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Molecular Profiling of Pakistani Selected Advance Lines of Rice for Amylose Content

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Abstract

Background: Pakistani rice is well-known for its quality. Its consumption increases with the increase in population. The gel consistency (GC) amylose content (AC) and gelatinization temperature (GT) are the most important rice characters, which are associated directly to eating and cooking attributes. But for its good taste and eating quality depends on its endosperm starch quality and quantity. Amylose, a chief determinant of rice attribute, is principally synthesized and controlled by a major gene (Waxy gene) encoding an enzyme called granule bound starch synthase (GBBS).

Methods: Current investigation was carried out to characterize advance lines of rice by both conventional and molecular approaches. In present study Waxy gene was identified in advance lines of rice.

Results: Show that out of 17 advanced lines, 9 lines were waxy or low amylose, and 1 line was non waxy or high amylose rice because of the presence of 425 bp fragment and 225 bp fragment of Wx gene respectively. For morphological data 14 morphological quantitative traits were studied.

Conclusion: Advance lines of rice analyzed during the present investigation showed better grain quality. A number of advance lines contain extra-long and medium slender grains which have intermediate to high gelatinization temperatures. Thus these advance lines are appropriate for the improvement of saline rice. Except one advance line 19 that showed Hard gel consistency and the majority of advance lines fall in the category of soft gel consistency and thus are of excellent quality.

Introduction

Rice is one of the most important staple crops that provide food for more than half of the world's population [1]. As a major cereal crop, it is one of the most diversified crop species due to its adaptation to a wide range of geographical, ecological, and climatic regions [1]. It is a vital food of the enormous proportion of population of Pakistan, but it is economically major crop of this country. Rice stands next to cotton in exports and contributes major part of the total export's earnings. More than 90% of rice is consumed and produced in Asia. The world's population is estimated to be over 9 billion by 2050, as large in universal raise food production will be needed [2]. (FAO, 2009). The increase in rice yield had beginning to slow by the start of the present century [1, 3]. Through genetic development through the green revolution in the previous half century rice production dramatically improved along with application of a greater quantity of chemical fertilizer [4], it is a regular Asian diet generally used as an entire grain after cooking and contribute of the total calorie intake for 40% to 80% [5, 6, 7, 8]. The priority of rice cooking and eating traits within a definite region and culture may not be accepted by other cultures. In general, the Japanese like better little grain, sticky rice that is normally used in creation sushi. On the other hand, Basmati rice is well-accepted in Pakistan, and India, the Middle East, because to its aroma and it's lengthens, dry grains when cooked [9].

Rice is a staple starchy food, which gives a large portion (~ 90% in Asia) of nutritional energy. Starch is the main component of rice grain, thus the starch-synthesizing capability of endosperm is mainly determined by the capacity of grain filling. Therefore Starch properties are a significant factor to determine the grain quality. Milled and Brown rice contains about 75-85% and 90% carbohydrates, respectively. The starch properties are mainly determined from Eating and cooking characteristics that creates up 90% of milled rice. The significant starch properties that influence eating and cooking properties are Gelatinization temperature, amylose content, and gel consistency. The most important forecaster is considered amylose of sensory attribute in rice [10]. Starch is consisting of two polysaccharides: amylose and amylopectin, which is primarily influenced by Rice quality. The amylose is linked glucose polymer by_ (1,4)– a relatively less branched while in amylopectin the branching enzyme generates 1,6 linkages. The percentage of amylose on, apparent Amylose Content (AAC) as measured, is the important determinant of cooking rice properties. High amylose content variety like risotto varieties are becoming dry and separated of grains after cooking, whereas low amylose cultivars (cvs) after cooking are glossy and tender cohesive or waxy. Lesser amylose rice is preferable than (over 20%) higher amylose rice because when they cooked it does not become dry and hard. Recommended classification on the basis of amylose content milled rice is classified in to the following five classes such as high (25-33%) intermediate (20-25%) low (12-20%) very low (5- 12%) waxy ((0-5%), even considering that commercially amylose content by rice is classified as either low (below than 20% amylose), medium (21–25%) and high (26–33%) [11, 12].

Waxy gene is responsible for amylose synthesis in rice, which encodes granule- bound starch synthase I (OsGBSSI). While amylopectin is synthesized by the action of four classes of enzyme: starch synthase, branching enzyme, ADP-glucose pyrophosphorylase, and debranching enzyme [13, 14]. In non- waxy rice cultivars GBSS quantity depend on the amylose content accumulating during grain filling process, in the Wax locus two functional alleles are present, Wxa and Wxb [15]. Therefore, present study is proposed for evaluation of Pakistani selected rice advanced lines for physical grain quality, yield and yield attributing traits and identification of waxy gene.

Methods

Plant materials

Plant materials were included of seventeen genotypes of rice varieties that have been developed at Hazara University, Mansehra, Pakistan. These following genotypes were growing at experimental field NTHRI, Shinkiari, Mansehra (Table 1 and Figure 1).

Sr. No.	Genotypes	Sr.	Genotypes
1	Line-60	10	Line-19
2	Line-61	11	Line-20
3	Line-62	12	Line-21
4	Line-65	13	Line-22
5	Line-66	14	Line-25
6	Line-11	15	Line-28
7	Line-12	16	Line-29
8	Line-15	17	Line-31
9	Line-16		

Table 1: Advance lines of rice used in this Study

Morphological characterization

Seventeen genotypes of rice were morphologically evaluated. All the genotypes were grown in the research fields of Hazara University, Mansehra. Environmental conditions were same for all the genotypes under investigation. Morphological traits studied in these genotypes of rice were categorized as quantitative traits. Total 14 morphological traits were investigated, such as number of tillers per plant, flag leaf length, grain length, grain chalkiness, plant height, flag leaf width, panicle length, number of branches per panicle, number of filled grains per panicle, unfilled grains, yield per plant, grain width, grain thickness and 100 grains weight.

Figure 1: 17 genotypes of rice cultivated at experimental field at research institute (NTHRI), Mansehra, Pakistan

Identification and extraction of genomic DNA for waxy gene

Extraction of Genomic DNA

Genomic DNA was extracted from new seeds of 17 genotypes of rice by using CTAB technique, with small alterations high quality of genomic DNA was obtained. With the help of spectrophotometer the concentration of DNA samples was maintain from 20 to 50ng/µl (Figure 2).

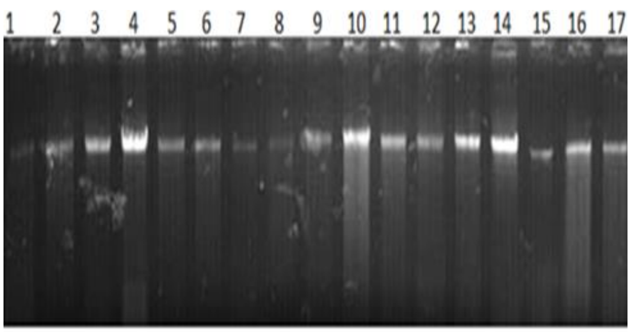

Figure 2: Genomic DNA extracted from new seed of growing varieties of rice

Furthermore, the waxy gene were further confirmed in the gnomic DNA of selected advance lines of rice by PCR using WxF and WxR primers. Moreover the amplified product was further confirmed by 1.5% Agarose gel electrophoresis.

Data analysis

The collected data from the selected lines of rice was further analysed by using Statistical software while the waxy gene presence was confirmed by PCR and Agarose gel electrophoresis.

Results

Morphological characterization

Total 14 morphological traits were investigated, such as number of tillers per plant, flag leaf length, grain length, grain chalkiness, plant height, flag leaf width, panicle length, number of branches per panicle, number of filled grains per panicle, unfilled grains, yield per plant, grain width, grain thickness and 100 grains weight.

There is variation in plant height and the range recorded was 77.33 to 134.33 cm. Minimum height (77.33) was recorded for line 16, while maximum height (134.33) was recorded for line 65-66. There are variations in the number of productive tillers per plants the range was recorded from 4.66 to 25.33. Minimum tillers (4.66) were recorded for line 61, 62. While maximum tillers (25.33) were recorded for line12. Length of flag leaf ranged from 19.16 cm to 48.66 cm. The smallest value (19.16 cm) was recorded for line 21 and the maximum value (48.66 cm) was recorded for line 62. Width of flag leaf ranged from 1.10 cm to 2.36 cm. least value (1.10 cm) was recorded for line 11and extreme value (2.36 cm) were calculated for line 22. Ranged of Panicle length from 21.66 cm to line 29 and the maximum value (53.33 cm) was calculated for line 62. Branches per panicle were ranged from 9.33 to 18.66. Least value (9.33) was recorded for line 11 while the maximum value (18.66) was calculated for line 62 respectively (Table 2 and 3).

Filled grains per panicle ranged from 93.33 to 277. The lowest amount of filled grains for each panicle (93.33) was recorded for line 29 whereas the maximum quantity of filled grains of each panicle (277) was recorded for line 19. Unfilled grains per panicle ranged from 12.33 to 322.

The minimum value (12.33) was recorded for line 16 and

the maximum value (322) was recorded in line 62. Yield per plant ranged from 22.33 to 69.33g. The minimum yield value was (22.33g) recorded in advance line 15 and the maximum yield value (69.33g) was calculated in line28 (Table 4 and 5).

100 grain weight ranged from 1.86 g to 3.30 g. Minimum value (1.86 g) was recorded for line 12 and maximum value (3.30 g) was recorded for line 61. Grain length ranged from 5.36 mm 8.20 mm. Minimum value (5.36 mm) was recorded for line 19 and 25 while the maximum value (8.20mm) was recorded for line 66. Grain width recorded from 1.50 mm to 2.30 mm. The smallest value (1.50 mm) was recorded for line 29 and the maximum value (2.30 mm) was calculated for line 61. Grain Length and breadth ratio ranged from 2.62 mm to 4.24 mm. the least value (2.62mm) was recorded for line 28 and the maximum value (4.24) was recorded for line 16. Grain chalkiness recorded as 26.03 mm to 66.97 mm. the minimum value (26.03 mm) was

Sr. No.	Genotype lines	Plant height (cm)	No. of productive tillers/plant	Length of flag leaf (cm)	Mid width of flag leaf (cm)	Panicle length (cm)	Branches/ panicle
1	60 line	105.33d	5.667f	32.50bcd	1.5e	29.00cde	12.7def
2	61 line	113.00c	4.670f	43.0ab	1.87bcd	33.83ab	17.33 c
3	62 line	124.67b	4.67f	48.67a	1.93bc	35.33a	18.7bc
4	65 line	134.33a	5.00f	36.00bcd	2.10ab	31.633bc	17.33c
5	66 line	131.33ab	7.00ef	41.67abc	2.17ab	33.50ab	21.333ab
6	11 line	78.00g	20.00b	35.83bcd	1.100f	29.33cd	9.33ef
7	12 line	80.00g	25.33a	33.33bcd	1.500e	27.67def	9.67f
8	15 line	90.00ef	8.67cdef	29.833cde	1.533de	24.067ghi	12.67def
9	16 line	77.33g	13.33c	27.50de	1.43ef	24.97fgh	10.33ef
10	19 line	89.33f	11.33cde	32.47bcd	1.533de	25.67efgh	12.33ef
11	20 line	104.67d	5.67f	26.33de	1.633cde	27.167defg	13.333de
12	21 line	106.67cd	7.67def	19.167e	1.967bc	26.400defgh	16.00cd
13	22 line	88.33 f	6.67ef	29.40cde	2.3667a	26.633defgh	17.67c
14	25 line	89.67 f	9.00cdef	30,00cde	1.967bc	25.833defgh	23.33a
15	28 line	96.67e	25.00a	37.06abcd	67cde	26.30defgh	12.00ef
16	29 line	88.33f	11.00cde	33.67bcd	1.500e	21.67i	12.00ef
17	31 line	94.67ef	12.00cd	34.067bcd	1.6333cde	23.367hi	11.00ef

Table 2: Grain quality characters of seventeen rice advance lines

Traits	D.F genotypes	Sum Square	Mean squares	F value	CV%	Grand mean	P value
Plant height	16	15178	948.623	56.88	4.1	99.549	0
Number of tillers	16	2149.02	134.314	16.16	26.83	10.745	0
Length of flag leaf	16	2247.25	140.453	2.57	22.02	33.559	0.0112
Mid width of flag leaf	16	4.94588	0.30912	6.64	12.47	1.7294	0
Panicle length	16	713.314	44.5821	9.77	7.69	27.786	0
Branch per panicle	16	826.039	51.6275	11.37	14.66	14.529	0

Table 3: ANOVA results for advanced lines of Rice

Sr. No.	Advance lines	Filled grains no	Un filled grains no	Yield per plant(g)
1	60 line	127.00de	194.00bc	35.00cdefg
2	61 line	133.33de	287.67ab	29.33efg
3	62 line	151.33cde	322.00a	38.33cdef
4	65 line	119.33de	143.00cde	14.33h
5	66 line	149.00de	285.00ab	23.33gh
6	11 line	128.33de	15.67h	46.00bcd
7	12 line	147.00de	22.67h	52.00b
8	15 line	127.67de	152.67cde	22.33gh
9	16 line	122.67de	12.33h	42.67bcde
10	19 line	277.00a	62.00efgh	25.67fgh
11	20 line	193.33bcd	73.67defg	33.67defg
12	21 line	152.67cde	125.33cdefg	36.33cdefg
13	22 line	241.33ab	133.33cdef	48.67bc
14	25 line	226.67abc	161.67cd	46.00bcd
15	28 line	182.00bcd	42.00fgh	69.33a
16	29 line	93.33e	29.33h	28.33efgh
17	31 line	143.33de	38.67h	49.00bc

Table 4: Grain quality characters of seventeen advanced lines

Traits	D.F genotypes	Sum. Square	Mean squares	F value	CV%	Grand mean	P value
Total grain	16	589203	36825.2	11.21	20.23	283.33	0
Filled grains	16	115138	7196.13	3.51	28.37	159.73	0.0012
Unfilled grains	16	489955	30622.2	9.65	45.58	123.59	0
Yield per plant	16	8797.3	549.833	6.88	23.73	37.667	0

Table 5: ANOVA Result for advanced lines of rice

Sr. No.	Advance Lines	100 grains wt.(g)	Length of 1 grains (mm)	Width of 1 grains (mm)	Mean L/B ratio	Chalkiness % mean (mm)
1	60 line	3.100ab	7.5667ab	1.900abcde	3.99a	2.16def
2	61 line	3.300a	7.533ab	2.300a	3.28abc	3.15abcde
3	62 line	3.200ab	7.600ab	2.00abcde	3.82ab	2.83abcdef
4	65 line	2.93bc	7.4667abc	1.833abcde	4.17a	1.99f
5	66 line	2.900bcd	8.200a	2.1667abc	3.78ab	2.25def
6	11 line	2.400fgh	7.0667bcd	2.00abcde	3.53abc	1.82f
7	12 line	1.87i	6.533cde	1.700bcde	3.84ab	2.28def
8	15 line	3.0667ab	6.200defg	2.200ab	2.82bc	3.38abc
9	16 line	2.600defg	6.500de	1.533de	4.24a	2.54bcdef
10	19 line	1.933i	5.3667g	1.600cde	3.52acb	2.28 def
11	20 line	3.27a	6.733bcde	2.200ab	3.23abc	3.79a
12	21 line	2.57efg	6.400def	2.00abcde	3.41abc	3.19abcd
13	22 line	2.67cdef	5.533fg	2.10abcd	2.79bc	3.57ab
14	25 line	2.733cde	5.3667g	1.70bcde	3.27abc	3.72a
15	28 line	2.133hi	5.500fg	2.10abcd	2.62c	2.52cdef
16	29 line	2.300gh	5.500fg	1.500e	3.72ab	2.79abcdef
17	31 line	2.400fgh	5.800efg	1.733abcde	3.40abc	2.14ef

Table 6: Grain quality characters of seventeen advanced lines.

Traits	D.F genotypes	Sum square	Mean squares	F value	CV%	Grand mean	P value
100grains weight	16	9.8298	0.61436	17.36	7.05	2.6686	0
Length of grains	16	41.1063	2.56914	7.79	8.81	6.5216	0
Width of grains	16	3.04078	0.19005	1.57	18.15	1.9157	0.135
L.B ratio	16	10.5321	0.65826	1.59	18.36	3.5	0.1279
Grains chalk%	16	9110.9	569.432	6.67	21.05	43.91	0

Table 7: ANOVA result for advanced lines of Rice

Sr. No.	Genotypes	Band size bp	Amylose content	Category
1	60	425	LOW	Waxy
2	61	425	LOW	Waxy
3	62	425	LOW	Waxy
4	65	425	LOW	Waxy
5	66	425	LOW	Waxy
6	11	425	LOW	Waxy
7	12	425	LOW	Waxy
8	15	425	LOW	Waxy
9	16	425	LOW	Waxy
10	19	228	HIGH	Non-waxy

Table 8: PCR results for Waxy gene

Sr. No.	Genotypes	Length of blue gel (mm)	Gel consistency (GC)	Digestion of Alkali	Gelatinization temperature (GT)
1	60	68	Soft	Partial effect	I(Intermediate)
2	61	32	Soft	Unaffected	H (High)
3	62	82	Soft	Unaffected	High
4	65	76	Soft	Unaffected	High
5	66	98	Soft	Unaffected	High
6	11	64	Soft	Unaffected	High
7	12	77	Soft	Partial effect	Intermediate
8	15	95	Soft	Partial effect	Intermediate
9	16	97	Soft	Unaffected	High
10	19	82	Hard	Partial un	H(High)
11	20	78	Soft	Partial effect	Intermediate
12	21	90	Soft	Complete Effect	L (Low)
13	22	86	Soft	Unaffected	High
14	25	80	Soft	Complete Effect	L
15	28	82	Soft	Unaffected	High
16	29	85	Soft	Unaffected	High
17	31	82	Soft	Unaffected	High

Table 9: Gel consistency and gelatinization temperature of genotypes of rice

recorded for line 11 and the maximum value (66.97 mm) was recorded for line22 (Table 6 and 7).

Molecular analysis of waxy genes

Waxy gene expressed differentially in various rice cultivars as its role is to control amylose synthesis and amylose content variation in rice endosperm. In the present study waxy gene was identified in the advanced lines of rice. The amplified PCR products of 17 samples were 228 bp and 425 bp in length. Products of PCR were generated by using the verified primers WxF (forward) and WxR (reverse). The PCR reaction was executed in 10 µl volume (Figure 3).

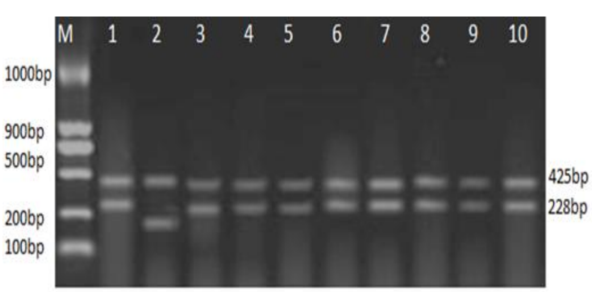

Figure 3: Banding pattern of targeted fragment of waxy gene in advance lines of rice. M = marker, (1) 60, (2) 61-(3) 62-, (4) 65, (5)66, (6)11, (7)12, (8) 15, (9) 16, (10) 19.

According to the PCR results, out of 17 advanced lines, only 10 advance lines showed results. 1 showed 425bp of Wx gene and 9 showed 228bp of Wx gene. It indicates that varieties with 425bp of Wx gene have high expression level for amylose content, while varieties with 228bp have low expression level for amylose content. Therefore varieties with high amylose content in their endosperms are known as non- waxy rice varieties and varieties with low amylose content in their endosperm are known as low amylose or waxy rice varieties. Conferring the above results, investigated advanced lines 60, 61, 62, 65, 66, 11, 12, 15, 16, are considered waxy rice varieties having Waxy gene and because of it contain low amylose in their endosperms and advance line19 showed high expression level of amylose content are considered non-waxy rice variety because of high amylose content in their endosperm (Table. 8).

Gelatinization temperature

Advanced lines of rice show the following gelatinization value of lines. 62, 65, 66, 11, 16, 19, 22, 28, 29, 31 showed high gelatinization temperature (GT).While line 60 and line 61- showed partial Intermediate gelatinization temperature while line12- and line 15 show high intermediate gelatinization temperature (GT) and line21- line25 show low gelatinization temperature (Table 9).

Gel consistency

Advance lines 60, 61, 62, 65, 66, 11, 12, 15, 16, 20, 21, 22, 25, 28, 29, 31 showed above than 60mm length of blue gel and thus have soft gel consistency (GC). Advance line line-19 show less than 40 mm of blue gel length and thus have hard gel consistency (GC) (Table 9).

Discussion

Pakistan has been traditionally an agricultural country. Rice is an important food crop of Pakistan. Pakistan rice holds second position in consumption after wheat. For better economy, export and consumption it is necessary to improve the grain quality and yield. For this aim it is necessary to improve plant both morphologically and genetically. Improving architecture of plant can support to protect the plant from lodging, salinity and any type of biotic and abiotic stresses. Investigation conducted on 17 advanced lines of rice designated differences in morphology and level of amylose due to the presence and absence of waxy gene. According to the morphological analysis, maximum and minimum values were recorded among these lines of rice. Maximum plant height 134.33 cm, number of productive tillers 25.33, yield per plant 69.33, and length of flag leaf 48.66 cm, width of flag leaf 2.36 cm and panicle length 35.33 was recorded in lines 65, 12, 28, 62, 22, and 62, respectively. Maximum numbers of primary branches 23.33 were recorded in lines 25 correspondingly. Maximum value for grain length 8.20 mm, and grain width 2.30 mm was recorded in lines 66, 61, respectively. Maximum filled grains 277, unfilled grains 322 and 100 grains weight 3.30g was recorded in lines 19, 62 and 61, respectively. Advance Line 62 and 66 have a good grain quality because of extra-long grain and maximum grain width. Variety 28 significantly exhibits high yield. The gel consistency (GC) amylose content (AC) and gelatinization temperature (GT) are main rice character, which are directly associated to cooking and eating quality. Advanced lines of rice analyzed during the present study showed better grain quality. The majority of the advance lines contained long slender grains and intermediate to high gelatinization temperatures. Thus these advance lines are appropriate for the development of high quality saline rice. Except one advance line that showed hard gel consistency and the majority of advance lines fall in the category of soft gel consistency and thus are of excellent quality. Gel consistency is a good directory of cooked rice texture, particularly among rice of high amylose content. Rice differs in gel consistency from soft to hard [7, 8]. Cooked rice with hard gel consistency solidifies earlier than those with a soft one. Rice with soft gel consistency cook rapidly or tender and stay soft even upon cooling [11]. Soft gel consistency rice is favored by most rice consumer. Breeder is therefore demanding to develop high yielding varieties with soft gel consistency [16]. Amylose content is the single core vigorous feature for rice eating and cooking quality. Classifying rice varieties amylose is considered as the significant and best indicator [11]. Rice varieties are categorized as high, intermediate, low and waxy rice varieties with amylose concentration >25%, 20 – 25%, 11 – 19%, 3 – 10%, and 0 – 2%, respectively [17]. Amylose is synthesized by an enzyme called the starch synthase (GBSS) which is the product of waxy gene. Variation in amylose content is because of high and low expression of Waxy gene. G to T point mutation in the first intron of the waxy gene alleles interrupts the formation of mature mRNA which causes low synthesis of amylose in the cultivated rice varieties [18, 19].

Using SDS – PAGE, Sano (1984) first studied the accumulation of rice waxy protein in rice seeds and illustrated its molecular weight i-e 60 kDa. Two alleles Wxa and Wxb of Wx locus were recognized by RFLP analysis (Sano et al., 1986). Information regarding genetic diversity in a crop plant is important for selection of parental lines and for construction of populations for various purposes. To evaluate genetic diversity of different crop species DNA markers were used [19]. In present study Waxy gene was recognized in advanced lines of rice. Variations in amylose content mainly depend on particular rice varieties and it is also proposed that oscillations in cold weather cause higher amylose content in the similar variety [20, 21]. Amylose variation was also observed in advanced lines because two different fragments of 425bp and 228 bp were appeared after a PCR reaction. Varieties with high amylose content in their endosperms are known as non-waxy rice varieties and varieties with low amylose content in their endosperm are known as low amylose or waxy rice varieties. Varieties having 425bp length fragments are non- waxy because of having negative results for Waxy gene and these includes line 19 while variety having 228 bp length fragments are waxy because of having positive results for waxy gene this include 60, 61, 62, 65, 66, 11, 12, 15 ,16, which showed low amylose content in their endosperm.

Pakistani rice is one of the best quality rice. For improved yield rice grain should be healthy and for good cooking and flavor quality an excellent amount of starch should be present in rice grain. High amylose content variety are becoming dry and separated of grains after cooking whereas low amylose cultivars (cvs) after cooking are glossy and tender, cohesive or waxy. Lesser amylose rice is preferable than (over 20%)

higher amylose rice because when they cooked it does not become dry and hard. For better economy, export and consumption it is necessary to advance the grain quality and yield. Genotypes 62 and 66 have a good quality grain because of extra-long grain and a maximum grain width. Variety 28 significantly exhibits high yield. A gene that controls the amylose is known as Waxy gene and in present study out of 17 advanced lines 9 (nine) lines ,(1) 60, (2) 61-(3) 62-, (4) 65, (5)66, (6)11 , (7)12 , (8) 15, (9) 16,) exhibited the Waxy gene. All the good quality grain varieties also contain a Waxy gene. So these varieties can be further used for improving better grain quality and yield of low yielding rice cultivars with low grain quality hence improving and increasing the country‘s economy and export values.

Competing Interest

The authors have declare that there is no conflict of interest.

Author Contributions

SF, SUR, ZH, WA, AI, Performed experiment, MAR, SA write the manuscript, HS and RA performed Analysis, KM review the manuscript.

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