



Assessment of agro-morphological traits, grain physical and physico-chemical properties in the Indian aromatic rice (*Oryza sativa*) germplasm

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ABSTRACT

Information on genetic diversity with respect to various agro-morphological traits and eating and cooking quality traits plays a key role in the improvement of rice (*Oryza sativa* L.). With this aim, we have characterized a set of 98 indigenous aromatic rice germplasm accessions for four agro-morphological, two physical and two physico-chemical traits. Significant variation was observed among the cultivars for all the traits. Correlation analysis showed negative correlation of DFF with KLBC ($r = -0.29$; $p = 0.022$) and kernel L/W ratio ($r = -0.25$; $p = 0.003$), while PL was significantly and positively correlated with KLBC ($r = 0.38$; $p = 0.000$) and kernel L/W ratio ($r = 0.43$; $p = 0.0649$). KLBC showed highly significant positive correlation with GC ($r = 0.22$; $p = 0.614$) while between AAC and GC a significant negative correlation was observed ($r = -0.38$; $p = 0.000$).

Keywords: Agro-morphological, Amylose, Gel consistency

Rice (*Oryza sativa* L.) is the major staple food for more than half of the global population providing about 19% per capita energy and 13% of per capita protein. The worldwide dependency on rice has led to the development of thousands of varieties varying for their morphological and grain quality traits. Morphological characterization is simple and economical as it involves less labour, easy to score, does not need any sophisticated technology, and a reliable method for classification of genotypes (Din *et al.* 2010). A large number of studies have been conducted on genetic diversity using agro-morphological characterization, which helps in identification of the phenotypic variability available in the rice germplasm (Ogunbayo *et al.* 2005, Bajracharya *et al.* 2006, Barry *et al.* 2007) that can be utilized for its improvement. There is an increasing demand for rice varieties with excellent grain quality characteristics throughout the world, since cooking and eating quality plays a major role in determining the market and consumer acceptability. Rice grain quality is determined by its physical and physico-chemical properties. The physical appearance is determined by grain shape and size, while

physico-chemical properties including apparent amylose content (AAC), gel consistency (GC) and gelatinization temperature (GT), determine the eating and cooking quality of rice. Both of them determine the nature of cooked rice which has great influence on the consumer preference. Rice with intermediate amylose content (20–25%) cooks soft and retains its tenderness for a longer duration due to which it is preferred the most among the major rice consumers in India. GC measures the cold paste-viscosity of milled rice flour on cooking. It complements AAC in determining the cooked rice texture of high amylose rices (Cagampang *et al.* 1973, Juliano 1979, Juliano 1985). Overall, the eating and cooking quality traits are vital characteristics of rice to meet the demands of consumers both domestic as well as international. Aromatic rice is one of the major subgroups of rice, which includes the long slender Basmati rice with exceptional grain elongation on cooking and the short-slender aromatic rices. They possess pleasant aroma and exquisite taste preferred around the world since ages and occupies a prime position in the Indian society. The information on the agro-morphological, physical and physico-chemical qualities of aromatic rice germplasm is still limited. Keeping this in view, the present investigation was undertaken to evaluate and characterize agro-morphological and assess the physico-chemical parameters of 98 aromatic rice germplasm, which will provide an understanding of the extent of diversity that would help their utilization in rice improvement.

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MATERIALS AND METHODS

The plant materials used in the present study include a set of 98 aromatic rice genotypes including both Basmati and non-Basmati accessions. The genotypes were coded as Aro.1 to Aro.98 serially and the details of these germplasm lines are given in Supplementary Table 1. These accessions have been collected from various places across the country and are being maintained in the Rice section of the Division of Genetics, ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi. The experiment was laid out in an 'Augmented Randomized Experimental Design' (Federer 1956) during *Kharif* 2017–18 at the Division of Genetics, ICAR-IARI, New Delhi, (Latitude 28°38' 23"N, Longitude 77° 09' 27" E and Altitude 228.61 m amsl).

Recording of agro-morphological data: The seeds were sown on a raised seedbed and 30-days-old seedlings were transplanted in the main field. Each genotype was grown in a single row of 4 m length with a row-to-row spacing of 20 cm and plant to plant spacing of 15 cm. Standard cultural practices for rice were followed to raise a good crop. Agro-morphological data of the genotypes were recorded at appropriate growth stage of rice plant following Standard Evaluation System (IRRI 1996). Five random plants were selected per genotype and data for the following agro-morphological traits were recorded; Days to 50% flowering (DFF), Plant height (PH, cm), Numbers of tillers (NT, no) and Panicle length (PL, cm).

Grain Physical Characteristics

The harvested grains were threshed, cleaned and dried to the moisture percentage of 14%. The samples were then dehusked using a hand operated paddy Palm Dehusker and the brown rice thus obtained was polished in rice polisher (Mini Lab Rice Polisher Model K-710, Krishi International) to obtain the milled rice.

Grain Dimensions: Ten polished whole kernels per accession were lined up end-to-end and placed under a 10x photo-enlarger for measuring the average kernel length before cooking (KLBC). Also, kernel width before cooking (KWBC) was measured by aligning the kernels sidewise. The average length and width of 10 kernels in millimeter (mm) was recorded for further analysis. On the basis of their length, IRRI (2013) classified rice cultivars as extra-long (>7.5 mm), long (6.61–7.5 mm), medium (5.51–6.60 mm), and short (<5.5mm).

Grain Shape: Grain shape is determined based on the length-width ratio (L/W), which was calculated as:

$$L/W = L \text{ (mm)}/W \text{ (mm)}$$

Where L, the average length of milled rice (mm); and W, the average width of milled rice (mm). Based on the L/W ratio, rice grains were further classified into slender (>3.0), medium (2.1–3.0), bold (1.1–2.0), and round (<1.1) grain types (IRRI 2013).

Physico-chemical characteristics

Apparent amylose content (AAC): For AAC

determination, the modified assay of Juliano *et al.* (1981) was used. Milled rice flour (100 mg) was weighed in 100 ml volumetric flasks. Then 1 ml of 95% ethanol and 9 ml of 1N NaOH were added. The suspension was heated in a boiling water bath for 10 min to gelatinize the starch. Then, it was cooled for 1 h at room temperature. Samples were diluted to volume 100 ml with distilled H₂O. Then 2 ml of iodine solution (0.2% I₂, 2% KI) was added to 5 ml of the starch solution. The solution was made up to 100 ml with distilled H₂O, shaken well, and allowed to stand for 20 min in dark. Spectrometric determination of the absorbance of the formed colour was calculated at 620 nm. The calibration was performed measuring the absorbance of standard amylose sample, supplied by Sigma (SIGMA-ALDRICH, A0512-1G) and a calibration curve was build up to obtain the percentage of AAC in our samples. Based on the AAC, rice cultivars were divided into 5 amylose groups, viz. waxy/glutinous (1–2%), very low (2–9%), low (9–20%), intermediate (20–25%) and high (>25%).

Gel consistency (GC): The GC of rice samples was determined by following Cagampang *et al.* (1973) method. For this, polished kernels were finely powdered in a micronizer mill. 100 mg of rice flour from each genotype was weighed and placed in a 20 mL (1.6 mm × 15 mm) slim borosilicate glass test tube. A total volume of 0.2 ml of 95% ethanol containing 0.25% thymol blue was added to each tube and mixed sufficiently, so as to prevent clumping of the rice flour. The mixture was vortexed gently, and then 2 ml of 0.2 N KOH was added and vortexed again. Each tube was covered with glass marbles to prevent steam loss and reflux of the samples and heated in a vigorously boiling water bath at 90–100°C for 8 min. The tubes were removed from the water bath, cooled to room temperature for 5 min, vortexed and then finally transferred to an ice bath (0–2°C) for 20 min. The tubes were wiped off moisture and then laid horizontally on a graph paper on a flat laboratory bench for 1 h. The total length of the blue coloured gel was measured from the bottom of the test tube to the end of the gel in millimeters. Genotypes were grouped into three classes based on the length of the gel: Hard (length of gel <40 mm), Medium (length of gel 41–60 mm), and Soft (length of gel >61 mm) (Graham 2002).

Statistical analyses: The descriptive statistics of the data collected from the experiment was analyzed using Microsoft Excel. The correlation coefficient among morphological, physical and physiochemical data of 98 genotypes were calculated using the online statistical package of CCSHAU, Haryana, Sheoran *et al.* (1998) (OPSTAT, <http://hau.ernet.in/about/opstat.php>).

RESULTS AND DISCUSSION

Agro-morphological characteristics: The summary of the agro-morphological data of the rice genotypes are presented in Table 1. The minimum DFF of 83 days after sowing was recorded for the genotype, UPRI 93-101 while the accession Kalimuchh recorded a maximum DFF of 129 days. The mean DFF of the 98 accessions was found to

be 101 days. Almost 78.5% of the accessions were in the medium duration (91-110 days) category based on DFF, while the remaining were of late duration type (111-130 days). Similar types of results were reported previously by Katsuta *et al.* (1996), Shah *et al.* (1999), Santhy (1999) and Pragnya *et al.* (2018). The PH of the set ranged from 65.80 cm (IR 62873-224-1-6) to 176.40 cm (Basmati 5853) with a mean value of 126.10 cm. Following the IRRI (2002) scale, 41 of the 98 genotypes were categorized as tall (>130 cm), 30 genotypes as medium (110-130 cm) and 27 genotypes as short (<110 cm). Similar results based on the morphological characters (plant height) were also reported by Moukoumbi *et al.* (2011) in rice.

The trait NT ranged from 7–26 with an average of 14 tillers per plant. The highest tiller number was observed in Ambemohar-157 (26) followed by DulhaBhog (24), RAU 3076 (23) and KDML 105 (23) respectively, while Elayachi showed poor tillering ability with only 7 tillers per plant. Similar types of results were also reported by Shah *et al.* (1999) and Moukoumbi *et al.* (2011) in rice. The mean PL in the rice genotypes was 25.4 cm ranged from 16.8–32.4 cm. IET 21953 possessed the longest panicle (32.4 cm); while RAU 3048 possessed the shortest panicle (16.8 cm). Four genotypes (IET 21953, Basmati 5853, Ranbir Basmati and IGSR -3-1-5) showed very long panicles of length >30 cm, 44 genotypes had long (26-30 cm), 45 genotypes had medium (21–25 cm) and five genotypes (RAU 3048, AS GPC-38, IR 62873-224-1-6, RRB 2005-1 and Bas Sufaid 187) had short panicles (16-20 cm). Pragnya *et al.* (2018) also classified the rice genotypes based on this categorization. Haryanto *et al.* (2008) and Akinwale *et al.* (2011) observed that the genotypes with long panicles have higher number

of grains and high yield, because of a positive correlation of PL with number of grains per panicle and weight of 1,000 grains, which was also later confirmed by Rohini Devi (2000), Dhanaraj (2001), Anitalakshmi (2002) and Sharma *et al.* (2004) in rice.

Grain physical characteristics

Grain Dimensions: Significant variation was observed for both grain length and width among the landraces used in the present study. The KLBC ranged between 3.07 mm (Mayur Kranti) and 8.80 mm (IET 22778) with an average of 6.10 mm. The accessions were classified into four groups on the basis of grain length with 34 accessions in the short grain type, 23 in medium, 29 in long and 12 in extra-long grain groups. In extra-long grain genotypes, the maximum length was seen in genotype IET 22778 (8.80 mm) and the minimum length in IR 62873-224-1-6, UPR 3429-2-1-1, IET 21953 and Pusa Basmati 1 (7.53 mm). In long grain genotypes, the maximum length was obtained in Pusa 834 (7.47 mm) and the minimum length in Sharbati and IR 74718-1-1-3-2 (6.67 mm). In genotypes with medium grain, the maximum length was seen in IET 16327 and Pusa 44 (6.60 mm) and the minimum length was seen in BasSufaid 187 (5.53 mm) and in short grain genotypes, the length ranged from 3.07–5.47 mm in MayurKranti and Samundchini, respectively. The variation for KWBC was significant in the landraces, ranging from 1.33 mm (Pant Sugandh Dhan-15, Super Basmati, Basmati Ravi, Kankjeer A, DulhaBhog, Pusa Basmati-6, Kalanamak 1) to 2.53 mm (Koliha) with an average of 1.68 mm. Bollinedi *et al.* (2020) and Pokhrel *et al.* (2020) in rice also observed such diverse range for kernel width before cooking. KLBC and KWBC were found to be the most discriminating quantitative traits and Sie (1991) also observed similar trend in the traditional rice varieties in Burkina Faso.

Grain Shape: The shape of the rice grain was expressed on the basis of L/W ratio and significant variation was observed for the L/W ratio among the landraces. The L/W ratio was maximum for genotype Pusa Basmati 6 (6.30 mm) and minimum for Elayachi (1.7 mm). The average L/W ratio was 3.70. The 98 landraces from the present study were grouped into three grain shape classes, bold (2), medium (23) and slender (73). According to Pokhrel *et al.* (2020) grain shape and size influences the rice cooking quality and they have also presented the similar type of results in landraces of Nepal.

Physico-chemical characteristics

AAC: AAC was found maximum in genotype IGSR-3-1-5 (35.88%) and minimum in Manipur Black rice (4.81%) with an average of 25.46%. We found 2 accessions in very low (2–9%), 12 in low (9–20%), 33 in intermediate (20–25%) and 51 accessions in high amylose category (25–32%). Manipur Black rice and RIL NJ-72 were found to have very low amylose content, while ANP RAU 3061 and IGSR -3-1-5 were found to have high amylose content and cooked hard and dry. The results reveal predominance

Table 1 Descriptive statistics for the agro-morphological, grain physical and physico-chemical quality traits of the 98 aromatic rice accessions

Trait	Minimum	Maximum	Mean±SD
<i>Agro-morphological characters</i>			
DFF	83	129	101.0±8.79
PH	65.8	176.4	126.10±23.40
NT	7	26	14.0±3.76
PL	16.8	32.4	25.4±2.92
<i>Grain Physical Characteristics</i>			
KLBC	3.07	8.80	6.10±1.32
KWBC	1.33	2.53	1.68±0.22
L/W	1.7	6.3	3.70±0.95
<i>Physico-chemical Characteristics</i>			
AAC	4.81	35.88	25.46±5.21
GC	27	118	65.00±22.88

DFF, Days of 50% flowering; PH, Plant height in cm; NT, No. of tillers; PL, Panicle length in cm; KLBC, Kernel length before cooking in mm; KWBC, kernel width before cooking in mm; L/W, length-width ratio; AAC, Apparent amylose content %; GC, Gel consistency in mm; SD, Standard deviation.

Table 2 The Pearson correlation of coefficient among morphological, physical and physiochemical traits

Parameter	DFF	PH	NT	PL	KLBC	KWBC	L/W	AAC	GC
DFF	1								
PH	0.165	1							
NT	0.024	-0.174	1						
PL	0.013	0.524**	-0.274**	1					
KLBC	-0.297**	0.003	-0.078	0.386**	1				
KWBC	0.012	-0.005	0.056	-0.247*	-0.071	1			
L/W	-0.254*	0.004	-0.074	0.434**	0.883**	-0.508**	1		
AAC	-0.059	-0.098	0.224*	-0.095	-0.124	0.123	-0.158	1	
GC	-0.025	0.084	-0.193	0.148	0.224*	0.151	0.125	-0.382**	1

DFF, Days of 50% flowering; PH, Plant height in cm; NT, No. of tillers; PL, Panicle length in cm; KLBC, Kernel length before cooking in mm; KWBC, kernel width before cooking in mm; L/W, length-width ratio; AAC, Apparent amylose content (%); GC, Gel consistency in mm.

** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level.

of intermediate and high amylose genotypes in the aromatic rice cultivars. AAC is largely genetically controlled by the *Wx* locus on chromosome 6, or specifically by the amount of *Wx* protein present. Waxy rice has nearly zero amylose, and is used for special foods such as desserts and snacks. Low amylose cultivars (9–20%) are soft and sticky, and include nearly all-temperate *japonica* cultivars. High amylose cultivars (>25%) becomes dry and hard on cooking, often becoming hard after cooling, common in *indica* rice, and their pasting properties related to hardness and inversely related to stickiness (Juliano 1971). Therefore, high AAC will lead to gumminess, softness, gloss and savory. Intermediate amylose (20–25%) rice cooks moist and remains soft and non-sticky and widely preferred by most consumers (Singh *et al.* 2005).

GC: The landraces varied significantly for their average GC. A total of 16 genotypes showed hard GC; 32 genotypes had medium and 50 genotypes showed soft GC. Manipur Black rice showed the longest gel length (118 mm), and IET 15831 showed minimum gel length (27 mm) with an average of 65 mm. GC in complementation with AAC helps in differentiating the high amylose rice for their cooked rice texture (Juliano 1985). When cooked, rice with hard GC hardens faster compared to those having soft GC. In the present study, almost 50% of the genotypes including Manipur Black (118 mm), RIL NJ-72 (113 mm), Pant Sugandh Dhan-15 (105 mm), Hasan Serai and IET 22778 (104 mm) etc. were found to be soft in GC, indicating their soft texture on cooking. Selected Kenyan and Tanzanian rice genotypes were also exhibited similar variation for GC reported by Chemutai, Musyoki and Kioko (2016). Rice with soft GC is highly preferred by the consumers, therefore, breeders prioritise to develop rice genotypes with soft GC (Wang *et al.* 2007). Negative correlation between AAC and GC, contrary to the correlated responses in selection for these traits (Oko *et al.* 2012). In the present study also, genotypes such as Manipur black rice and RIL NJ-72 showed the extremes of these traits with high GC (118 mm and 113 mm) and low AAC (4.81% and 7.36%), respectively.

Correlation studies: The correlations among agromorphological, grain physical and physico-chemical traits were calculated for 98 genotypes of rice (Table 2). PH showed significant positive correlation with the PL ($r=0.52$; $p=0.181$). DFF showed negative correlation with KLBC ($r=-0.29$; $p=0.022$) and kernel L/W ratio ($r=-0.25$; $p=0.003$). NT showed significant positive correlation with AAC ($r=0.22$; $p=0.614$), while significant negative association was observed with PL ($r=-0.27$; $p=0.599$). PL was significantly and positively correlated with KLBC ($r=0.38$; $p=0.000$) and kernel L/W ratio ($r=0.43$; $p=0.0649$), while negatively correlated with KWBC ($r=-0.24$; $p=0.027$). KLBC showed highly significant positive correlation with kernel L/W ratio ($r=0.88$; $p=0.0456$) and GC ($r=0.22$; $p=0.614$). AAC was negatively correlated with GC ($r=-0.38$; $p=0.000$). The other parameters were not significantly correlated for the present set of characters.

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