RESEARCH ARTICLE

Roshan and Shahryar, two mutant cultivars of rice (*Oryza sativa* L.) with distinguished agronomic and grain biochemical properties

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ABSTRACT: To better understand the genetic relationships between quantitative and qualitative characteristics of rice, the most important agronomic traits, seed physicochemical properties, and storage protein compositions were evaluated in different quantitative and qualitative cultivars including two new mutant cultivars, their parents and their common ancestor. The result of agronomic traits shows that two mutant cultivars, Roshan and Shahryar, have the highest yield (8500 and 8000 kg/ha respectively) accompanied by some desirable agronomic characteristics such as lodging resistance, early maturity period, a high number of tillers and high panicle length. The qualitative properties evaluation also revealed these two novel mutant cultivars were characterized by good cooking and eating properties. Roshan has moderate amylose content (AC), and both were similar to Sangetarom, a qualitative cultivar, in terms of gelatinization temperature (GT) and gel consistency (GC). The recessive variant of the *BADH2* gene was also observed in Roshan and Sangetarom. Moreover, the Shahryar cultivar showed a significant decrease in prolamin protein when compared to the parent and the other cultivars. Generally, no significant relationship was detected between agronomic characteristics and yield with seed biochemical properties, such as cooking and eating quality, fragrance and storage protein content. These results promise that by identifying and pyramiding desirable alleles, especially alleles involved in starch synthesis, fragrance and prolamin protein biosynthesis, can lead to obtaining rice cultivars with higher quantitative and qualitative properties.

KEYWORDS: Agronomic characteristics, Cooking and eating quality, Physicochemical properties, Storage proteins.

INTRODUCTION

After wheat, rice (*Oryza sativa* L.) plays an important role in providing food needs, particularly in Asia, including Iran. China and India are the largest rice producers, while Indonesia, Nigeria, Bangladesh and Iran are the largest importers. Seed yield is one of the important breeding goals in many crops, including rice, but cooking and eating quality is one of the determining factors for rice cultivation and consumption [16,57]. Iranian indigenous rice varieties, known as qualitative cultivars, comprise good cooking and eating quality, but their high height

leads to lodging and also has a low yield. On the other hand, imported cultivars which are known as quantitative cultivars, they have shown high yield, long maturity periods as well as low cooking and eating quality. Therefore, introducing of new cultivars with desired agronomic properties like lodging resistance, shorter maturity period, high yield, as well as favorable cooking and eating properties, is one of the most valuable goals in rice breeding [15,45].

Many efforts have been made to produce new varieties of rice through classical breeding or gene pyramiding, nonetheless, these bred cultivars either have low yield like local cultivars or have low cooking and eating quality like quantitative cultivars. For example, the Gohar cultivar was introduced in the Agricultural Research Center of Gilan Province in 2012 as a cross between two quality foreign varieties, although have a good yield and eating quality, did not enough develop due to their long growing period. Brown rice yield, starch quality, fragrance and protein content are the most important factors involved in the quality properties of rice. Brown rice conversion efficiency (BRCE) is one of the most important physical characteristics of rice seed yield, which is obtained from the ratio of the weight of husked seed to paddy seed [1,4]. Starch properties and fragrance play a key role in cooking and eating quality, while nutritional value depends on their protein content [49,15,38,8].

Starch, which contains the largest part of rice seed (80-90%), is synthesized in amyloplast by an enzymatic complex from two polymers of glucose, amylose (linear) and amylopectin (branched) [49,24]. In this enzymatic process, glucose will be synthesized by the GBSSI enzyme (Granule-Bound Starch Synthase I) to amylose and by SSS (Granule-bound starch synthase), SBE (Starch branching enzyme) and DBE (Starch debranching enzyme) enzymes to amylopectin [54]. GBSSI enzyme encodes by the Waxy (*Wx*) gene and differences in amylose content (AC) have been reported in relation to the allelic diversity of this gene [13,20,43,49]. Among the enzymes that synthesize amylopectin, the SSS family plays a key role compared to the others. SSS enzymes with four isoforms I, II, III and IV are involved in the synthesis of short, semi-long and long chains of amylopectin respectively [20,49]. Semi-long chain distributions (SSSII) play a more important role in cooking and eating properties such as gelatinization temperature (GT) and gel consistency (GC) [18,47,11].

Fragrance (Fgr) also plays an important role in rice eating quality, which is specifically associated with 2-acetyl-1-pyrroline (2AP). 2PA levels are associated with the activity of the betaine aldehyde dehydrogenase-2 enzyme (BADH2), inhibiting the synthesis of 2AP [10,8].

Since AC, GT, GC and Fgr are the most important determining factors in rice quality, the activity of GBSSI, SSSII and BADH2 enzymes can be considered the most important targets involved in cooking and eating quality of rice.

In 2019, two new mutant cultivars of rice, Roshan and Shahryar, were introduced in the Genetics and Agriculture Biotechnology Institute of Tabarestan (GABIT) from two quantitative cultivars, which were superior compared to common cultivars in terms of agronomic characteristics, including plant height, days to maturity and yield as well as cooking and eating properties.

In order to better understand breeding relationships, the most important agronomic traits, physicochemical properties and storage protein compositions in these two mutant cultivars, their parents and their ancestors were studied. The study of these traits in this population will not only increase our understanding of the relationship between quantitative and qualitative properties, but will also pave the way for the production of new rice cultivars.

MATERIALS AND METHODS

Plant material

Five commercial cultivars included: Sangetarom, (a local quality cultivar), Amol 3, (an imported quantitative cultivar), Nemat, (a quantitative cultivar improved via classical breeding from Sangetarom and Amol3) and two mutant cultivars Roshan and Shahryar derived from

mutation breeding of Nemat and Amal3 respectively were studied for agronomic and seed physicochemical properties (Figure 1). The homogenized seeds of these cultivars were obtained from the Genetics and Agriculture Biotechnology Institute of Tabarestan (GABIT).
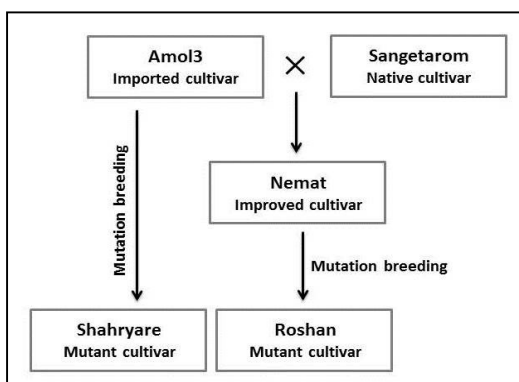

Figure 1. Source of quantitative and qualitative cultivars studied in this research.

Morphological assessment

All of the cultivars were planted in the experimental field of GABIT in the spring of 2020. After complete growth and elimination of marginal effect, ten plants were randomly selected in each plot and evaluated for some of the most important agronomic traits such as day to maturity, plant height, tiller number, panicle length, seed weight and yield.

Seed physicochemical assessment

For seed physicochemical properties, BRCE, AC, GC, GT, Fgr as well as protein components were studied. To execute these experiments, the seeds collected from each plot were first peeled by LTJM-2099, rice Polisher machine. BRCE was calculated in percentage in terms of the weight ratio of husked seed to paddy seed [1,4].

For chemical properties, the peeled seeds were ground in a mortar and pestle with liquid nitrogen. The AC by spectrophotometer [11], GT by dissolution in alkaline KOH solution [18] and GC by gelatinization percentage in 0.2 N KOH solutions [18] were measured.

The characteristic of the fragrance was determined qualitatively using a 3-point scoring scale of 1 (poor aroma), 2 (moderate aroma) and 3 (good aroma) based on Sood and Siddiq, (1978) instruction [46].

Seed storage protein composition assessment

To assess the storage protein the crude protein content was determined by the Dumas Nitrogen Analysis method. Protein composition is also compared by total soluble protein and fractionation based on solubility. The extracted proteins were analyzed by SDS-PAGE. Total soluble protein was extracted by Urea/Thiourea extraction buffer (7M Urea, 2M Thiourea, 3% CHAPS, 1% Triton-X100, 1% DTT) in ratio of 10:1 w/v after washing with 10% TCA/acetone solution [25]. Storage protein also was fractionated in 10mM Tris-HCl pH: 7.5, 1M NaCl, 60% n-propanol, 1mM EDTA-2Na and 0.05 M NaOH in a ratio of 1:10, w/v for albumin, globulin, prolamin and glutelin, respectively [26]. Extraction of each fraction was done by three times repeated stirring for 30 min at 4°C, followed by centrifugation at 20,000 g for 15 min. The extracted protein concentration was determined by the Bradford method [5] with bovine serum albumin (BSA) as the standard.

To evaluate protein subunits, 10 μl of total protein samples and 40 μl of albumin and globulin fractions were resuspended in an equal volume of sample loading buffer (Tris 0.5M, SDS 1gr, Glycerol 5ml, β-Mercaptoethanol 2.56 ml, BromoPhenol Blue 0.001%). For prolamin and glutelin, 1ml of each fraction was mixed with an equal volume of cold acetone and incubated overnight at -20°C. The proteins precipitated by centrifugation at 16,000 g at 4 °C for 30 min and the pellet dissolved in 80 μl of sample loading buffer. All of the total volumes of each protein fraction were loaded in the wells of SDS-PAGE after being heated at 95°C for 5 min.

The electrophoresed gels were stained by Coomassie Brilliant Blue R250 and then scanned separately with a resolution of 600 dpi and 16-bit TIFF grayscale pixel depth (Bio-Rad Calibrated Densitometer GS-800). The position of each protein and their subunits based on their

molecular weight was matched by Iida et al., [25], Kim et al., [28], Nishimura et al., [36], Jiang et al., [25] and Kawakatsu et al. [26] reports. Based on protein subunits, Proglutelin by two subunits 55-60 kDa, glutelin proteins with acidic subunits (30-35 kDa) and alkali (18-20 kDa) and prolamin proteins with 10-13 kDa subunits were characterized.

Molecular assessment

Molecular studies were performed by AC, GT and Fgr related gens. A designed specific primer set of the waxy gene for moderate amylose was used to evaluate the quality of amylose [58]. For GT, two primers that were designed based on Ex8 SNPs of the *SSSIIa* gene (4342: GC/TT and 4211: A/G) for low gelatinization temperature were used [11]. Three specific primers of the *BADH2* gene were used for Fgr, one primer was selected based on Ex7 SNPs [5] and two, were designed for Ex2 and Ex7 based on Japonica and Indica germplasm data (www.ncbi.nlm.nih.gov) by Primer 3 software.

The specifications of primer pairs are shown in Table 1. Genomic DNA was extracted from young leaf tissue using CTAB, and the quantity and quality of the extracted DNA were confirmed by 1% agarose gel and spectrophotometer. Amplification of the studied genes was performed by Taq DNA Polymerase Master Mix RED (Ampliqon, Denmark).

Data analysis

Agronomic and biochemical characteristics were analyzed using SAS statistical software and Excel program. Field experiments were performed in a randomized block design and physicochemical experiments were performed in a completely randomized design with three replications. The means were also compared using Duncan's multiple-range tests.

RESULTS

Agronomic characteristics

Significant differences existed for all agronomic traits, plant height, tiller number, panicle length, seed weight and yield (Table 2). Among the cultivars, Nemat, a quantity cultivar, showed the longest day-to-maturity periods (137 days) and Sangetarom, a quality cultivar, with two mutant cultivars, Roshan and Shahryar, showed the shortest (125, 124 and 125 days, respectively).

Based on mean comparisons, Sangetarom was characterized by the highest plant height (160 cm), whereas Amol3 followed by Roshan and Shahryar cultivars showed the lowest (105, 119 and 114.3 cm, respectively). On the other hand, Shahryar followed by Roshan and Nemat cultivars showed more tillers when compared to the other. Although, the panicle length was the same in Shahryar, Nemat and Amol3, the highest was observed in Roshan (32.5 cm) and the lowest in Sangetarom cultivars (24 cm). The highest seed weight belonged to Nemat (29.8 g) and the lowest was observed for Sangetarom, Amol3 and Roshan cultivars (23.73, 23.92 and 24.20 g, respectively).

Table 1. Specific primers for detecting polymorphism of seed quality traits

Traits	Gene name	Primer Name	Primer Seq (F)	Primer Seq (R)	Reference
Amylose	Waxy	WaxEx6 A/C	CTGGAGAAGGTGGAGTCAT CAACCCATACTTCAAAGGAACATC	GATCTTGAGATCAATTGTAACTCACGAT GGCGGTGATGTACTTGTCC	58
Fragrance	BADH2	BADH2 Ex7	TCCTGTAATCATGTATACCC	AATTTGGAAACAAACCTT	Designed by Primer3 software
		BADH2 Ex2	CCTCCTCTTCACCCTCTCCA	GCGATTGCGCGGAGGTACT	
		ASA	TTGTTTGGAGCTTGCTGATG CTGGTAAAAAGATTATGGCTTCA	AGTGCTTTACAAAGTCCCGC CATAGGAGCAGCTGAAATATATACC	5
Gelatinization Temperature	SSSIIa (SSSII-3/ALK)	ALK 4342 GC/TT	CAAGGAGAGCTGGAGGGGGC TCGGCGGGCTGAGGGACAC	CATGCCGCGCACCTGGAAA TCGCATCAATGGACATAACAAACAC	11
		ALK 4211 A/G	GCGGGCTGAGGGACACCG AACAGCAAGGTGCGCGGGTG	AACGGGTCGAACGCCGACGT CCACCGAGTCGCACCTCCACA	

BADH2: Betaine aldehyde dehydrogenase 2; ASA: Allele specific application; ALK: Alkaline

Table 2. Analysis of variance of some agronomic characteristics in different quantitative and quality cultivars

S.O.V	df	Plant height	Number of tiller	Panicle length	seed weight	Yield
Genotypes	4	1356.05 **	20.38 **	27.76 **	21.30 **	14994750.00 **
Repeat	2	292.86 ns	11.73 ns	39.76 *	20.00 ns	32000 ns
Error	8	452.03	6.79	9.37	7.39	5062083
CV		16.74	20.04	11.33	10.87	32.74

**, * and ns Significant at 1%, 5% and no significant probability levels.
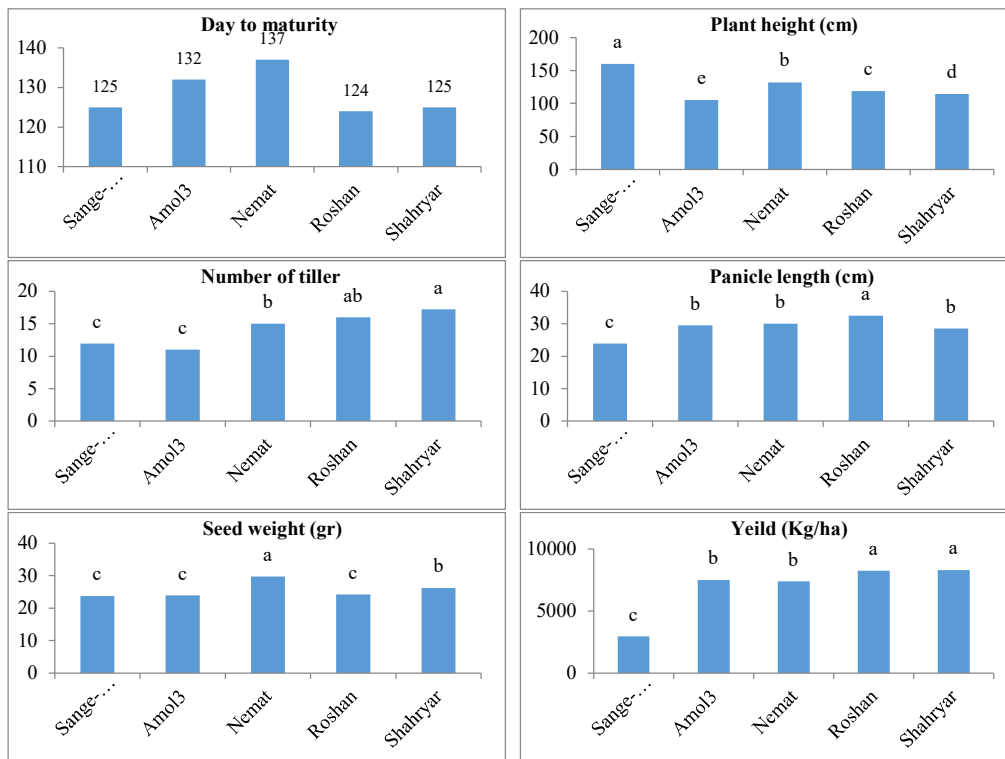

Figure 2. Comparisons of the means of some agronomic characteristics in different quantitative and qualitative rice cultivars.

Finally, Shahryar and Roshan mutant cultivars with the highest number of tillers and the longest panicle length show the highest yield (8300 and 8250 kg/ha, respectively) and Sangetarom with the highest plant height and lowest panicle length shows the lowest (2950 Kg/ha) (Figure 2).

Seed physicochemical properties

There was a significant difference for all seed physicochemicals, BRCE, AC, GC, GT and Fgr (Table 3). The highest and lowest BRCE were observed in Amol3

and Roshan cultivars respectively (63.11 and 54%, respectively) (Figure 3). For AC, the studied cultivars were divided into two gropes, Amol3, Nemat and Shahryar cultivars were in the first class (27.04, 26.46 and 26.26%, respectively) and Sangetarom and Roshan cultivars were in the second class (19/10 and 18/50%, respectively). For GC, Sangetarom, Roshan and Shahryar cultivars show the highest (96.10, 98.00, 97.00 mm, respectively), whereas Nemat and followed by Amol3 cultivars show the lowest (30.00 and 11/40 mm respectively).

Table 3- Analysis of variance of some seed physicochemical properties in different quantitative and quality cultivars

S.O.V	df	BRCE	AC	GC	GT	Fgr
Genotypes	4	35.62 **	55.02 **	3496.87 **	6.22 **	2.76 **
Error	10	1.01	0.69	5.95	0.41	0.33
CV		1.68	3.53	3.37	11.23	26.11

** significant at 1% probability levels; BRCE: Brown rice conversion efficiency. AC; Amylose content. GC; Gel concentration. GT; Gelatinization temperature. Fgr; Fragrance.
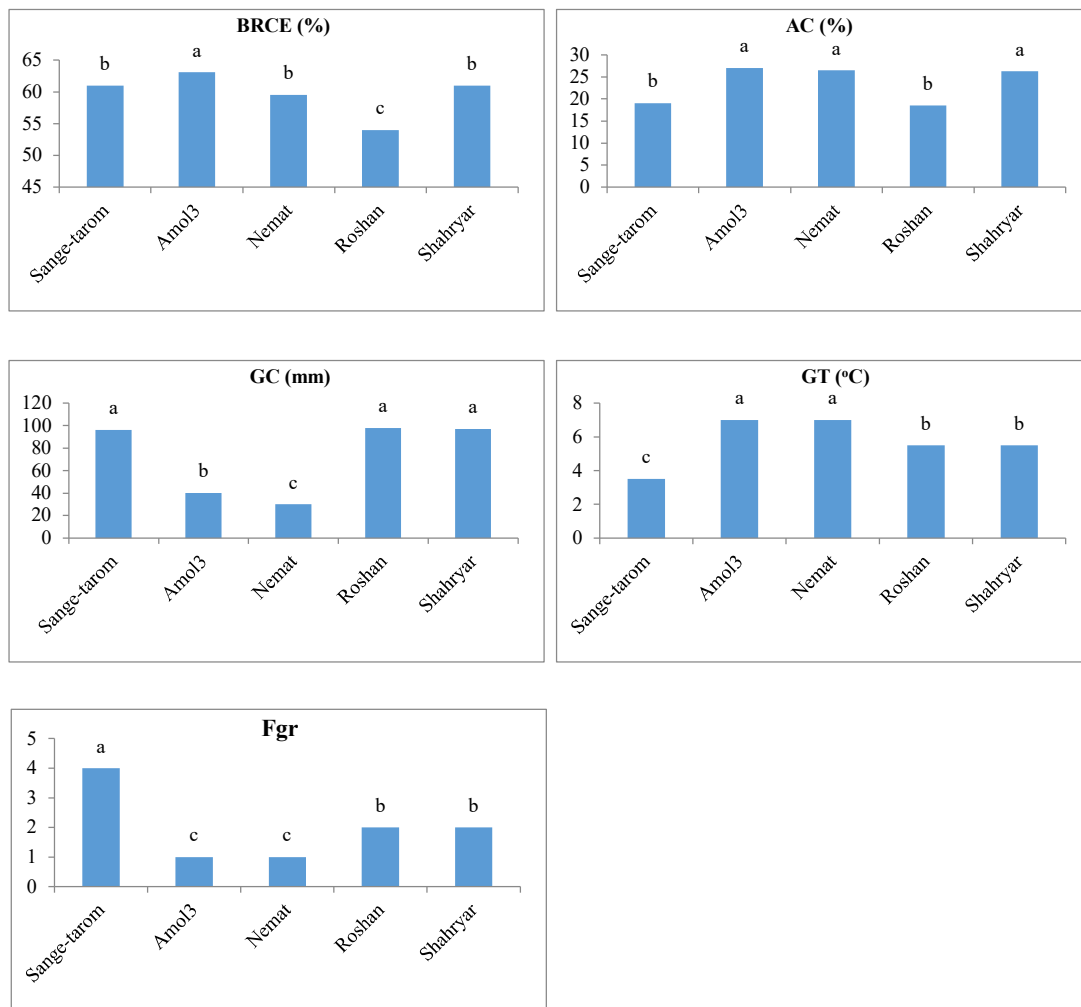

Figure 3. Comparisons of the means of some quality characteristics in different quantitative and qualitative rice cultivars. BRCE; Brown rice conversion efficiency. AC; Amylose content. GC; Gel concentration. GT; Gelatinization temperature. Fgr; Fragrance.
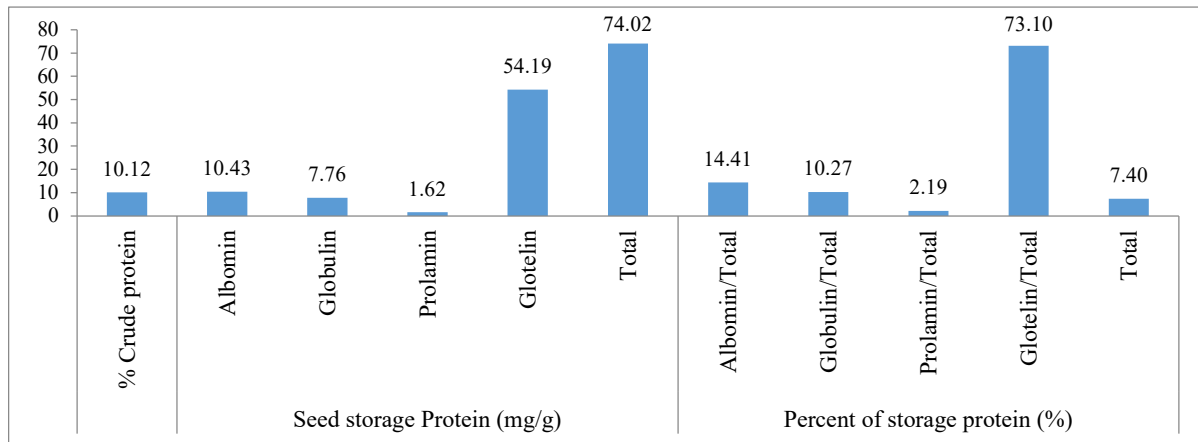
Figure 4. The means of seed storage proteins in different quantitative and qualitative rice cultivars.

The highest GT was observed in Amol3 and Nemat cultivars (7.01 and 7.00 °C, respectively), whereas the lowest was obtained in Sangetarom (3.50 °C). For Fgr, the cultivars were divided into three gropes, Sangetarom with the highest rank in the first group, Roshan and Shahryar cultivars in the second group and Nemat and Amol3 in the last group (Figure 3).

Seed storage protein studies

The average crude protein content and their solubility in the studied cultivars were 10.12% and 7.40% respectively. The fractionation results also showed that glutelin's protein content was 73%, albumin 14%, globulin 10%, and prolamin 2% (Figure 4).

Analysis of this storage proteins variance showed that except for the percentage of glutelin, there was a significant difference for other proteins based on solubility (Table 4). Among them, Sangetarom and Amol3 had the highest amount of crude protein (12.18 and 11.10%, respectively), while Nemat had the lowest (8.46%). Among the soluble proteins (Figure 5), the highest albumin content was obtained

in Amol3 and Roshan (11.74 and 11.55 mg/g respectively), In contrast, the highest globulin was obtained in Sangetarom and Amol3 (10.79 and 10.12 mg/g respectively). However, Nemat was characterized by the lowest albumin and globulin proteins (8.55 and 4.10 mg/g respectively). For prolamin and glutlin proteins, the highest was obtained in Amol3 (2.50 mg/g), while the lowest was observed in Shahryar (0.77 mg/g).

Typical electrophoretic patterns based on total protein and solubility are shown in Figure 6. These results demonstrated that mutant cultivars, Roshan and Shahryar, showed different banding patterns compared to parents and the other. Roshan by the absence of the 60kDa subunit of proglutelin and Shahryar by a low level of the 13kDa subunit of prolamin were differentiated from other cultivars. These results also show that the lack of 60 and 22 kDa subunits of albumin and 22kDa subunit of globulin in Roshan cultivar has increased the expression of some other protein subunits (Figures 6 C and B).

Table 4. Analysis of variance of seed storage proteins composition based on their solubility

S.O.V	df	Crude protein	Albumin	Globulin	Prolamin	Glutelin	Total protein	% Albumin	% Globulin	% Prolamin	% Glutelin
Genotypes	4	0.19 **	5.27 *	22.90 **	1.15 **	379.16 **	644.13 **	23.54 **	15.80 **	1.58 **	5.42 ns
Error	10	0.01	1.63	0.50	0.06	9.79	12.88	2.50	0.83	0.15	3.14
CV (%)		5.95	12.24	9.11	15.12	5.77	17.40	10.97	8.87	17.68	2.42

**, * and ns Significant at 1%, 5% and no significant probability levels.
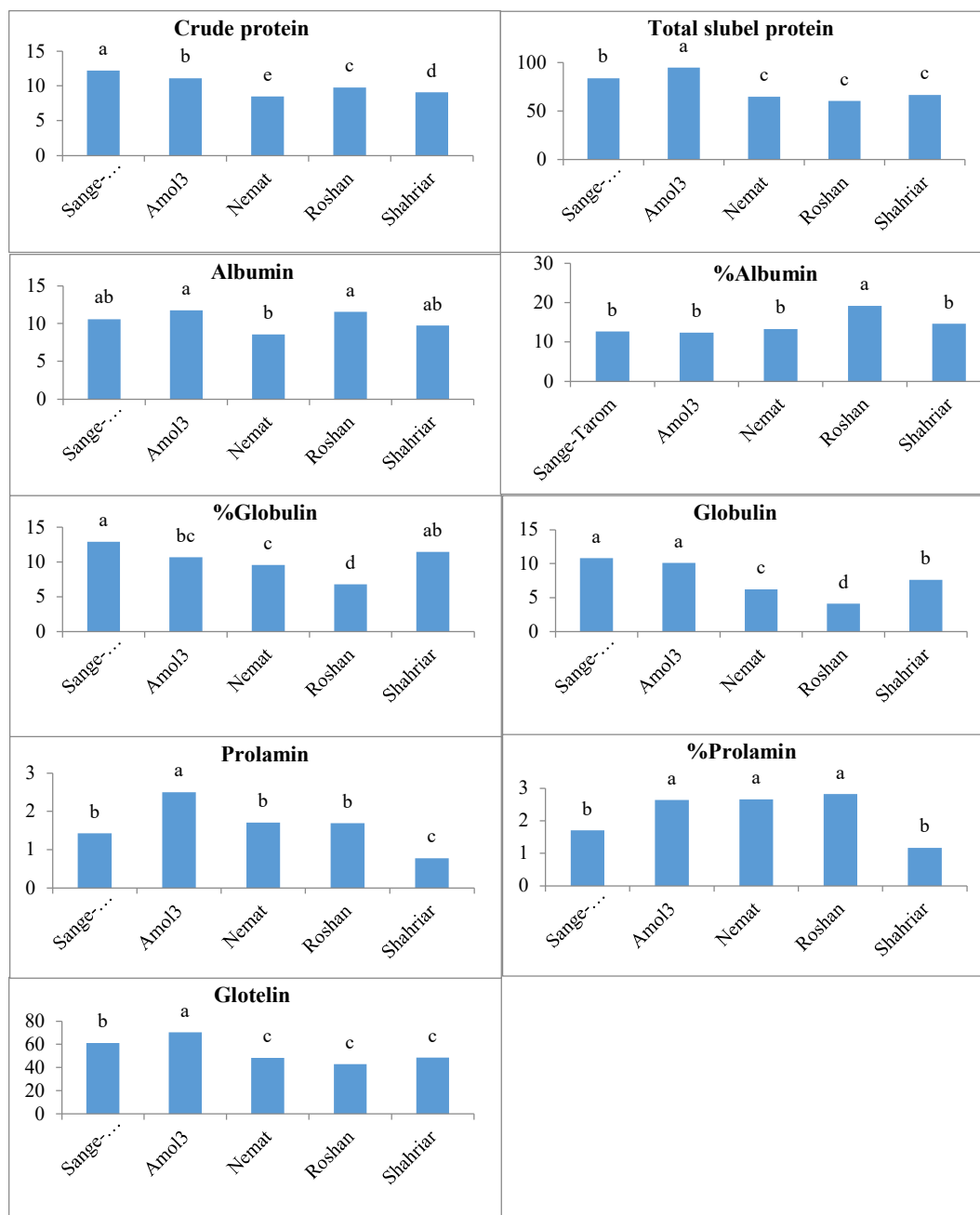
Figure 5. Mean comparisons of seed storage proteins in different quantitative and qualitative rice cultivars.
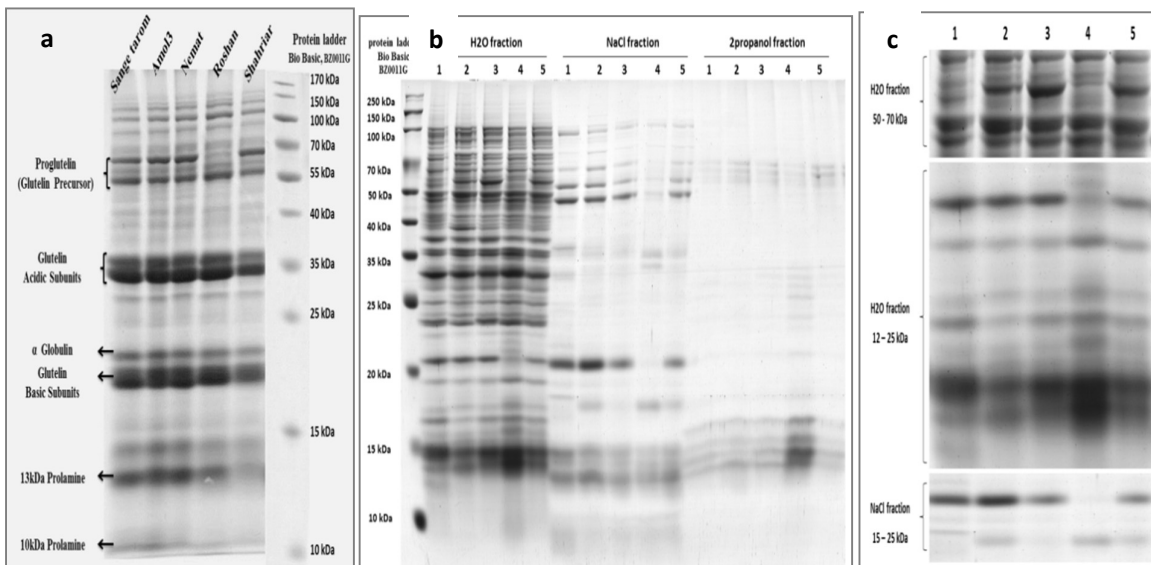
Figure 6. Typical electrophoretic profiles based on total protein and solubility of seed storage proteins in different quality and quantitative cultivars. A: Bond pattern of total proteins. B: Bond pattern of seed proteins based on solubility. C: Polymorphisms observed in the band pattern of albumin and globulin fractions .1: Sangetarom, 2- Amol 3, 3- Nemat, 4: Roshan, 5: Shahryar.

Molecular studies

Molecular studies were performed for waxy (*Wx*), GT (*SSSIIa* / Alkalin) and Fgr (*BADH2*) genes (Figure 7). The results of the waxy gene demonstrated that the quantity cultivar, Sangetarom, was distinguished from other cultivars according to Wx[c] a molecular marker, which indicates moderate amylose content (Figure 7 A). For the alkaline properties, Sangetarom was also distinguished from other cultivars for the ALKa allele, which indicates a low GT (Figures 7 B and C). For Fgr, although the studied cultivars were not different for Ex2 and Ex7 exons of the *BADH2* gene (Figure 7 E), Sangetarom and Roshan cultivars were characterized by a recessive allele of the *BADH2* gene based on allele-specific amplification (Figure7 D).

DISCUSSION

Agronomic characteristics

Iranian indigenous rice has a good eating quality, but due to high-plant height leads to lodging and on the other hand has a low yield. On the other hand, although quantitative cultivars produce a high yield, they have a

long maturation period and poor cooking and eating quality. Production of new cultivars with desired agronomic traits and favorable cooking and eating properties, is one of the most significant breeding programs in modern rice cultivars [15,45]. The results of agronomic traits showed that the cultivars with a higher number of tillers and panicle lengths had higher yields. On the other hand, there was no significant relationship between day to maturity and plant height. Many researchers have presented a significant relationship between agronomic traits and yield in different rice cultivars. Investigating the relationship between seed yield and agricultural characteristics for 40-year (1978-2017) in China, showed that in indica varieties, plant height, days to maturity, panicle length, number of seeds, and seed weight have positively correlated with yield, while in Japonica varieties number of panicles and days to maturity were positively correlated. Moreover, the effect of plant height on yield in inbred lines was positive and in hybrids was negative [31]. According to these results and other reports, it can be concluded that there are significant interactions between morphological
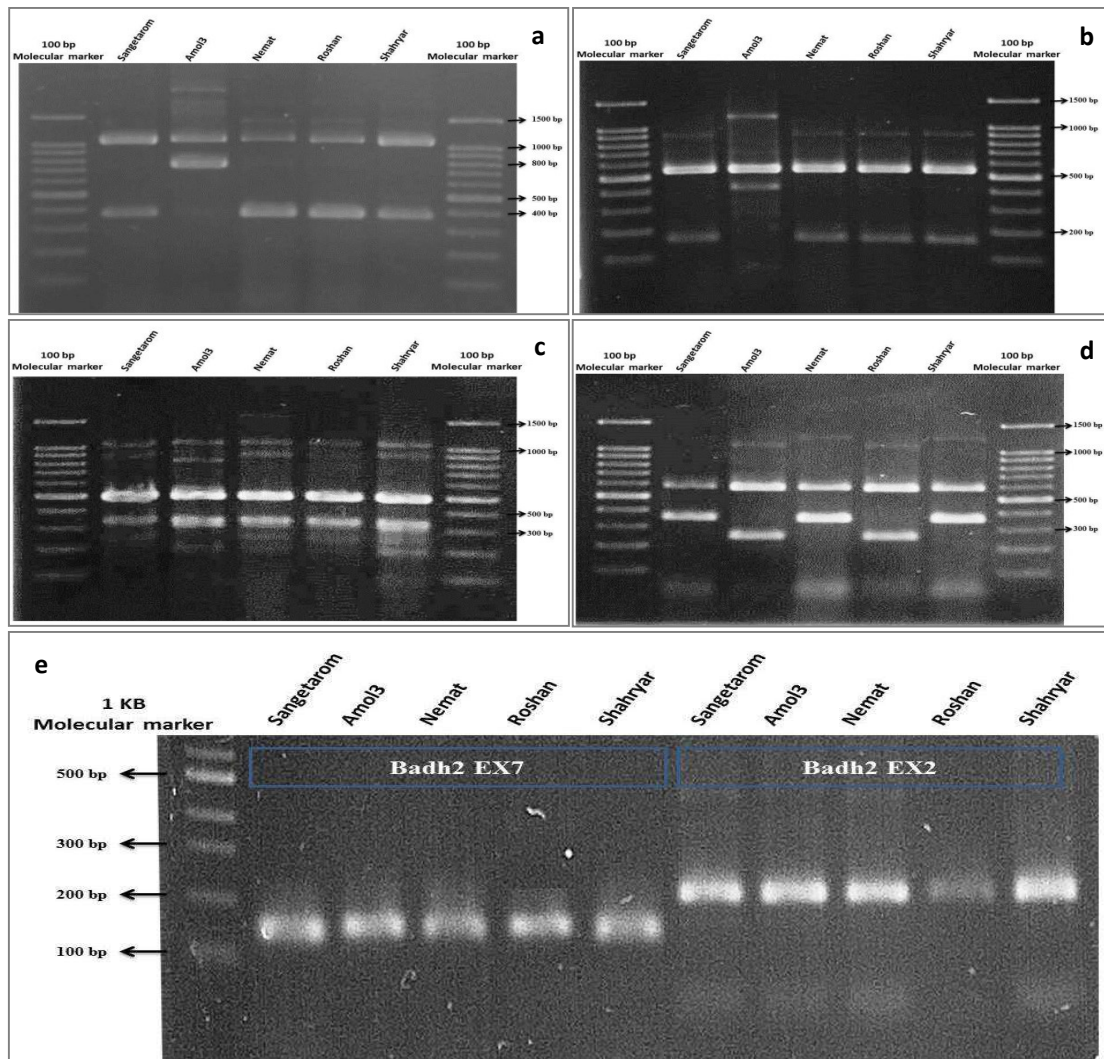
Figure 7. Molecular evaluations of genomic DNA of different rice cultivars for seed quality properties.
A: Waxy gene, B: SSSIIa ALK gene 4211. C: SSSIIa ALK gene 4342. D: BADH2 gene.

traits with yield in different rice genotypes. Therefore it is not possible to directly relate one or several specific traits with yield in all different genotypes of rice. For example, although two new mutant cultivars, Roshan and Shahryar, decreased in plant height and increased in tiller number and seed weight and were able to increase seed yield significantly, these two cultivars have a shorter maturity period than their quantitative parents.

Seed physicochemical properties

Although the cultivars introduced in recent years have been high-yield, they failed to satisfy farmers and consumers because of their low-quality properties.

Brown rice yield, cooking and eating quality, fragrance and protein content are considered to be the most important factors that determine the physicochemical properties of the seed. The properties of cooking and eating quality depend on starch quality. Starch consists of two polymers (amylose and amylopectin), each

playing a decisive role in cooking and eating quality. Different rice varieties are classified into three categories based on AC. The first category is low amylose (less than 10%), which is completely sticky after cooking and lacks volume expansion, the second category with medium amylose (between 20-25%), which is soft after cooking and watches keeps its softness, and the third category of high amylose (more than 25%) is completely separated after cooking and is dry and hard due to cooling.

The difference in amylose content in different rice varieties has been reported in different varieties of *WX* gene alleles [49]. The most important alleles of this gene are WX^a, WX^b, WX^c and *wx*, which synthesize high, low, medium and lack amylose phenotypes, respectively [56,58]. The Indica variety has Wx^a alleles and shows higher activity for the GBSSI enzyme and high amylose content. However, Japonica variety generally has Wx^b alleles and a low amylose phenotype [43, 23]. The results of this study demonstrated that Sangetarom, a local quality cultivar, is characterized by the Wx^c allele for moderate amylose content than the other. Nonetheless Roshan, with a moderate amylose phenotype like Sangetarom, was not differentiated with the Wx^c allele. The Waxy gene has 12 introns and 13 exons. The Wx^c primer is designed based on exon 7 for moderate amylose content. Alternatively, other alleles such as Wx^{op}, Wx^{mq}, Wx^{mp} and Wx^{mw} of this gene have also been reported for various amylose content [40,48,56,16,55]. Based on these results, it seems that the Roshan cultivar has a new allele for the Waxy gene with a moderate amylose phenotype that should be further studied.

Unlike amylose, amylopectin contains an important part of the starch and plays a decisive role in the alkaline properties (Alkali) of rice such as GT and GC [20,11]. GT is the temperature range in which starch molecules begin to swell. Different rice cultivars for this characteristic are divided into three categories of cultivars low (55-69°C), medium (70-74°C), or high (above 74°C) GT [12]. If the GT is high, the cooked rice

will harden and dry, and in low GT, the cooked rice will soft and sticky. The GC also indicates the amount of gel movement in cooked rice. Cultivars with hard GC harden quickly, while rice with soft GC cooks gently and slowly and remains soft even after cooling. In general, quality rice refers to rice that has moderate amylose (20-24%), medium GT (70-74°C) and medium GC (41-60 mm) [19]. The results of biochemical characteristics showed that Roshan and Shahriar cultivars like Sangetarom, a quality cultivar, were in the category of cultivars with high GC, and quantitative cultivars, Amol3 and Nemat, were in the category of cultivars with low GC. Furthermore, Roshan, Shahryar and Sangetarom cultivars were in the category of medium GT, and quantitative cultivars, Amol3 and Nemat, were placed in the category of low GT.

The activity of SSS, SBE and DBE enzymes in amyloplast will lead to the synthesis of amylopectin from glucose [54]. SSS family enzymes are involved in the distribution of chains and SBE and SDE enzymes are involved in the distribution of branches. Among these enzymes, SSS family, which has four isoforms I, II, III and IV, plays a key role in amylopectin synthesis [49]. SSSI and SSSII enzymes are involved in the synthesis of short and semi-long chains with degree polymerization (DP) less than 12 (DP≤12) and 13≤DP≤24 respectively, and SSSIIIa and SSSIV enzymes are involved in the synthesis of long-chain amylopectin (DP≥25) [20,49]. Studies have shown that the distribution of semi-long chains of amylopectin (13≤DP≤24) plays a decisive role in the alkali properties of rice, such as GT and GC [18,11].

The *SSSIIa* gene is located on chromosome 6, near the Waxy gene. The linkage between these two genes in Nemat, originates from a crossing between Sangetarom and Amol3 cultivars, displays clear evidence that the AC and GT in Nemat are the same as in Amol3 (Figure 4). Despite the linkage between Waxy and *SSSIIa* genes, an inverse relationship was observed between AC and GT in transgenic and mutant plants for these two genes [18,32,11]. Evaluation of the alkaline properties in different rice germplasms has shown that

Japonica varieties have a higher GT than Indica [51,52], this difference has been reported to be associated with SNPs of exon 8 of the *SSSIIa* gene [11]. So far, three haplotypes have been reported for Ex8 of this gene, as ALKa and ALKb alleles give a phenotype with low GT and ALKc phenotype with high GT [11]. Studied of ALKa and ALKb alleles showed that the differences between these two alleles are in the 4211(G/A) and 4342(GC/TT) of exon 8 SNPs respectively [11]. The results of SSSIIa allelic diversity showed that although Roshan and Shahryar cultivars, like Sangetarom, were in the category of moderate GT, only Sangetarom cultivar was characterized by the ALKb allele. Evaluation of enzymatic complex in eight varieties of Japonica with different qualitative characteristics showed that in addition to the SSSIIa enzyme, SSSIa, SBE1 and SBE3 enzymes play a decisive role in the quality of cooking and eating quality [47]. On the other hand, an inverse relationship has been reported between the activity of amylose synthesis enzymes (GBSSI) and the enzymes involved in the synthesis of amylopectin SSSIa [31], SSSIIa [32], SSSIIIa [57], SBE and SDE [53,2]. Moreover, the evaluation of *SSSIIa* gene SNPs in 60 different cultivars of rice reported two new mutant alleles for this gene [50]. Therefore, it is suggested that in order to better understand the genetic relationships involved in amylopectin synthesis, the allelic diversity of these genes should be further studied in Roshan and Shahryar mutant cultivars.

The most important factor in the presence of aroma in rice is related to 2-acetyl-1-pyrroline (2AP), which is present in all plant tissues except roots [34,39]. 2AP synthesis is related to Betaine Aldehyde Dehydrogenase II (BADH2) activity [10]. Since the BADH2 enzyme is the key factor in determining aroma in rice, silencing of this gene by RNA interference [37] and CRISPR-cas9 [3] has been able to successfully increase the fragrance of rice. Among the studied cultivars, although Sangetarem was characterized by the highest aroma, the amount of aroma in Roshan and Shahryar mutant cultivars was more than their parents. The results of BADH2 primer by specific molecular marker [4] showed that Roshan, like to Sangetarom cultivar characterized by a recessive allele than the other cultivars. Although the phenotypic evaluation of aroma in the Roshan cultivar was less than Sangetarom, this cultivar shows the same allele as Sangetarom. In addition, Shahriar, which was in the same category as Roshan, was not distinguished from the recessive allele of this gene.

BADH2 SNPs have shown that the knockout mutation in exons Ex7 and Ex2 has differentiated aromatic from non-aromatic cultivars. In addition to the Ex7 and Ex2 exons, various other alleles have been identified for the *BADH2* gene [41,8]. Therefore, it seems that the Shahryar cultivar, which shows the same phenotypic Fgr as Roshan, probably has a new allele for this gene.

Seed storage protein studies

Storage proteins are the most important determinant of seed nutritional value. Although the seed storage protein content of rice is relatively low (7-10 %), it has the highest protein efficiency [9,25].

Among the cultivars studied, Sangetarem, Amol3, Roshan, Shahryar and Nemat were divided into five separate groups with the protein content of 12.18, 11.20, 9.78, 9.09 and 8.46 % respectively. The seed storage protein profiles based on total protein and fractionation showed that except for two new mutant cultivars, there was low variability for the storage proteins profile. So the 60kDa subunit of proglutelin in Roshan and the 13kDa subunit of prolamin in Shahryar are different than the others. Accordingly, this polymorphism can be used as a protein marker for these two new cultivars (Figures 6).

Various reports have shown that there is a low variation in the storage protein pattern of rice [17,41,27]. Evaluation of storage protein in different varieties of Japonica and Indica as well as three wild varieties of Oryza genius (*O. rufipogon, O. officinalis* and *O. meyeriana*) indicated that different varieties have a similar banding pattern of seed proteins [25]. Considering the low diversity of seed storage proteins in rice, these researchers argued that relatives' species

could be used as a new genetic resource in rice. In addition, many studies have shown that mutation breeding [29,26,36] or genetic engineering [28] has been able to induce high variability in the seed storage proteins. Due to the limitations of cross-breeding for undesirable genes in interspecific crossing, it seems that mutation breeding and genetic engineering can be used as more efficient techniques to diversify and increase protein quality in rice.

Among rice seed proteins, albumin and a part of globulin are stored in the aleurone layer and glutelin and prolamin are stored in the endosperm. Because the aleurone layer separates during the bleaching process, the nutritional value of rice is related to the globulin, glutelin and prolamin proteins [45]. Among the endosperm proteins, prolamin is indigestible and reduces the protein nutritional quality of rice, while gluten protein is rich in lysine, an essential amino acid. Since the most important limitation in the nutritional value of rice protein is related to lysine, the nutritional value of glutelin is estimated to be higher than that of prolamin protein [25]. Based on this research, Amol 3 cultivar with high prolamin level and Shahryar and Sangetarom cultivars with low prolamin level were identified (Figures 5 and 6).

The result of storage protein based on solubility and protein subunits also showed that the change in the expression of some protein subunits leads to the change in the expression of some other protein subunits. For example, in Roshan, the absence of the 60kDa subunit of albumin has led to an increase in the expression of some other protein subunits. Likewise in Shahryar, a decrease in the 13kDa subunit of prolamin has led to an increase in globulin proteins (Figure 6). Evaluations of stored proteins in some mutant cultivars also show that the expression of some subunits has changed with the expression of certain protein subunits [19,26]. Prolamin protein has three subunits of 10 kDa, 13kDa and 16kDa, among which the 10kDa and 16kDa subunits are rich in sulfur-amino acids. Evaluation of seed proteins in a rice mutant with a reduced of the

10kDa subunit in prolamin showed that the 16kDa subunit of this protein was increased to compensate for sulfur amino acids. In another study, in which the 13kDa subunit of prolamin was suppressed by RNA interference (RNAi), its glutelin protein level was significantly increased, leading to a 28% increase in lysine [28]. Therefore, according to these results, it seems that there is a significant relationship between different protein subunits.

Relationship between quantitative and qualitative characteristics

Due to the importance of quantitative and qualitative characteristics in rice, various results have been reported regarding the relationship between these traits. Evaluation of seed yield and physicochemical traits in the Japonica mutants' population showed that there was no significant relationship between seed yield with protein and amylose levels [1]. The result of this study also shows that no relationship exists between protein content with quantitative characteristic, so that Sangetarom, a quality cultivar, and Amol3, a quantity cultivar, are distinguished by the highest protein content.

On the other hand, Chandi and Sogi, (2008) reported traditional basmati rice was low in albumin, globulin and prolamin and high in glutelin when compared to developed basmati rice [7]. Chen et al., (2018) also identified a lead SNP, sf0601764762, for albumin content in the waxy locus, which causes a negative relationship between albumin and AC [9]. In addition, Peng et al., (2020) also reported that prolamin caused a decline in eating quality, the reason is that prolamin hindered the development of starch reticular structure in rice [38]. Based on these reports, it seems that there is a negative relationship between albumin and prolamin proteins with eating and cooking quality in rice. In this study, although there was no significant relationship between albumin and amylose, the increased albumin in the Roshan cultivar compared to the parent (Nemat) led to a decrease in AC. Moreover,

Sangetarem, which is a quality variety, also showed a low prolamin level. It seems that probably the reduction of prolamin in the Shahryar cultivar has also increased its cooking and eating quality.

CONCLUSION

The results of this study demonstrated that Roshan and Shahryar mutant cultivars, in addition to favorable agronomic characteristics and high yield, also have good cooking and eating quality. Roshan, showed moderate AC and both were in terms of GT and GC properties similar to Sangetarom, a quality cultivar. Moreover, these mutant cultivars showed higher fragrance compared to their parents. In addition, the seed storage protein profile in different quantitative and qualitative cultivars was similar, but this pattern is different in these mutant cultivars, so the lack of 60kDa subunit of proglutelin and low level of 13kDa subunit of prolamin can be used as protein markers in Roshan and Shahryar cultivars, respectively.

Based on these results, it seems likely that Roshan and Shahryar mutant cultivars have new alleles for waxy and *SSS* family genes related to starch synthesis, *BADH2* gene for fragrance and prolamin gene in storage proteins, which need more detailed confirmation in this regard.

This study's results generally show no significant relationship between agronomic characteristics and yield with seed biochemical properties such as cooking and eating quality, fragrance, and seed protein content. These results promise that rice cultivars with higher quantitative and qualitative properties can be obtained by identifying and pyramiding desirable alleles, especially alleles involved in starch synthesis and fragrance.

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دو رقم موتانت برنج (.Oryza sativa L) روشن و شهریار، با ویژگی‌های متمایز زراعی و بیوشیمیایی دانه

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چکیده

جهت درک بهتر روابط ژنتیکی بین خواص کمی و کیفی در برنج، مهم‌ترین ویژگی‌های زراعی، خصوصیات بیوشیمیایی دانه و پروتئین‌های ذخیره‌ای دانه در دو رقم جدید موتانت، والدین و جد مشترک آن‌ها مورد ارزیابی قرار گرفت. نتایج صفات زراعی نشان داد که ارقام موتانت روشن و شهریار ضمن خصوصیات زراعی مطلوب مانند مقاومت به ورس، دوره رسیدگی کوتاه‌تر، با تعداد پنجه و طول خوشه بیشتر، بالاترین عملکرد را به خود اختصاص دادند (به ترتیب ۸۵۰۰ و ۸۰۰۰ کیلوگرم در هکتار). ارزیابی خواص کیفی نیز نشان داد که این دو رقم موتانت ضمن خصوصیات زراعی مطلوب و عملکرد بالا، از خواص پخت و خوراک مناسبی نیز برخوردار بودند. بطوریکه رقم روشن از نظر محتوی آمیلوز (AC) همانند رقم کیفی سنگ طارم در دسته آمیلوز متوسط، و هر دوی آن‌ها از نظر خواص دمای ژلاتینه شدن (GT) و قوام ژل (GC) همانند رقم کیفی سنگ طارم بودند. همچنین رقم روشن همانند رقم سنگ طارم با آلل مغلوب ژن *BADH2* برای عطر متمایز شد. از طرفی دیگر رقم شهریار کاهش معنی‌داری را برای پروتئین پرولامین نیز نشان داد. بطور کلی نتایج این تحقیق نشان داد که ارتباط معنی‌داری بین خصوصیات زراعی و عملکرد با خواص بیوشیمیایی دانه مانند کیفیت پخت و خوراک، عطر و طعم و همچنین محتوی پروتئین دانه وجود ندارد. این نتایج نوید بخش آن است که با شناسایی آلل‌های مطلوب دخیل در کیفیت پخت و خوراک برنج و هرمی کردن آن‌ها می‌توان به ارقام جدیدی از برنج با خواص توام کمی و کیفی بالاتر دست یافت.

کلمات کلیدی: خصوصیات زراعی، کیفیت پخت و خوراک، ویژگی‌های فیزیکوشیمیایی، پروتئین‌های ذخیره‌ای.