul, 1970). The L/B ratio of raw rice kernel ranged from 2.34 for Kalanamak to 5.76 for varieties VB-22 respectively. According to the provisions of seed Act (1966), length, breadth and L/B ration are the first quality characteristic of aromatic basmati rice, which has establish standards. According to it, the minimum average of raw rice grain length should be 6.61 mm, the average length of raw rice grain breadth ≤ 2.00 mm and the minimum L/B ratio of raw rice grain should be 3.50. A length to breadth is used to classify the shape of rice kernel; the lower L/B ratio shows round or bold shapes of grains, the intermediate shows the medium and the higher L/B ratio shows the slender shapes (Verma and Sirvastava 2020).

Table 1: Morphological, Bio-chemical and cooked characteristics of aromatic rice genotypes.

Sr. No.	Genotypes	L (mm)	B (mm)	L/B ratio	Test-weight (gm)	KLAC (mm)	ER	GC	Amylose (%)	ASV	Size	Shape	Aroma (KOH method)	Aroma (IRRI method)
1.	Haryana Basmati-2	8.24±0.53	1.43±0.15	5.76	23.1±1.10	17.48±0.32	1.75	108.16±2.25	22.21	6 H	Extra Long	Slender	Strong	Strong
2.	Malviya Basmati	6.83±0.24	1.59±0.17	4.29	24.16±0.15	10.02±0.36	1.46	34.50±3.12	15.46	2 L	Long	Slender	Strong	Strong
3.	Pusa Basmati-1612	8.27±0.41	1.66±0.15	4.98	25.03±0.20	15.66±0.27	1.65	39.83±3.61	20.00	1 L	Extra Long	Slender	Strong	Strong
4.	Pusa Basmati-1	7.84±0.38	1.39±0.16	5.64	23.06±0.30	13.68±0.13	1.74	122.80±1.70	20.11	7 H	Extra Long	Slender	Strong	Moderate
5.	Kalanamak	4.8±0.20	2.05±0.14	2.34	19.76±0.35	5.68±0.17	1.18	89.53±1.58	15.55	7 H	Short	Medium	Strong	Strong
6.	Palam Basmati	6.51±0.17	1.49±0.07	4.36	21.73±0.66	9.20±0.20	1.41	84.83±1.75	15.28	3 I	Medium	Slender	Strong	Strong
7.	Vallabh Basmati-22	8.18±0.22	1.42±0.04	5.76	21.16±0.15	11.84±0.51	1.44	86.33±3.51	20.30	6 H	Extra Long	Slender	Strong	Strong
8.	Pusa Basmati-1609	8.55±0.13	1.62±0.11	5.27	22.03±0.92	14.90±0.10	1.74	24.16±1.89	23.79	4 I	Extra Long	Slender	Strong	Moderate
9.	Panjab Basmati-4	7.95±0.23	1.54±0.11	5.16	21.5±0.70	15.62±0.37	1.71	33.66±0.57	22.89	4 I	Extra Long	Slender	Strong	Strong
	Mean	**7.46**	**1.58**	**4.84**	**22.39**	**12.67**	**1.56**	**69.31**	**19.51**	-	-	-	-	-
	CD	0.283	0.114	0.078	0.874	0.243	0.085	6.521	0.166	-	-	-	-	-
	SE (D)	0.142	0.057	0.036	0.409	0.122	0.04	3.050	0.078	-	-	-	-	-
	SE (M)	0.100	0.040	0.026	0.289	0.086	0.028	2.157	0.055	-	-	-	-	-
	CV	4.246	8.48	0.92	2.16	3.165	4.175	4.551	0.538	-	-	-	-	-

Means± Standard deviation

ASV- Alkali Spreading Value, GC- Gel consistency, L- grain length, B-grain breadth, KLAC-Kernel length after Cooking, L/B ratio-length breadth ratio, ER-Elongation ratio

Most of the rice varieties was considered acceptable L/B ratio.VB-22, HB-2, PB-1612, PB-1, PB-1609 and Panjab Basmati-4 had extra-long size and slender shape, Malvya Basmati had long size and slender shape, Kalanamak had medium size and shape and Palam Basmati had medium size and slender shape. Test-weight of rice varieties was ranged from 29.03±0.92 gm to 19.76±0.35 gm among them, PB-1609 with 29.03±092 gm had the highest grain weight, the second highest grain weight was PB-1612 with 25.03±0.20 gm 1000-grain weight and Kalanamak had the lowest grain weight 22.03±0.92 gm (Fig. 2). Weight is important parameter to be used in the design of cleaning grains using aerodynamic forces (Oje and Ugbo 1991).

Physio-chemical characteristics of the rice varieties. Based on the alkali spreading value observed in the figure 1.0, the rice genotypes were divided into three groups; low, intermediate and high alkali spreading value (Table 1). The variety PB-1612 with low degree of alkali digestion while two genotypes namely; Palam Basmati and Malvya Basmati had intermediate alkali spreading value. High alkali spreading value was observed in the varieties namely; HB-2, PB-1, VB-22, PB-1609, Panjab Basmati-4 and Kalanamak. In gel consistency, varieties PB-1, HB-2, Palam Basmati, VB-22 and Kalanamak had soft gel consistency, variety PB-1612 had intermediate gel consistency and hard gel consistency was observed in varieties Panjab Basmati-4, PB-1609 and Malvya Basmati. The rice varieties with soft gel consistency remained soft when cooled after cooking.

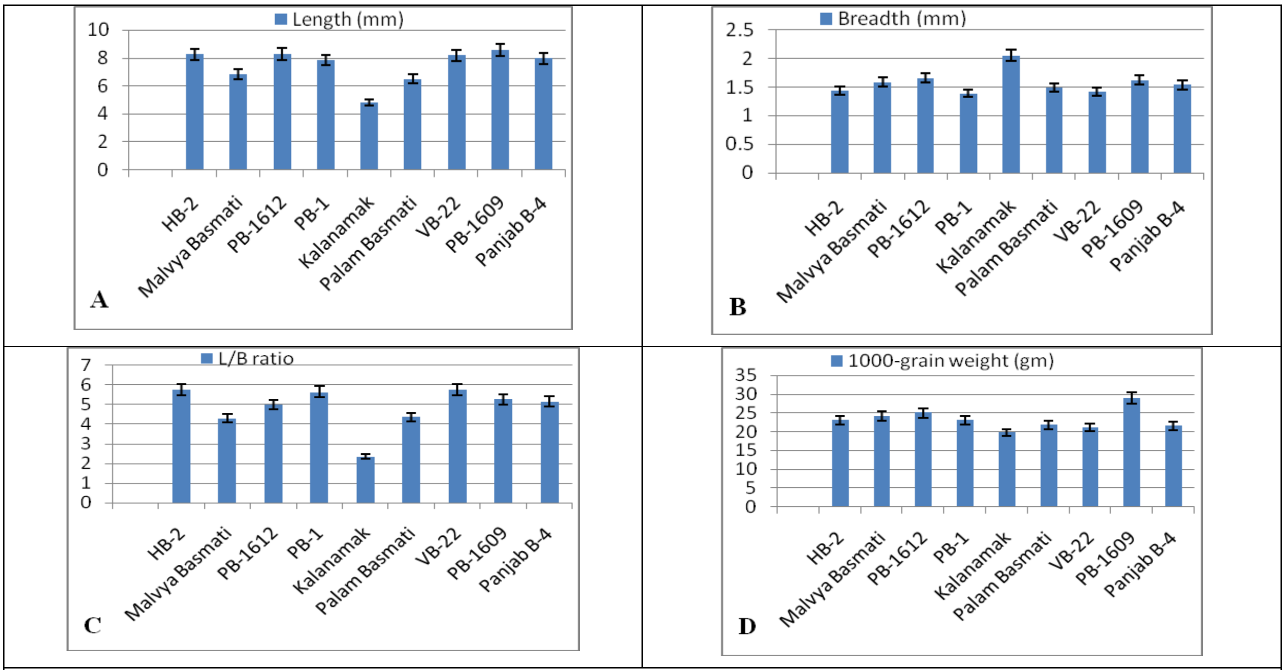

Fig. 2. Morphological appearance of characters (A) length of Kernal of rice (B) Breadth of kernel of rice (C) L/B ratio of selected of rice variety and (D) 1000 grain weight of rice variety.

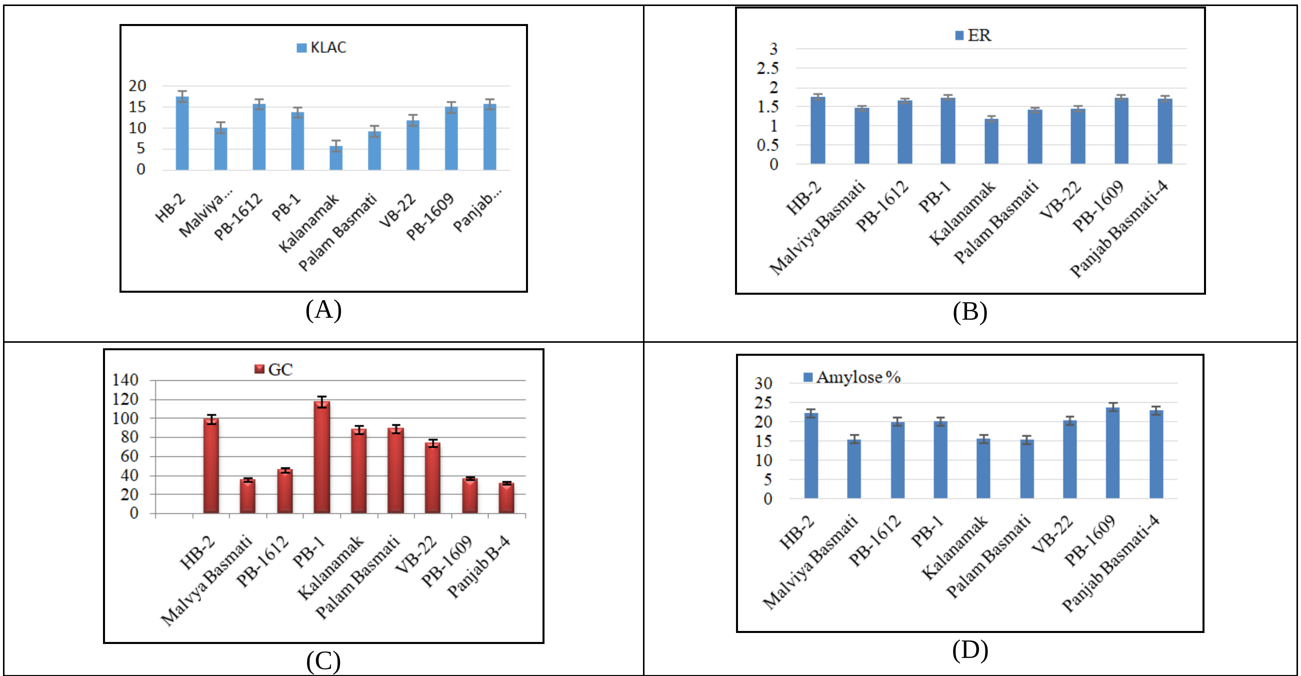

Fig. 3. Physiochemical traits of selected nine rice genotypes of rice (A) KLAC (B) Elongation ratio (C) Gel Consistency (D) Amylose percent in selected rice.

Amylose content is the most important determinant of rice quality and great influence consumer preference. Rice with low amylose is sticky and soft after cooking but as amylose content increases, the rice becomes firmer (Kennedy and Burlingame 2003). The lowest amylose content 15.46 %, 15.55 % and 15.28 % was observed in varieties Malviya Basmati, Kalanamak and Palama Basmati respectively (Table 1). Genotype Haryana Basmati-2 (22.21%), Pusa Basmati-1612 (20.00 %), Pusa Basmati-1 (20.11 %), Vallabh Basmati-22 (20.30 %), Pusa Basmati-1679 (23.79 %) and Panajab Basmati-4 (22.89 %) had intermediate amylose content. Indica rice varieties, when cooked that are hard but non-sticky, are generally preferred in India, Indonesia and Pakistan because of their stickiness and moderate elasticity (Bhattacharya *et al.*, 1982). Aroma is the most important desirable rice grain quality and there is high acceptance in local and global market. The rice varieties were subjected to two methods for aroma test i. e. cooking and reaction with KOH to detect aroma, all varieties had aroma. The observation was showed that all genotypes had strong aroma in both KOH and IRR methods except Pusa Basmti-1 and Panjab Basmati-4 genotypes had strong aroma in KOH method and moderate aroma in IRRI method. According to Hu *et al.* (2020) a mixture of compounds are responsible for aroma in rice. The quantities of 2AP present in rice genotype varied with type and climate conditions (Nadaf *et al.*, 2006; Bhonsle and Sellapan 2010). The rice varieties kernel length after cooking studied ranged from 17.48±0.32 mm in HB-2 to 5.48mm±0.15 in Kalanamak, therefore, the varieties HB-2, PB-1609, PB-1612, Malvya Basmati, Panjab Basmati-4, HB-2, and VB-22 had maximum KLAC value and variety Kalanamak and Palam Basmati had the least KLAC. Similarly, the elongation ratio after cooking was studied from 1.75 to 1.18. The highest elongation ratio was indicated by the rice variety HB-2 with 1.75 and the least elongation ratio was shown by variety Kalanamak with 1.18.

Correlation analysis was made among morphological and bio-chemical characteristics (Table 2), it was found that the kernel length of raw rice had high significant and positive correlation with kernel length after cooking ($r=0.972$, $p<0.01$), kernel length had significant positive correlation with elongation ration ($r=0.736$ $p<0.05$) and kernel length had negative correlation with breadth ($r=-0.707$ $p<0.05$). Kernel breadth had high significant and negative correlation with L/B ratio ($r=-0.905$ $p<0.01$) and the finding was similar to that of (Begum, 2006; Seraj *et al.*, 2013; Basri *et al.*, 2015; Shijagurumayum *et al.*, 2018). Kernel length had high significant and positive correlation with L/B ratio ($r=0.923$ $p<0.01$) similar that reported by Singh *et al.* (2022) also Nayak and Reddy (2005) found a favorable correlation between kernel length and L/B ratio. There is no correlation was observed between L/B ratio and ASV similar to that reported by (Vanaj and Babu 2003; Shijagurumayum *et al.*, 2018). Elongation ratio had significant correlation with kernel length ($r=0.736$ $p<0.05$). 1000-grain weight were not had correlation between any chosen physio-chemical characteristics. GC had negative correlation with elongation ratio ($r=-0.744$ $p<0.05$). ASV were not had correlation between any chosen physio-chemical characteristics. A high significant positive correlation was observed between KLAC and kernel length ($r=0.972$ $p<0.01$) similar outcome reported by (Roy *et al.*, 2021). Singh *et al.* (2022) also reported a significant and positive correlation between kernel length and the l/b ratio of the raw rice kernel and kernel length after cooking (KLAC) similarly, Singh *et al.* (2017) observed a significant and positive correlation between kernel length and the l/b ratio and KLAC.A high positive significant correlation was observed between kernel length after cooking and elongation ratio ($r=0.874$ $p< 0.01$) similarly that report by (Tamu *et al.*, 2017). Significant positive correlation between KLAC and L/B ratio ($r=0.837$ $p<0.05$). Singh *et al.* (2022) also reported that the kernel length after cooking had a significant positive correlation with the L/B ratio.

Table 2: Correlation among various morphological and bio-chemical characteristics.

Characters	Length	Breadth	L/B ratio	ER	1000-Grian weight	Amylose %	GC	ASV	KLAC
Length	1.00								
Breadth	-0.707*	1.00							
L/B ratio	0.923**	-0.905**	1.00						
ER	0.736*	-0.211	0.506	1.00					
1000-GW	0.487	0.032	0.211	0.496	1.00				
Amylose %	-0.373	0.136	-0.225	-0.118	-0.422	1.00			
GC	-0.245	-0.181	0.017	-0.744*	-0.447	0.100	1.00		
ASV	0.036	-0.281	0.242	-0.290	-0.329	-0.106	0.364	1.00	
KLAC	0.972**	-0.580	0.837**	0.874**	0.521	-0.308	-0.434	-0.075	1.00
*. Correlation is significant at the 0.05 level.									
**. Correlation is significant at the 0.01 level.									

ER= Elongation ratio; GC= gel consistency; ASV= alkali spreading value; KLAC= kernel length after cooking.

CONCLUSIONS

In the present investigation mostly rice genotypes were extra-long size, slender shape with strong aroma, and the most preferable characters of rice grain quality. All varieties had desirable L/B ratio >3 except Kalanamak which had L/B ratio <3. Based on gel consistency most of varieties found soft gel consistency and high ASV. Mostly aromatic Basmati genotypes had intermediate amylose content and aromatic non-Basmati genotype had low amylose content. Correlation among varieties under study showed high significant correlation between L/B ratio and kernel length and negative correlation between L/B ratio and breadth. Kernel length after cooking had high significant correlation between length of raw rice and elongation ration. There is no correlations observed between alkali spreading value and other studied characters.

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Conflict of Interest. None.

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