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Physiological characteristics of grain quality and yield attributing traits of advance rice lines (*Oryza Sativa. L*)

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Abstract

Aromatic rice is a special group of rice and considered the best in term of significant quality traits of rice and aroma. In this study sixteen advance lines of rice were evaluated for physical grain quality, aroma, gene of fragrance and yield attributed traits. Physical grain quality includes grain length, L/B ratio and grain width were significantly higher in Line 67 [7.73mm (SD±0.06)], Line 4 [1.601mm (SD±0.102)] and Line 4 [3.4mm (SD±0.02)]. Cooking time was positively correlated with gelatinization temperature (GT). The test advance lines were classified into, three groups: four at the advance lines were high GT, six with intermediate and six with low GT. High GT advance lines were recommended for parboiling. Out of sixteen advance lines showed aroma by conventional approaches; eleven lines were confirmed with the presence of fgr gene for aroma. The rest of the advance lines were non-aromatic with lacking fgr gene. The advance lines of rice were evaluated for yield attributing traits include, panicle length^{-1}, filled spikelet's panicle^{-1}, unfilled spikelet's panicle^{-1}, primary branch panicle^{-1} and secondary branch panicle^{-1} were significantly higher in Line 67 [33.153 cm (SD±0.73)], Line 4 [398.22 cm (SD±2.69)], Line 69 [109.78 cm (SD±8.27)), Line 36 [21.7 cm (SD±0.509)] and Line 182 [79.2 cm (SD±2.499927)] respectively. The productive tillers plant^{-1} and yield plant^{-1} were significantly higher in Line 21 [16.0 cm (SD±9)] and Line 4 [448.88g (SD±6.63)]. The results of this study indicated the excessive genetic potential of genotypes study. The promising genotypes recognized through the present study can be used in future breeding programs for the productive and quality result.

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Introduction

Rice (*Oryza Sativa)* considered the prime importance in the daily human life and therefore, the rice quality has gained the increasing attention by the researchers [1]. It is used as the primary food by more than 50% of the world's population [2]. Rice delivers 20 percent of the earth nutritional energy supply, while 19 prompt wheat deliveries and 5% maize. Fats carbohydrates, proteins and a reasonable amount of iron compounds, calcium, thiamine, riboflavin, and niacin which are the primary source of energy are found in rice [3]. In Pakistan rice is one of the most demanding cereal crops, status third in importance. During the year 2006-07, the area sown was 2581 thousand hectares, and production was 5438 thousand tons.

Moreover, being a vital diet of the people of Pakistan, rice has also gained a strong position as imported exchange grossing product. According to IRRI report, Pakistan is one of the top ten rice exporting country in the worldwide; its export performance has a regular improved in last two years [4, 5]. The earth population is estimated to enhance by about 2 billion in the next twenty years, and half of this increase will be in Asia where rice is the staple diet [6]. By the year 2025, about 760 million tons of rice will be compulsory to supply the increasing population at worldwide. This obligation is about 35% advanced than the total rice production [7].

In rice natural grain quality comprised of diverse characters that are directly or indirectly related to consumer preference [8, 9]. Rice grain quality comprises the grinding, recovery, gastronomic and nutritive qualities. The composite quality of rice grain quality is the number of total component traits, containing presence, eating quality and cooking, and nutritional quality [10]. The inheritance of rice cooking quality and eating characteristics are strongly influenced by amylose content, i.e., 25-30% is considered high amylose content lead to cooked rice grain rigid and hard, 20-25% is transition amylose content might be soft and sticky white rice with low amylose content less than 20% tends to have quite soft and sticky. Zero amylose content is found in waxy rice and generally referred to as sticky rice [11, 12].

In rice, only the dehulled grain (usually milled) is spent as food. Both hulled and un-hulled rice grains have great diversity in size and shape. The different widespread distribution in agro-climatic conditions diversified selection for a wide range of uses of rice grain [13]. A particular trend of grain cooking quality characters linked to grain shape [9]. Therefore, the variety in grain morphology needs to be examined. The mechanization of rice cultivation was based on an adaptation of improved varieties, and more recently the use of biotechnology for the incorporation of the gene for resistance to diseases and insect pests have come up in PARC [1]. However, recently it was realized at a national level that rice with better grain quality should be produced [14]. Since grain morphology between the first to be a visible character for selection and quality marking; and also keeping in view the importance of grain shape and the combination of the attributed characters for cooking quality as in US rice cultivars.

One of the essential traits in rice quality is aroma or fragrance, which is classified as the premium quality of rice in the worldwide market [15]. Aroma is emitting from aromatic rice during harvesting, cooking, milling, storage and in fields. Both genetic and environmental factor influences aroma rice that is why aroma rice is the best to harvest in those areas where the temperature is low (cooler) during maturity. Aroma rice constitutes a small but unique group of rice in the global business market because of it is marked, pleasant and unique aroma and mouth sense taste after cooking. Aroma rice is preferred more than non-aromatic rice due to different special occasions and for export from Pakistan, India, and Thailand, and thus aromatic rice has a high market price [16].

In a national financial system, the standard harvest is 1970 kg/hector, which is a lesser amount than other rice cultivating states of the earth. Many factors contribute towards yield. High speed and seedling vigor are required for a good stand establishment and successful crop performance in direct seeded rice. Varieties with high seedling vigor are also needed for better competitive ability against weeds [17].

Sixteen advanced lines of aroma rice were collected by the Department of Genetics Hazara University and the study was conducted with the aim to evaluate the advance lines of rice for grain quality, to identify the gene for fragrance and yield attributing traits.

Materials and Methods

In this study the genetic variations in grain quality and crop attributing traits in aromatic rice were determined via the following experiments during the harvest season 2017 at Hazara University Cultural Center, Mansehra. Sixteen locally developed rice genotypes were selected for the evaluation of the grain size, shape gelatinization temperature and identification of gene aroma. Moreover, morphological and physiological characteristics such as tillers plant^{-1}, panicle length^{-1}, and primary branches panicle^{-1}, secondary branched panicle^{-1}, filled spikelet's panicle^{-1}, unfilled spikelet's panicle^{-1}, and yield plant^{-1}**.**

Physical characteristics of rice grain variants and their correlations

This experiment was directed to regulate the relationships of grain physical traits among the varieties and possible difference between conventional varieties and advanced lines (NPT). For each variety, nine uniform grains were selected for determining the following parameters: grain length, width, thickness. For measuring the grain length, width and thickness electronic digital Vanier's Caliper were used. The correlations of rice grain physical traits among the varieties were performed. Sixteen varieties/advanced lines of rice were used in this study. The fifteen advance lines and control variety bars 385 are given in (**Table 1**).

The phenotypic characters of grain quality including grain size and shape were calculated by determining the grain length and width of ten whole crushed kernels. The average grain length and size were categorized according to scale (**Table 2 & 3**).

Table1: Advance lines used for correlations and variations of rice physical grain characteristics.

Sr. No	Name of advance lines	Sr. No	Name of advance lines
1	Line L4	**9**	Line 172
2	Line 26	**10**	69M
3	Line 187	**11**	Line 21
4	Line 80	**12**	Line Bas:385 (control)
5	Line 161	**13**	Line NPT:46
6	Line 7	**14**	Line 36
7	Line 151	**15**	Line 67
8	Line 182	**16**	Line L3

Table 2: The scale used for classification of grain length

Scale	Size Category	Length in mm
1	Extra-Long	More than 7.5
3	Long	6.61 to 7.5
5	Medium	5.51 to 6.6
7	Short	Less than 5.5

Table 3: The scale used for determination of grain shape

Scale	Shape	Length/width
1	Slender	Over 3.0
3	Medium	2.1 to 3.0
5	Bold	1.1 to 2.0
9	Round	1 or less

L/B Ratio

The L/B ratio was calculated by isolating the average length by the average breadth of the rice kernel. Constructed on the L/B ratio, grains were classified into short bold (SB), long bold (LB), medium slender (MS), short slender (SS) and long slender (LS) [9, 18].

Assessments for the aroma rice

The outermost shell was removed from ten whole dried seeds of each variety of rice and crushed. Crushed seeds were placed in a tightly packed box about 5x5 cm in size and then added 500 mL of diluted alkali (1.7%) in each particular box and covered directly. The preserved samples were placed at the optimum temperature for half an hour. After 30 minutes, each specific box was opened, and aroma was judged by smell.

Gelatinization temperature (GT)

Breaking down the intermolecular bonds of the starch molecule using water and temperature called starch gelatinization. To measure the GT of rice grains of different quality were placed in an extent of alkali spreading, i.e., 1.7% (KOH) Potassium hydroxide for twenty-three hours at optimum temperature. At low gelatinization temperature, rice grain disintegrated utterly, while at intermediate gelatinization temperature rice grain exhibited only partial disintegrated. At a high gelatinization temperature, rice grain remained largely unaffected in the alkali solution presented by (LSD) test at 5 % probability level.

DNA extraction and PCR analysis of fgr gene in aromatic advance lines of rice

Genomic DNA extraction was performed as previously described by [19]. Briefly, Five seeds of each genotype were collected in 1.5 mL Eppendorf tubes, and 500 µL of 2x CTAB buffer (50 mM Tris-HCl, pH 8.0, 25 mM EDTA, 300mM NaCl and 2% CTAB) was added and crushed with squeezing needles followed by the incubation at 65°C for 1 hourand. 500 µL Phenol: Chloroform: Isoamyl alcohol (25: 24: 1) was added and incubuated at room temperature for 30 minutes and then centrifuged at 8000 rpm for 15 minutes. The supernatant (400 µL) was transferred to new tubes and equal volumes of 2-propanol were added and incubated at – 20°C for 3 hours. DNA pellet was obtained by the centrifugation at 8000 rpm for 10 minutes. The pellet was washed with 70% ethanol and then air dried and then vortexed with 50 µL TE buffer. The DNA samples of each genotype were visualised on 1% agarose gel electrophoresis. The concentration of extracted genomic DNA was measured by spectrophotometer and then stored at 4°C for further analysis.

Amplification of *fgr* gene was carried out using allele-specific primers RG28L (F5'-GATCTCACTCCAAGTAAACTCTGAC-3' & R5'-ACTGCCATTGCTTCTGTTCTC-3'). Amplification reactions were carried out in 16 µL reaction volumes having 1 µl genomic DNA (20–50 ng/µL), 0.5 µL each of forward and reverse primers (10 µM / µL), 1.2 µL of dNTPs (25 mM each), 0.4 µL of Taq DNA Polymerase (2 U, Enzynomix), 1X Taq Buffer and 1.6µl MgCl2 (2.5 mM).

PCR amplification was carried out in DNA Thermal Cycler (Applied Biosystems) set at: an initial denaturation of 5 min at 94°C; 32 cycles of 94°C for 45 sec, 55°C for 45 sec, and 72°C for 1:30 sec. One additional cycle of 7 min at 72°C was used for final extension. Amplification products were resolved by electrophoresis on 3% agarose gel run in 1 X TAE buffer. The amplified products were observed below UV light after staining with ethidium bromide (10 ug/mL). The data were scored for the presence or absence of *fgr* linked DNA fragments.

Statistical analysis

The data were statistically analyzed for randomized complete block design using MSTATC software. The square of mean differences of sixteen rice genotypes was divided using the least–significance difference.

Results

Evaluation of physical grain quality of advance lines of rice

Sixteen advance lines of aroma rice were measured for physical gain quality. The materials showed considerable variability for grain characteristics. Following parameter were studied (**Table 4**).

The L/B ratio decreasing between (2.5 to 3 cm) has been significantly acceptable if the length is higher than (6 mm). In this study grain length and width ratio is determined by measuring grain length and width then taken as a percentage. Maximum grain L/B ratio was measured for the Line182 having (4.6 mm) and minimum L/B ratio (1.601 mm) was determined for the Line 4 (**Figure 1A; Table 4**). Grain length ranged from (5 - 7.73 mm). The maximum and minimum value was recorded; Maximum value was for the Line 67 having a length about [7.73cm (SD±0.06)] while minimum value was for Line 3 [5 cm SD±0.152)] (**Figure 1B; Table 4**). Grain width extended from (1.46 - 3.4 mm) in width. The maximum value was recorded for Line 4 [3.4 cm (SD±0.02)] and minimum value [1.46 cm (SD±0.01)] was set down for Line 182 (**Figure 1C; Table 4**).

The most significant characters associated with the purchaser selection are grain size. Depend on the length of grain; the selected samples were separated into four groups; one Extra-Long, two Long, three Medium and four Short grain varieties. The length of kernel seed advance lines extended from (5 - 7.73 cm). The highest grain length was verified for Line 67 [7.73cm (SD±0.065)]. The lowest grain length was recorded for Line 35 cm SD±0.152)]. Depend on grain length the advance lines were clustered into one Extra-Long, three Long, six Medium and six Short grain (6). Our analysis showed that the majority of advance lines categorized as medium grain and short grain followed by long and extra-long grain (**Figure1D; Table 4**).

The three-dimensional shape of grain was measured as length, width, and thickness. Based on Length: Breath ratio, the advance sixteen elite lines were classified into two groups: either slender or medium. The grain shape and size was recorded, the advance lines were subdivided into three groups: Slender, Medium, and Bold. In this experiment, nine advance lines were slender shape including Line 26, Line 161, Line 7, Line 182, Line 67, Line 69M, Line 21, Bas 385 and Line 36. Six lines were Medium including Line 187, Line 80, Line 151, Line 172, Line NPT 46 and Line 3. Only one advance Line 4 was Bold in shape. In these sixteen advance lines, there are no round shapes found (**Table 4**).

Gelatinization temperature

Gelatinization temperature is the variety of heat at which the starch granule of rice takes in water, drop their crystalline nature and begins a change that is irreversible. It is the reliable quality of the starch and is distinct as the series of temperature in which the starch granule begins to swell permanently in boiling water. The GT of starch in sixteen trial advance lines is specified in (**Table 4**). Based on the gelatinization temperature the advance lines were classified into three groups: Four advance lines were of high GT including Line 4, Line 7, Line 151 and Line 67, six with intermediate, including Line 26, Line 187, Line 182, Line 69M, Line NPT 46 and Line 3 while six line with low GT is Line 80, Line 161, Line 172, Line 21, Bas 385, and Line 36. These high GT advance lines could be used for parboiling (**Figure 2**).

The vital relationship was found among fragmentation of rice endosperm starch granule in alkali (KOH) solution and gelatinization temperature of milled rice. The milled grains were subjected to 1.7% potassium hydroxide at 30°C for 23 hours. Rice with low gelatinization temperature (18-21°C) was almost completely degraded in 1.7% KOH solution; however, rice with intermediate with gelatinization temperature (22-25°C) was partially degraded. Rice with high gelatinization temperature (26-30°C) was unaffected and mostly remains in 1.7% KOH solution shown in (**Figure 2**).

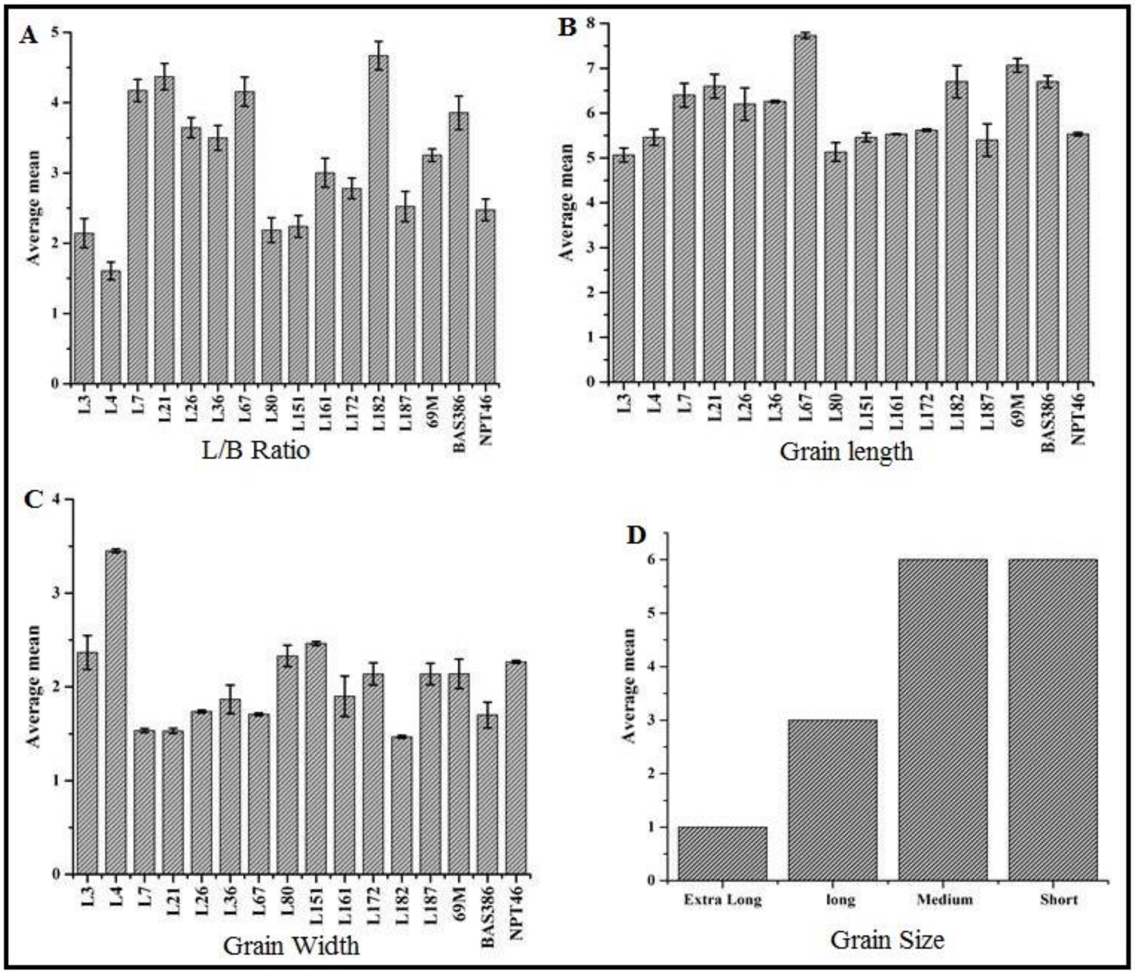

Fig. 1: Physical grain quality of advance lines of rice. (**A)** Variability of advance lines of rice for L/B ratio. (**B**) Genetic variability of advance lines of rice for grain length. (**C**) Variability of advance lines of rice for grain width. (**D**) The grain size of lines of rice. Based on grain size, Advance lines have been separated into four groups (Extra-long, Long, Medium, and Short).

Assessment for the Aroma

The seeds of sixteen different varieties were dehulled and crushed. Crushed rice of each grain was placed in a specific tightly covered plastic box about 5x5 cm in size. In an individual box, added 500 ml of diluted alkali (1.7%) and covered directly. The selected samples were kept at the optimum temperature for half an hour. After 30 minutes the boxes opened one by one and aroma was identified by smelling. In this test six advance lines including Line 4, Line 26, Line 80, Line 7, Line 36 and Line 3 showed strong aroma, six showed moderate aroma including Line 187, Line 172, Line 69 M, Line 21, Base 385 and NPT 46, while four lines including Line 161, Line 151, Line 182 & Line 67 showed no aroma smelling (**Table 4**).

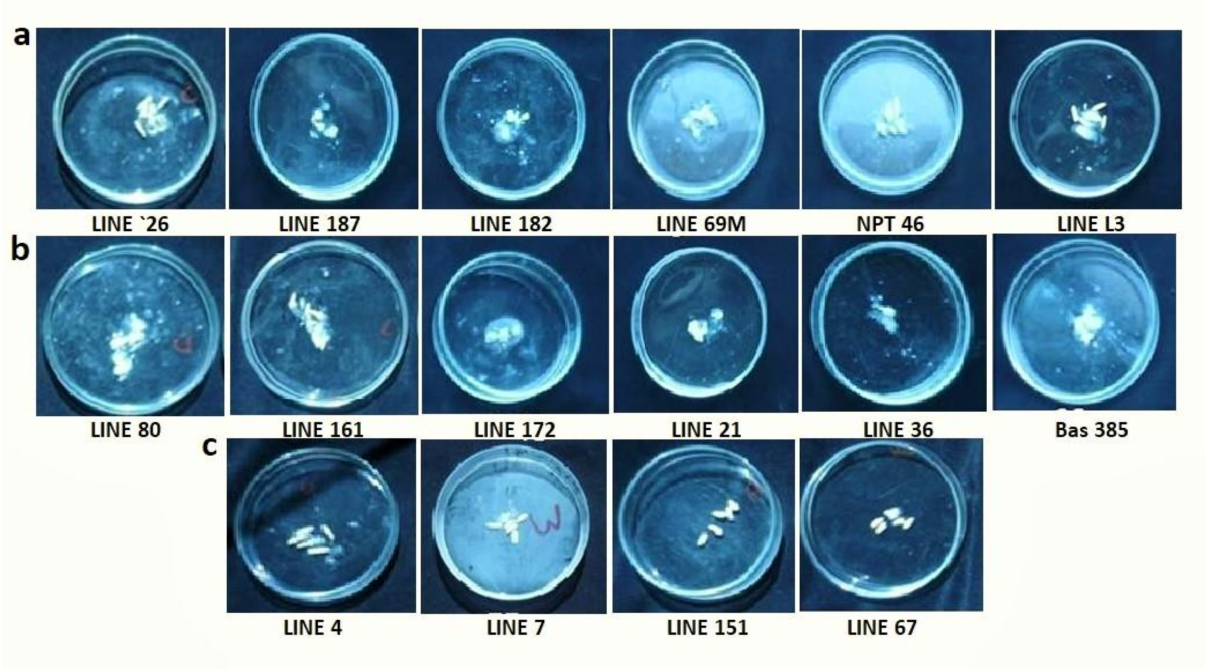

Fig. 2: Gelatinization temperature of advance lines of rice. The milled grains were subjected to 1.7% Potassium hydroxide for 23 hours. (**a**) Intermediate GT including Line 26, Line 187, Line 182, Line 69M, Line NPT 46 and Line 3 were partially degraded. (**b**) Low GT including Line 80, Line 161, Line 172, Line 21, Line Bas: 385, and Line 36 were completely degraded. (**c**) High GT including Line 4, Line 7, Line 151 and Line 67 were not degraded by Potassium Hydroxide.

Table 4: Grain quality of advance lines of rice

Sr. No	Name of advance lines	Grain Shape	Grain Size	Temperature	Aroma	Grain Length (mm)	Grain Width (mm)	L/B ratio
1	Line L4	Bold	Short	High	Strong	5.46	3.4	1.60
2	Line 26	Slender	Long	Intermediate	Strong	6.2	1.73	3.6
3	Line 187	Medium	Short	Intermediate	Moderate	5.4	2.138	2.52
4	Line 80	Medium	Short	Low	Strong	5.1	2.33	2.17
5	Line 161	Slender	Medium	Low	Nill	5.53	1.9	3
6	Line 7	Slender	medium	High	Strong	6.4	1.53	4.18
7	Line 151	Medium	Short	High	Nill	5.46	2.46	2.21
8	Line 182	Slender	Long	Intermediate	Nill	6.7	1.46	4.6
9	Line 67	Slender	Extra long	High	Nill	7.73	1.7	4.15
10	Line 172	Medium	Medium	Low	Moderate	5.6	2.1	2.71
11	Line 69M	Slender	Long	Intermediate	Moderate	7	2.133	3.25
12	Line 21	Slender	Medium	Low	Moderate	6.6	1.533	4.34
13	Line Bas:386	Slender	Long	Low	Moderate	6.7	1.7	3.88
14	Line NPT46	Medium	Medium	Intermediate	Moderate	5.53	2.26	2.44
15	Line 36	Slender	Medium	Low	Strong	6.26	1.8	3.5
16	Line 3	Medium	Short	Intermediate	Strong	5♦	2.3♦	2.12

Note: Significant association between grain length and width ♦ ($P<0.05$), Chi-Square = 10.44

Morphological and physiological characteristics

Fifteen advance lines by the side of a general check were assessed for yield and yield contributing characters under the similar harvest conditions of Hazara Mansehra. Several morphological and physiological traits were studied such as, panicle length^{-1}, number of tillers plant^{-1}, primary branches panicle^{-1}, secondary branches panicle^{-1}, filled spikelet's panicle^{-1}, unfilled spikelet's panicle^{-1}, yield plant^{-1}.

Records concerning panicle length^{-1} are accessible in (**Table 5**). Among the tested genotypes, significant levels of variability ($p \leq 0.05$) were observed. For panicle length^{-1}, the coefficient of variation was

4.44%. The statistics for panicle length^{-1} ranged from (33.153 cm) to (23.80 cm). The lowest amount of panicle length^{-1} was recorded for Line 161 while the highest panicle length^{-1} were determined for Line 67. Variability for various traits under the genomic control was used in the collection of a required characteristic. Crop performance is affected by photoperiod, leaf area index, sink and source relationship, inter-competition among plant inhabitants and plant density. The analysis of variance exposed that the genotype was highly significantly different for a total number of panicle length^{-1}. Maximum panicle length^{-1} exhibited by Line 67 having [33.153 cm (SD±0.73)], while the lowest panicle length^{-1} was observed in Line 161 [23.8 cm (SD±1.11) as shown in (**Figure 3A; Table 5**).

Productive tillers plant^{-1} have significant role in the yield attributing traits of rice. High number of tiller per plant produces the high yields. Based on the analysis of variance highly significant differences were found among tillers plant^{-1}. Line 21 shows a maximum number of tillers plant^{-1}, i.e. [16 cm (SD±9)] in (**Figure 3B; Table 5**) While Line 3 shows the minimum number of tillers plant^{-1}, i.e. [5.33 cm (SD±2.30)].

Based on the analysis of regulation it was noted that the advance lines were highly significant for the number of filled spikelets panicle^{-1}. Significantly different maximum filled spikelet's panicle^{-1} were recorded for Line 4 [398.22 cm (SD±2.69)], and minimum filled spikelet's [85.77 cm (SD±2.21)] were recorded for Line 161 (**Figure 3E; Table 5**).

Highly substantial differences among the advance lines were recorded for unfilled spikelet's Panicle^{-1}. The data for unfilled spikelet's per panicle ranged from (109.78) and (25). Highest unfilled spikelet's per panicle have been recorded for Line 69M [109.78 cm (SD±8.27)] while the lowest unfilled spikelet's Panicle^{-1} [25 cm (SD±2.03)] were recorded for Line 187 (**Figure 3F; Table 5**).

The analysis of variance revealed that the advance lines were highly significant by different for Primary branches per panicle (21.773 cm). Maximum numbers of primary branches panicle^{-1} were recorded for Line 36 [21.773 cm (SD±0.509)] while the lowest number of primary branches panicle^{-1} [9.330 cm (SD±1.017)] were recorded for Line NPT.46 (**Figure 3C; Table 5**).

The coefficient of variance for secondary branches panicle^{-1} showed highly significant difference among the advance lines. Maximum secondary branches [79.217cm (SD±2.499)] were recorded for Line 182, while the lowest secondary branches panicle^{-1} [33.107 cm (SD±0.502)] were recorded for Line Bas: 385 (**Figure 3D; Table 5**).

Analysis of difference shown a significant difference for yield plant^{-1}. The Line 4 has the highest values of yield per plant [448.88g (SD±6.63)] while the Line 161 has the lowest yield [114.11g (SD±2.673)] plant^{-1} (**Figure 3G; Table 5**).

DNA extraction and PCR analysis of fgr gene in aromatic advance lines of rice

Genomic DNA was extracted from sixteen cultivated verities of advance lines of rice from fresh seeds with the help of CTAB method [19]. We were extracted high quality of genomic DNA was obtained as shown in **figure 4A**. The concentration of sixteen extracted DNA sample were standardized from 20 to 50 ng/µL with the help of spectrophotometry.

Confirmation of fgr gene in elite aromatic lines of rice

Molecular and conventional approaches were used for confirming the presence of *fgr* gene. The molecular analysis was showed for the identification of *fgr* gene in sixteen cultivated verities of advance lines of rice. STS marker was used in this study that amplified 180 bp fragments in aromatic and nonaromatic genotypes, respectively. Out of sixteen lines showing aroma by conventional approach, 11 lines were confirmed with the presence of *fgr* gene. These elite lines included Line Base:385, Line NPT 46, Line 3, Line182 and Line 187, Line 161, Line 80, Line 69M, Line 4, Line 36, and Line 172 have the gene for aroma. The rest of advance lines for example Line 26, Line 7, Line 8 and Line 151 are nonaromatic as lacking *fgr* gene. This study discriminated the aromatic and nonaromatic advance lines based on the sequence-tagged site marker data (STSs) of RG28.

The statistics were counted using "+" symbol for the presence of a gene (*fgr*) and "–" symbol for the absence of (*fgr*) gene (**Table 6 and Figure 4B**).

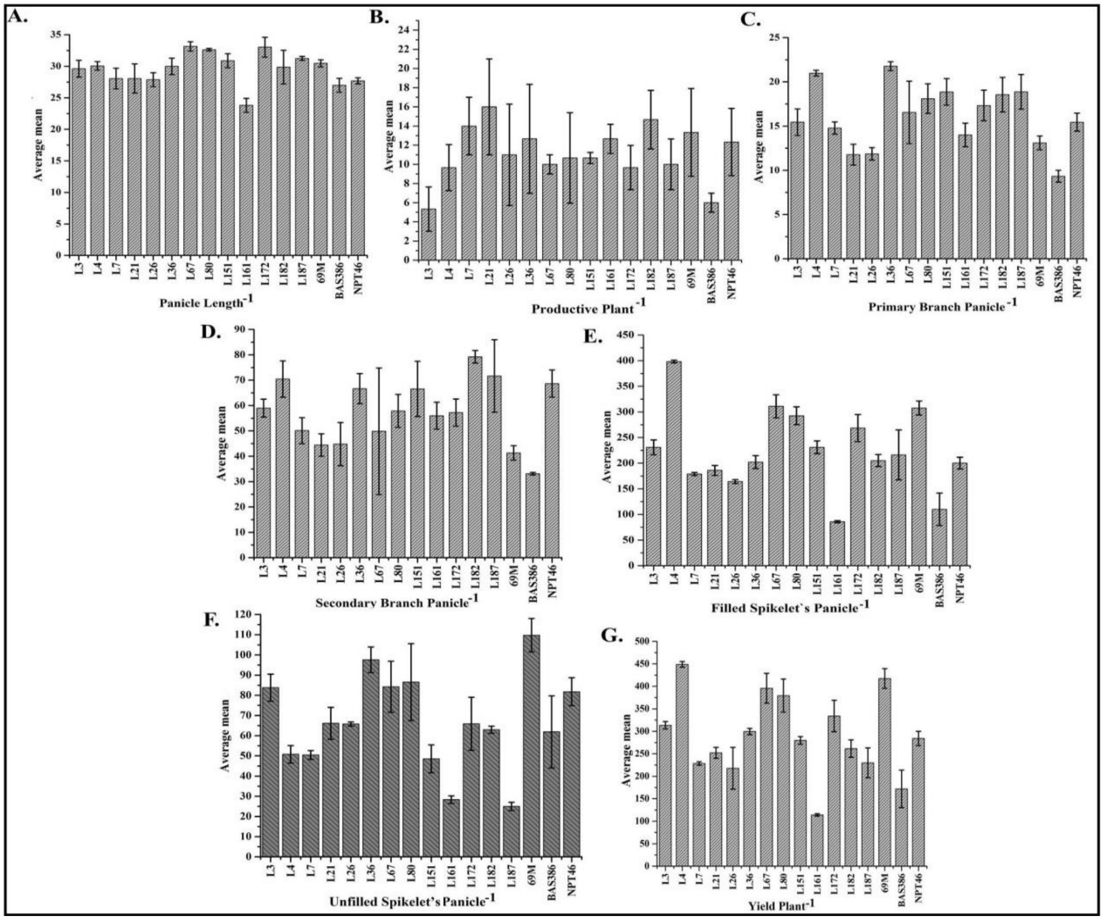

Fig. 3: Morphological and Physiological Parameters. (**A**) Panicle Length. (**B**) Productive tiller Plant^{-1}. (**C**) Primary Branch Panicle^{-1}. (**D**) Secondary Branch Panicle^{-1} (**E**) Filled Spikletes Panicle^{-1} (**F**) Unfilled Spikletes Panicle^{-1} (**G**) Yield Plant^{-1}.

Table 5: Mean values for yield attributing lines of rice

Sr. No	Name of advance lines	Panicle Length plant1 (cm)	Primary Branches panicle1 (No)	Secondary Branch panicle1 (No)	Spikelet filled panicle1 (No)	Spikelet unfilled panicle1 (No)	Productive Tillers plant (No)	Yield Plant (g)
1	Line4	30.1	20.9	70.4	398.2	50.7	9.6	448.8
2	Line 26	28	11.8	44.7	164.2	65.7	11	218
3	Line 187	31.2	18.8	71.6	216	25	10	230
4	Line 80	32.6	18.1	57.8	292.5	86.5	11	379.4
5	Line 161	23.8	13.9	56	85.7	28.3	13	114.1
6	Line 7	28.0	14.7	50.1	179	50.4	14	228.3
7	Line 151	31	19	66.5	231	48.5	11	279.5
8	Line 182	30	18.5	79.2	205.1	63	13	261.4
9	Line 67	33.1	16.5	49.8	311.1	84.2	10	395.7
10	Line 172	33.0	17.3	57.2	268.6	65.8	10	334
11	Line 69M	30.4	13.1	41.3	307.6	109.7	13.3	417.4
12	Line 21	28.0	11.7	44.4	186	66.1	16	252.1
13	Line Bas:385	27	9.3	33.1	110	61.8	6	171.8
14	Line NPT46	28	15.4	68.6	200.2	81.7	12.3	284
15	Line 36	30	21.7	66.6	201.9	97.5	13	299.5
16	Line 3	30	15.4	59	230.7	83.7	5.3	313.3
MCP*		28.80	24.51	44.13	76.22	31.25	19.5	80.26♦

Note: Multiple comparison proportions*, Significant ♦ (P<0.002).

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The statistics were counted using "+" symbol for the presence of a gene (*fgr*) and "–" symbol for the absence of (*fgr*) gene (**Table 6 and Figure 4B**).

Table 6: Presence (+) and absence (-) *fgr* gene in advance lines of rice

Sr.#	**Advance lines**	***fgr* Gene**	**Sr. #**	**Adv. lines**	***fgr* Gene**
1	Line 4	+	9	Line 172	+
2	Line 26	-	10	Line 69M	+
3	Line 187	+	11	Line 21	-
4	Line 80	+	12	Bas:385 (control)	+
5	Line 161	+	13	Line NPT:46	+
6	Line 7	-	14	Line 36	+
7	Line 151	-	15	Line 67	-
8	Line 182	+	16	Line L3	+

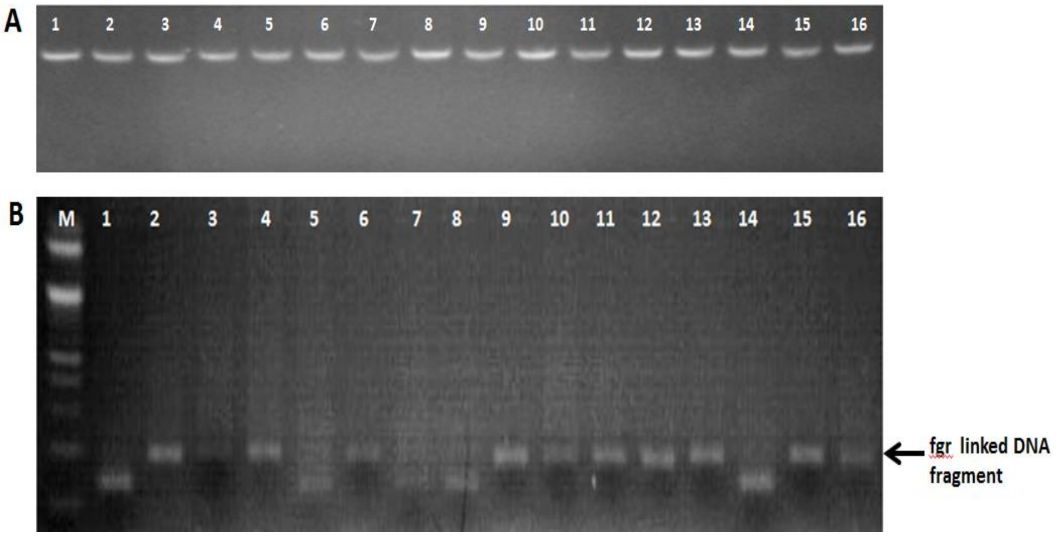

Fig. 4: DNA extraction and PCR analysis of *fgr* gene in aromatic advance lines of rice. (**A**) Genomic DNA extracted from fresh seeds of cultivated varieties of rice using CTAB method and fixed on 1% agarose gel. 1=Line 21; 2=Line Bas:385; 3=Line NPT46; 4=Line 3, 5=Line 26; 6=Line 182; 7=Line 7; 8=Line 67; 9=Line187; 10=Line 161; 11=Line 80; 12=Line 69m; 13=Line 4; 14=Line 151; 15=Line 36; 16=Line 172. (**B**) Banding pattern of the presence and absence of fgr gene for aroma in advance lines of rice. M= 100 bp DNA ladder; 1=Line 21; 2= Line bas385; 3= Line NPT-46; 4= Line 3, 5=Line 26; 6=Line 182; 7=Line 7; 8=Line 67; 9=Line187; 10= Line 4; 11=Line 80; 12=Line 69m; 13=Line 4; 14=Line151; 15=Line 74; 16=Line 72. Out of sixteen advance lines showed aroma by conventional approaches; eleven lines were confirmed with the presence of *fgr* gene for aroma. The rest of the advance lines were non-aromatic with lacking *fgr* gene.

Discussion

Genetic modification of seed control is the primary goal of rice breeding program since it increases the whole sale price of rice varieties. The present study aims were to evaluate the variation in grain quality traits among advance lines of rice (*Oryza sativa L*). Two approaches were used for assessing the grain quality: a physical method of evaluating the aroma by organoleptic test and a molecular procedure by identification of *fgr* gene in the elite lines. Rice grain characteristics such as morphological (Grain size, length (L) and breadth (B), L/B ratio, grain shape, gelatinization temperature and morphological traits like plant height, panicle length, spikelets filled /panicle, spikelet's unfilled/panicle, grain classification and overall acceptability and variability were also studied for Sixteen advance lines of rice. The appearances of milled rice including grain size and shape are the initial criteria of rice quality which breeders select in evolving innovative varieties and release it for commercial production [20]. The physical dimensions of rice independently classified into grain-type categories depend upon physical parameters such as Length and shape, as length is the length determines the measure of grain rice in its greatest dimensions and shape: width ratio. The Kernel shape as well as Length: width ratio is significant features for grain quality determination [21]. According to [9] the length: breadth ratio decreasing 2.5 to 3.0 has been measured usually suitable as well as the length is more than 6mm. Based on physical parameters and L/B ratio the sixteen advance lines were categorized into three groups such as, slender, medium and bold. In this research, the slender shape rice exhibits maximum 3mm L/B ratio, 2mm is for medium, and 1.6 mm is bold grain. The highest value for grain length was found in Line 67 (7.73 cm) and lowest value in Line 3 (5 cm). The smallest grain length-breadth ratio was recorded in Line 4 (1.601 cm), and the highest value was measured in Line 182 (4.6 cm). The grain width maximum value was recorded for Line 4 (3.4 cm), and the minimum value was recorded in Line 182 (1.46 cm), respectively (**Table 4; Figure 1**). In an earlier study, the cultivated varieties were used for evaluating grain quality for identification, genetic discrepancy and varietal classification [12, 113, 33, 31]. Rice was collected for seven quantitative and eight qualitative characters from different parts of Pakistan. A significant amount of genetic variation was displayed for most of the traits examined [22]. The above length breath ratio 3 mm is usually measured as slender. The L/B ratio was analyzed to determine the individual shape of rice grain. The buyer select rice with a glowing endosperm and pay the best worth for it, while dullness dissolves throughout cooking and does not adjust eating quality. One group of consumers disagree with the other because of the ideal grain size and shape [23].

Gelatinization temperature and alkali digestion value affect the quality of eating and cooking of rice [24]. The alkali digestibility assessment can guide gelatinization temperature and restrained by alkali digestion value [25]. According to [26] gelatinization properties of starch significantly different as the ripening temperature different, supporting finding suggest that rice starch grown at a high temperature would require a higher cooking temperature and longer time. In the present study rice with low gelatinization temperature (18-21^{o}C) were almost completely degraded in 1.7% KOH solution; rice with intermediate with gelatinization temperature (22-25^{o}C) was partially degraded, and rice with high gelatinization temperature (26-30^{o}C) was unaffected and largely remains (**Figure 2**) [27]. Eenvironmental aspects such as temperature throughout grain development and developing grain stages affect gelatinization temperature. According to [28] rice with high gelatinization temperature expands extremely soft and tends to fragment when overcooked require more time for cooking compared to low or intermediate gelatinization temperature. Elongated chains need a higher temperature, so more energy, to separate and unravel the chain to initiate gelatinization than binary helices with smaller chains [29].

Some genetic variations were displayed for most of the characters examined. The diversity within 20 rice varieties used as progenitors in Cuban rice breeding program concerning agro-morphological traits, pedigree and DNA markers. By conventional approach of assessment of aroma, 14 elite lines viz. Bas. 370, Line-4, Line-15, Line-75, Line-18, Line-80, Line-77, Line-97, Line-87, Line-148, Line-99, Line-172, Line-129, Line -26 and Line 10 were considered as aromatic elite lines [30]. The cultivated varieties Basmati-385 and Swat-1 existence of *fgr* gene (140 bp amplicon) while IRBB 59, JP-5, Fakhre Malakand, and IR24 were absent this gene. Between the advance lines, 12 genotypes presented the existence of *fgr* gene (140bp), two genotypes (NPT-86 and Line 36) were segregating whereas the remaining 16 genotypes were missing this gene [31]. In this present study, sixteen elite lines were selected as aromatic by aroma tests. These lines were reconfirmed for the presence of fragrance gene (*fgr*) by using STS marker. Around 180 bp fragments was elevated in eleven elite lines viz. Line Bas:385, Line NPT 46, Line 3, Line182 and Line 187, Line 161, Line 80, Line 69M, Line 4, Line 36, and Line 172 have the gene for aroma confirming that these elite lines had the gene for fragrance (**Figure 4**). The expression of this gene led to the synthesis of 2-acetyl-1-pyrroline that is responsible for fragrance in rice. STS marker could be used to identify new genes or quantitative trait loci in rice that enable the breeder for using in crossing and selection to increase the genetic variability for this trait. Further studies are needed to compare the *fgr* gene in rice with the fragrance gene in popcorn, mung bean, and green tea.

Characterization of crop germplasm concluded different morphological traits is a significant phase for valuation of its genetic perspective. The advance lines were evaluated for yield attributing traits including, productive tillers^{-1}, panicle length^{-1}, filled and unfilled grain panicle^{-1}. The highest number of panicle length^{-1} Line 67 (33.153cm), filled spikelet's panicle^{-1} Line 4 (398.22 cm), number of unfilled spikelet's panicle^{-1} Line 69M (109.78 cm), primary branch panicle^{-1} Line 36 (21.773 cm) and secondary branch panicle^{-1} Line 182 (79.217 cm). The highest number of productive tillers plant^{-1} exhibited by Line 21 having (16.000 cm) and yield plant^{-1} was obtained by Line 4 (448.88 cm). The lowest number of productive tillers plant^{-1} was recorded by Line 3 (5.33 cm), and yield plant^{-1} was recorded by Line 161 (114.11g) respectively (**Table 5; Figure 3**). The objective of this investigation was to identify the required morphological characters that further used in breeding and other rice kernels. These lines could be used in breeding varieties for medium tillers. Highly significant variability among the different rice of genotypes was recorded by [19]. Grain yield constituent such as dry grain weight and 1000 grain weight verified positive and vital link with some productive tiller per plant [26]. This study result shows the excessive genetic potential of the studied genotypes. The favorable genotypes recognized during the present study can be used in future breeding programs for the productive and quality result.

Conclusion

In this study sixteen advance lines of rice were evaluated for physical grain quality, aroma, gene of fragrance and yield attributed traits. Some of rice lines were exhibits their high grain quality and further confirmed the presence of *fgr* gene for aroma. The constructive genotypes were recognized and can be further used in rice breeding programs for further improvement purpose.

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