Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Heritability and amylose content in hybrid lines of late-generation rice with colored pericarp

Herdabilidade e teor de amilose em linhagens híbridas de arroz de última geração com pericarpo colorido

G. Baiseitova[a,b], Kh. Berkimbay[a,b], D. Mynbayeva[a], A. Nussupova[c], A. K. Amirova[a,c], B. Usenbekov[a,c*], Z. Kulakhmetova[a], G. Yernazarova[c], D. Yussayeva[c], D. Kazkeyev[a] and S. Mukhambetzhanov[a,c]

[a]Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan
[b]Kazakh National Agrarian Research University, Almaty, Kazakhstan
[c]Al-Farabi Kazakh National University, Almaty, Kazakhstan

Abstract

Improving grain quality in rice breeding is one of the main tasks. This concerns the creation of rice varieties with colored pericarp uncommon in the Republic of Kazakhstan, and the assessment of its quality is an important stage of breeding. Rice with colored pericarp is an important dietary crop, more useful for the human body than white rice. Regardless of the type of rice, the amount of amylose in rice grain is a crucial indicator that determines the quality of rice. The paper presents the results of electrophoretic separation of spare grain proteins of rice hybrids and dihaploids with colored pericarp and their parent forms obtained as a result of the hybridization of varieties with colored pericarp (Black Rice (China), Mavr (Russia), and Yir 5815 (Ukraine)) with white rice varieties zoned in Kazakhstan. The hybridization of the rice varieties with colored pericarp with white rice varieties was carried out to obtain rice varieties with colored pericarp oriented to the soil and climate of Kazakhstan. Analyzing the results of electrophoresis and the amount of amylose, it was found that hybrid lines differed in amylose content. One of the studied hybrids was high in amylose, four had a medium amylose content, ten had a low amylose content, three had a very low amylose content, and six were glutinous. According to the results of electrophoretic separation of spare rice grain proteins, the spectrum of the enzyme determining amylose was detected in five hybrids, which corresponds to the results of spectrophotometric determination of amylose: high amylose in one hybrid and medium amylose content in four. The results show that the hybrids obtained as a result of hybridization are true hybrids and as a result of long-term selection, the amylose content in the F7-F8 hybrids stabilized. The hybrids can be used in further breeding of rice with colored pericarp.

Keywords: rice breeding, colored pericarp, Kazakhstan, grain proteins, hybridization.

Resumo

Melhorar a qualidade dos grãos no cultivo do arroz é uma das principais tarefas da indústria. A criação de variedades de arroz com pericarpo colorido e a avaliação da sua qualidade é uma etapa importante do melhoramento e são pouco comuns na República do Cazaquistão. O arroz com pericarpo colorido é uma cultura alimentar importante, mais útil para o corpo humano do que o arroz branco. Independentemente do tipo de arroz, a quantidade de amilose no grão é um indicador crucial que determina a qualidade do arroz. Este trabalho apresenta os resultados da separação eletroforética de proteínas de grãos de arroz híbridos e diploides com pericarpo colorido e suas formas parentais obtidas como resultado da hibridização de variedades com pericarpo colorido (Arroz Preto, China), Mavr (Rússia) e Yir 5815 (Ucrânia) com variedades de arroz branco zoneadas no Cazaquistão. A hibridização das variedades de arroz com pericarpo colorido com variedades de arroz branco foi realizada para obter variedades de arroz com pericarpo colorido orientadas para o solo e clima do Cazaquistão. Analisando os resultados da eletroforese e a quantidade de amilose, constatou-se que as linhagens híbridas diferiram no teor de amilose. Um dos híbridos estudados apresentava alto teor de amilose, quatro tinham teor médio de amilose, dez tinham baixo teor de amilose, três tinham teor muito baixo de amilose e seis eram glutinosos. De acordo com os resultados da separação eletroforética das proteínas sobressalentes do grão de arroz, o espectro da enzima determinante da amilose foi detectado em cinco híbridos, o que corresponde aos resultados da determinação espectrofotométrica da amilose: alto teor de amilose em um híbrido e médio teor de amilose em quatro. Os resultados mostram que os híbridos obtidos como resultado da hibridização são híbridos verdadeiros e como resultado da seleção a longo prazo, o teor de amilose nos híbridos F7-F8 foi estabilizado. Híbridos podem ser usados para melhorar arroz com pericarpo colorido.

Palavras-chave: melhoramento de arroz, pericarpo colorido, Cazaquistão, proteínas de grãos, hibridização.

*e-mail: bakdaulet7@yandex.ru
Received: November 28, 2023 – Accepted: December 20, 2023

1. Introduction

Rice (*Oryza sativa* L.) is an important cereal that provides nutrition for more than half of the world's population. In addition to grain yield, improving grain quality is also important for rice breeders (Cordero-Lara, 2020; Tong et al., 2019; Custodio et al., 2019; Nair, 2019; Kamrul et al., 2020). The main areas of rice sowing in the world are used for growing white-grained varieties (Ahmad et al., 2023; Javed et al., 2022). Wild-growing ancestors of cultivated rice had a colored grain pericarp. In the countries of traditional rice growing, along with white-grain varieties, red- and black-grain rice is grown for use as a dietary and medicinal product (Blagov et al., 2024; Tambunan et al., 2016; Kerboua et al., 2016). It is eaten without pre-grinding of the grains, so all the nutrients and biologically active substances that are valued in this crop are preserved (Valarmathi et al., 2020). Rice with colored grain pericarp has a higher antioxidant activity than white-grain rice (Zelenskaya et al., 2018).

In the leading rice-growing countries, along with white-grain rice, varieties with colored grain pericarp are created and cultivated. The collection of rice genetic resources for use in breeding is carried out in most rice-growing countries. When creating new varieties with a colored grain pericarp, both anc

]ient varieties and wild (ruderal) forms of rice with non-crumbling spikelets with several economically valuable features serve as the starting material. In the breeding practice of many rice-growing countries, both targeted artificial hybridization and the selection of spontaneous hybrids between white-, red-, and black-grain rice samples are widely used (Zelenskaya et al., 2018).

O. sativa L. is used in many diets (Vieira et al., 2024). Rice husk is mostly indigestible to humans because it is enriched with fibrous components. Rice is usually sold as white rice, and brown unprocessed rice is polished. The ground pericarp, seed coat, nucellus, and aleurone layer are called rice bran, and the oil obtained from bran is called rice bran oil (RBO). RBO has gained popularity as a heart-healthy oil because it is a good source of valuable nutrients, including gamma-oryzanol, vitamin E, and phytosterols, which have antioxidant and cholesterol-lowering effects (Karimov, 2009).

The consumption of pigmented rice as a staple food is growing rapidly due to its health benefits and the fact that it is considered a functional food ingredient. More interest was shown in many varieties of colored rice due to their multiple biological activity (Bhat et al., 2020). The ancestors of the cultivated *O. sativa L.* are the wild species *O. nivara* and *O. rufipogon,* the seeds of which have a colored pericarp. White rice is the result of introduction into the crop, that is, the white color of rice shells appears in representatives of this genus as a result of domestication. The color of the rice grain (a sign associated with antioxidant activity) is conditioned by the pigmentation of the sclerenchyma and (or) the pericarp and can vary widely: purple (violet/black), red, brown, yellow; variants are possible with uneven color (spotting) (Tumanian et al., 2020; Semeskandi et al., 2024). Rice grains rich in anthocyanins are recognized as an excellent source of natural and safe food dyes (Papillo et al., 2018; Anuyahong et al., 2020; Yi et al., 2020).

Starch, protein, and lipids are the most important nutrients in rice, and their composition and content play a crucial role in grain quality (Seregina et al., 2022). The quality of rice (including appearance, cooking quality, nutritional value, grinding and processing quality, etc.) is affected not only by the composition of nutrients in rice grains but also by the physical and chemical characteristics and their content in the grain (Peng et al., 2020; Iqbal et al., 2022). Starch and protein together make up more than 90% of the dry matter mass of rice grains (He et al., 2021). Starch in rice can be divided into amylose and amylopectin, and the structure and ratio of the two types of starch have an important influence on many qualitative characteristics of rice (Amagliani et al., 2016).

In the Republic of Kazakhstan, studies on rice with colored pericarp have not been conducted, which led to the absence of Kasakh varieties of red and black rice. Imported rice with colored pericarp (in particular, black rice) is 5-6 times more expensive than ordinary white-grain rice, which makes it unaffordable for the general population. The relevance of the study and creation of Kazakh varieties of rice with colored pericarp using classical and modern breeding methods is beyond doubt. The paper presents the results and discussion of the studies of the electrophoretic separation of spare proteins in an alkaline medium and the amylose content of the initial varieties and hybrid lines F_7-F_8 generations of rice with colored pericarp.

The study aims to identify the nature of inheritance of the presence of a protein with a molecular weight of 60 kDa from the initial forms to the hybrids of the later generation and to determine the amylose content.

2. Materials and Methods

Breeding work (2013-2023) was carried out at Birlik Agrofirma, Balkhash district, Almaty region, and the determination of amylose was carried out at the Laboratory of Plant Physiology and Biochemistry of the Institute of Plant Biology and Biotechnology of the National Academy of Sciences of the Republic of Kazakhstan.

The following rice varieties and their hybrids were used as the study material:

black: Mavr, Black rice;

red: Yir 5815;

white: Yantar, Marjan, Bakanassky, PakLi, Kurchanka, Sprint;

late hybrid lines obtained with their participation and dihaploids from hybrids:

a) red grain: F_7 Yir 5815/Pak Li *(var. sundensis Koern);* F_7 Yir 5815/Pak Li *(var. subpyrocarpa Gust);* F_7 Yir 5815/Bakanassky *(var. sundensis Koern);* F_7 Yir 5815/Bakanassky *(var. pyrocarpa Alef);* F_7 Yir 5815/Marjan *(var. pyrocarpa Alef)*; DG2 F_2 Yir 5815/Marjan *(var. pyrocarpa Alef)*; Almavita.

b) anthocyanin-colored (AC) grain: F_8 Black Rice/Yantar AC; F_8 Black Rice/Yantar *(var. pseudovialonica Vasc);* F_8 Black Rice/Yantar *(var. nigrispina Port);* F_6 Black Rice/Sprint *(var. pseudovialonica Vasc);* F_6 Black Rice/Sprint *(var. pyrocarpa Alef);* F_8 Black Rice/Bakanassky *(var. pseudovialonica Vasc);* F_8 Black Rice/Bakanassky *(var. Desvauxii Koern);* F_8 Black

Rice/Bakanassky *(var. Eediana Koern);* F_8 Black Rice/Bakanassky *(var. para-Gastrol Port);* F_8 Black Rice/Marjan; F_8 Black Rice/Marjan *(var. pyrocarpa Alef);* F_8 Black Rice/Marjan *(var. subpyrocarpa Gust);* F_8 Mavr/Kurchanka *(var. pyrocarpa Alef);* F_8 Mavr/Kurchanka *(var. sundensis Koern);* F_8 Mavr/Pak li *(var. bansmatica Koern);* F_8 Mavr/Bakanassky *(var. Desvauxii Koern);* DG2 F_2 Black Rice/Bakanassky.

2.1. Biochemical analysis of spare rice proteins

The extraction of spare proteins was carried out with tris-HCl and pH 6.8 phosphate buffers containing sodium dodecyl sulfate (SDS) Na, mercaptoethanol, glycerin, and bromophenol blue dye. The fractionation was carried out in a polyacrylamide gel (PAAG) of 10% concentration using the modified Laemmli method (Laemmli, 1970).

The analyses were carried out on single grains. The grain was ground into flour in a porcelain mortar and transferred to Eppendorf-type test tubes, and 0.26 ml of an extraction solution prepared based on 25 samples was poured on it. The solution contained: phosphate buffer, pH 6.7: 6 ml, 2.25 ml of 8.3% DDS Na solution, β-mercaptoethanol: 0.28 ml, bromophenol blue: at the tip of a scalpel, glycerin: 0.7 ml The samples were placed on a rocking gear for 2 hours, and extraction was carried out at room temperature. To stop the action of β-mercaptoethanol, the samples were alkylated and heated for 2 minutes in a boiling water bath. Then 25-30 µl of the protein sample was transferred to the pocket of the gel plate using a micro-syringe. Since the extraction is carried out with solvents containing β-mercaptoethanol, all operations were performed under a draught. Protein extracts were prepared on the day of application; for reuse, they were stored in a frozen state for only a few days.

Preparation of 12.0% separating gel: 10 ml 48% solution of acrylamide and bis-acrylamide, 5 ml 0.03 m tris HCl (pH 8,9), 10 ml 0.1% polysialic acid (PSA), 5 ml 0.8% DDS Na, 10 ml H_2O, 200 µl of tetramethylethylenediamine (TEMED).

Preparation of 3.5% concentrating gel: 1.2 ml 48% solution of acrylamide and bis-acrylamide, 2 ml 1M Tris-HCl (pH 6.9), 4 ml 0.1% PSA, 5 ml 0.8% DDS Na, 6.8 ml H_2O, 0.08 ml TEMED.

The gel solution was mixed and poured into prepared cassettes. After this, distilled water was carefully poured over the gel with a syringe to form a smooth edge surface of the gel. After complete polymerization of the lower separating gel, the water was drained and then the concentrating gel was topped up. To do this, the components of the solution were mixed and poured over the separating gel, and combs were inserted to form pockets (the air bubbles formed on the edges of the teeth were removed). The comb was carefully removed from the polymerized gel, after which the formed wells were washed with distilled water, and the remaining water was removed with filter paper. The prepared protein samples were applied to the wells.

Buffers were prepared for electrophoresis: the upper buffer (21.6 g glycine, 4.5 g tris, 0.45 g DDS Na brought to 1,500 ml with H_2O) and the lower buffer (2.88 g glycine, 0.6 g tris, 0.45 g DDS Na brought to 1,000 ml with H_2O).

Electrophoresis: after the samples were put into the wells, the chamber was filled with appropriate electrode buffer solutions, and the device was connected to a power source. First, the current strength was set at 100 mA per plate. After the proteins migrated into the separating gel, the current was increased to 180-200 mA.

Fixation and staining of gel plates. At the end of electrophoresis, gel plates were placed in 10% trichloroacetic acid with the Coomassie brilliant blue dye K-250 for 12-15 hours. The gel plates were washed from excess dye with running water. The spectra were photographed and visually analyzed.

The quantitative content of amylose was determined by the Giuliano method (Shih, 2003). 1 ml of 96% ethanol (C_2H_5OH) and 9 ml of 1N NaOH were added to 100 mg of a rice sample ground into flour. They were mixed and put in a water bath (100°C, 10 minutes). Then they were brought to 100 ml with distilled water. The substance was mixed, then 5 ml of the sample was taken from this flask, and 1 ml of 1 n acetic acid and 2 ml of iodine reagent (KI+2) were added to it. It was mixed and brought to 100 ml with distilled water. Then it was left for 20 minutes in a dark place. The amylose content in the sample was determined by a standard curve. To build it, 40 mg of potato amylose (amylose, from potato, Sigma A 0512, USA) was added to 1 ml of 96% ethanol and 9 ml of 1N NaOH. It was heated for 10 minutes in a boiling water bath, cooled, and brought to 100 ml 1, 2, 3, 4, and 5 ml of amylose solution were measured with a pipette, then 1 ml of 1N acetic acid and 2 ml of iodine reagent was added, and the mixture was brought to 100 ml with distilled water. The amylose content was mixed and measured after 20 minutes at a wavelength of 620 nm. The quantitative content of amylose in the sample was determined by a standard curve constructed for potato amylose.

We used 1 ml of 1 N NaOH + 1 ml of 1N acetic acid + 2 ml of iodine reagent with the volume adjusted to 100 ml with distilled water as a control sample. After 20 minutes, the measurement was carried out at a wavelength of 620 nm.

3. Results

To study the composition of spare proteins of rice hybrids and dihaploids with colored pericarp and their parent forms, electrophoretic separation of spare proteins in an alkaline medium was performed (Figure 1).

The analyzed hybrid lines of F_7 and F_8 generations are hybrids from the crossing of 10 parental genotypes: Yir 5815, Bakanassky, PakLi, Marjan, Mavr, Kurchanka, Yantar, Black Rice, and Sprint.

The presence of a protein with a molecular weight of 60 kDa was analyzed using electrophoregram by visual evaluation of the gel. This protein is a product of the *Wx* gene that controls the amylose content. The intensity of this protein band is associated with a high or low amylose content (Zelenskaya et al., 2018).

No. 2 and 3 originate from combinations of the Yir 5815 genotype with variants of the Pak Li variety. Judging by the intensity of the component with a molecular weight of 60 kDa, both genotypes have a low amylose content.

The genotype Yir 5815 is a low-amylose sample, whereas Bakanassky is a sample with a high amylose content. Hybrid line No. 5 inherited low amylose content from the parent form Yir 5815, while line No. 6 is a sign of the allele of the *Wx* locus of the variety of the parent form Bakanassky. Line No. 8 is a hybrid obtained from combinations of the genotypes Yir 5815 and Marjan, and it shows average amylose content, as well as the Marjan parent form (Figure 1).

M-marker; 1: Yir 5815; 2: F_7 Yir 5815/Pak Li *var. sundensis Koern.*; 3: F_7 Yir 5815/Pak Li *var. subpyrocarpa Gust.*; 4: Pak Li; 5: F_7 Yir 5815/Bakanassky *var. pyrocarpa Alef.*; 6: F_7 Yir 5815/Bakanassky *var. sundensis Koern.*; 7: Bakanassky; 8: F_7 Yir 5815/Marjan *var. pyrocarpa Alef.*; 9: Marjan; 10: F_8 Black Rice/Marjan *var. pyrocarpa Alef.*; 11: F_8 Black Rice/Marjan *var. subpyrocarpa Gust.*; 12: F_8 Black Rice/Marjan; 13: Black Rice; 14: F_8 Black Rice/Bakanassky *var. para-Castrol Port.*; 15: F_8 Black Rice/Bakanassky *var. Pseudovialonica Vasc.*; 16: F_8 Black Rice/Bakanassky *var. Desvauxii Koern.*; 17: F_8 Black Rice/Bakanassky *var. Eediana Koern.*; 18: DG 2 F2 Black Rice/Bakanassky; 19: Bakanassky; 20: Almavita; 21: Black Rice; 22: F_8 Black Rice /Yantar AC; 23: F_8 Black Rice/Yantar *var. Pseudovialonica Vas.*; 24: F_8 Black Rice/Yantar *var. nigrispina Port.*; 25: Yantar; 26: F_6 Black Rice/Sprint *var. Pseudovialonica Vasc.*; 27: F_6 Black Rice/Sprint *var. pyrocarpa Alef.*; 28: Sprint; 29: Mavr; 30: F_8 Mavr/Kurchanka *var. sundensis Koern.*; 31: F_8 Mavr/Kurchanka *var. pyrocarpa Alef.*; 32: Kurchanka; 33: F_8 Mavr/Pak Li *var. bansmatica Koern.*; 34: Pak Li; 35: F_8 Mavr/Bakanassky *var. Desvauxii Koern.*; 36: Bakanassky; 37: Yir 5815; 38: DG 2 F2 Yir 5815/Marjan *var. pyrocarpa Alef.*; 39: Marjan; 40: Almavita.

Hybrid lines No. 10, 11, and 12 obtained from the Black Rice and Marjan genotypes were low-amylose in terms of the intensity of the marker protein band (60 kDa). They inherited the allele of the *Wx* locus from the Black Rice genotype, whereas the Marjan variety (the other parent form) contained an intense marker band in the spectrum of spare proteins. A similar inheritance of the amylose index (low content) was observed in lines No. 14, 15, 16, and 17, the other parent forms of which are varieties of the Bakanassky variety.

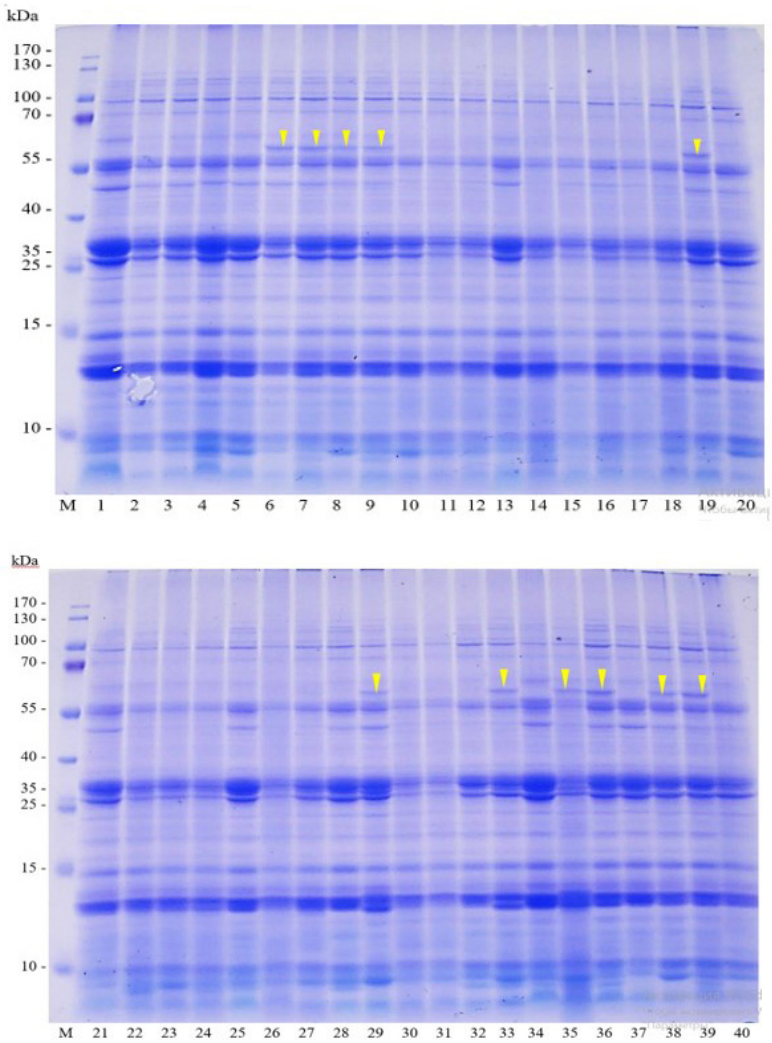

Figure 1. Electrophoretic spectrum of spare seed proteins of rice hybrids and dihaploids with colored pericarp.

Line No. 18 was also low-amylose, inheriting this trait from the parent form of Black Rice, whereas in the spectrum of spare proteins, the Bakanassky variety had a marker band with a molecular weight of 60 kDa. Line No. 20: the future Almavita variety belongs to glutinous samples according to the spectra of spare proteins (Figure 1).

Hybrid lines No. 22, 23, and 24 obtained with the participation of the Black Rice genotype and varieties of the Yantar variety were low-amylose in terms of the intensity of the marker protein band (60 kDa), and both initial parent forms also belonged to low-amylose types.

Lines No. 26 and 27 obtained from crossing the Yantar and Sprint genotypes according to electrophoretic patterns were low-amylose and both initial forms also belonged to the low-amylose genotype.

Hybrid lines No. 30 and 31 were also low-amylose, inheriting the allele of the *Wx* locus from the Kurchanka genotype of the Chicken, as the Mavr variety (the other parent form) contained a fairly intense marker band in the spectrum of spare proteins, which determines the amylose content of the genotype. Another line No. 33 was obtained by crossing the Mavr genotype with the Pak Li genotype, which had a high amylose content, like the Mavr genotype. Hybrid line No. 35, obtained by crossing two highly amylotic genotypes, namely, the Mavr and the Bakanassky, had an amylose content as high as the parent forms (Figure 1).

Line No. 38 on the electrophoretic spectra of spare proteins showed high amylose content, inheriting this feature from the Marjan parent form, while the second parent form (Pak Li) showed low amylose content on the electrophoretic spectra. The possible future variety Almavita (No. 40) turned out to be glutinous, like on the first electrophoregram.

At the next stage of the study, the amylose content was determined in 35 varieties of rice where electrophoretic separation of spare proteins was carried out. The obtained results were analyzed and compared with the obtained electrophoregram data (Table 1).

Table 1. Amylose content in rice varieties and their hybrids.

No.	Genotypes	C_a, %	Amylose content	Protein with m.m. 60 kDa
1	**2**	**3**	**4**	**5**
1	Yir 5815	18.5	low amylose content	-
2	Pak Li	18.0	low amylose content	-
3	Bakanassky	20.0	medium amylose content	+
4	Marjan	20.0	medium amylose content	+
5	Black Rice	2.3	glutinous	-
6	Yantar	17.0	low amylose content	-
7	Kurchanka	19.6	low amylose content	-
9	Sprint	15.0	low amylose content	-
10	Mavr	33.0	highl amylose content	+
11	Almavita	1.0	glutinous	-
12	F_7 ♀ Yir 5815 x ♂ Pak Li var. sundensis Koern.	10.8	low amylose content	-
14	F_7 ♀ Yir 5815 x ♂ Pak Li var. *subpyrocarpa Gust.*	11.6	low amylose content	-
15	F_7 Yir 5815/ Bakanassky *var. pyrocarpa Alef*	16.6	low amylose content	-
16	F_7 Yir 5815/ Bakanassky *var. sundensis Koern*	22.2	medium amylose content	+
17	F_7 Yir 5815/ Marjan *var. pyrocarpa Alef*	20.5	medium amylose content	+
18	F_8 Black Rice/Marjan *var. pyrocarpa Alef*	12.0	low amylose content	-
19	F_8 Black Rice/Marjan *var. subpyrocarpa Gust*	15.0	low amylose content	-
20	F_8 Black Rice/Marjan	19.0	low amylose content	-
21	F_8 Black Rice/ Bakanassky *var. para-Gastrol Port*	1.1	glutinous	-
22	F_8 Black Rice/ Bakanassky *var. pseudovialonica Vasc*	0.8	glutinous	-
23	F_8 Black Rice/ Bakanassky *var. Desvauxii Koern*	0.1	glutinous	-
24	F_8 Black Rice/ Bakanassky *var. Eediana Koern*	4.0	very low amylose content	-
25	DG 2 F_2 Black Rice / Bakanassky	1.5	glutinous	-
26	F_8 Black Rice/ Yantar AC	14.7	low amylose content	-
27	F_8 Black Rice/ Yantar *var. pseudovialonica Vasc*	1.5	glutinous	-
28	F_8 Black Rice/ Yantar *var. nigrispina Port*	2.2	very low amylose content	-
29	F_6 Black Rice/Sprint *var. pseudovialonica Vasc*	2.4	very low amylose content	-
30	F_6 Black Rice/Sprint *var. pyrocarpa Alef*	17.0	low amylose content	-
31	F_8 Mavr/ Kurchanka *var. sundensis Koern*	19.2	low amylose content	-
32	F_8 Mavr/ Kurchanka *var. pyrocarpa Alef*	19.4	low amylose content	-
33	F_8 Mavr/ Pak Lee *var. bansmatica Koern*	27.5	highl amylose content	+
34	F_8 Mavr/ Bakanassky *var. Desvauxii Koern*	23.0	medium amylose content	+
35	DG2 F_2 Yir 5815/ Marjan *var. pyrocarpa Alef*	20.0	medium amylose content	+

Note: glutinous (from 0 to 2%), non-glutinous (2%), very low amylose content (from 2 to 9%), low amylose content (from 10 to 19%), medium amylose content (from 20 to 24%), high amylose content (24%).

4. Discussion

Evaluation of breeding material based on a protein marker makes it possible to quickly and efficiently select and control the transfer of desired traits from parental forms to hybrid populations (Karimov, 2009). The advantage of protein as a genetic marker is conditioned by the fact that protein is the primary and direct product of the genetic system. Proteins are least susceptible to phenotypic variability and, accordingly, have a well-expressed biological specificity. Among the protein markers in plants, seed proteins occupy a special position. These highly polymorphic proteins make it possible to identify the gene pool of varieties with sufficient completeness, distinguish and register their biotypes and hybrid lines, and analyze varietal, hybrid, and natural populations (Gubareva et al., 2015).

Rice proteins consist of four traditionally classified fractions depending on solubility: albumin (water-soluble), globulin (salt-soluble), glutelin (alkali-soluble), and prolamine (alcohol-soluble). Globulin (about 12%) and glutelin (about 80%) are the main components of rice protein (Shih, 2003).

In our studies, we performed the electrophoretic separation of glutelins, which make up most rice proteins. The presence of a protein with a molecular weight of 60 kDa was analyzed by electrophoregram by visual evaluation of the gel. This protein is a product of the *Wx* gene that controls the amylose content. The intensity of this protein band is associated with a high or low amylose content (Zelenskaya et al., 2018). The protein spectrum with a molecular weight of 60 kDa was present in the following hybrids: F_7 Yir 5815/Bakanassky *var. sundensis Koern.*; F_8 Mavr/Pak Li *var. bansmatica Koern;* F_8 Mavr/Bakanassky *var. Desvauxii Koern*; F_7 Yir 5815/Marjan *var. pyrocarpa Alef.*; and DG2 F2 Yir 5815/Marjan *var. pyrocarpa Alef*. The results of the study of the amylose content in flour show that these five hybrids are high- and medium-amylose hybrids. The remaining hybrids have a low amylose content or none.

Variations of amylose content in cultivated rice samples range from 1 to 35% (Jin et al., 2006). By the amylose: amylopectin ratio, rice varieties can be classified depending on their amylose content, for example, wax-colored rice (1-2% of amylose), rice with a very low amylose content (2-12% of amylose), rice with a low amylose content (12-20% of amylose), rice with a medium amylose content (20-25% of amylose) and rice with a high amylose content (25-33% of amylose). The texture of boiled rice and the functional properties of rice starch are primarily affected by the amylose content (Bhattacharya et al., 1982).

Rice grains with a high amylose content increase in volume and become flaky when cooked but become harder when cooled. In contrast, rice grains with a low amylose content remain moist and sticky after cooking (Calingacion et al., 2015).

According to the obtained data, the studied rice hybrids were divided into five groups according to the quantitative content of amylose:

In F_8 Black Rice/Bakanassky *var. para-Gastrol Port*; F_8 Black Rice/Bakanassky *var. pseudovialonica Vasc*; F_8 Black Rice/ Bakanassky *var. Desvauxii Koern.;* DG 2 F_2 Black Rice/Bakanassky; F_8 Black Rice/Yantar *var. pseudovialonica Vasc.*, the amount of amylose varied from 0 to 2% and these varieties were assigned to the glutinous group.

F_7 Yir 5815/Pak Li *var. sundensis Koern.*, F_7 Yir 5815/Pak Li *var. subpyrocarpa Gust.;* F_7 Yir 5815/Bakanassky *var. pyrocarpa Alef.;* F_8 Black Rice/Marjan *var. pyrocarpa Alef.;* F_8 Black Rice/Marjan *var. subpyrocarpa Gust.;* F_8 Black Rice/Marjan*;* F_8 Black Rice/Yantar AC; F_6 Black Rice/Sprint *var. pyrocarpa Alef.;* F_8 Mavr/Kurchanka *var. sundensis Koern.;* and F_8 Mavr/Kurchanka *var. pyrocarpa Alef.* belong to the low-amylose group and the amylose content in them ranges from 10 to 20%.

F_7 Yir 5815/Pak Li *var. pyrocarpa Alef.*, F_7 Yir 5815/ Bakanassky *var. sundensis Koern*, F_8 Mavr/Bakanassky *var. Desvauxii Koern*; F_7 Yir 5815/Marjan *var. pyrocarpa Alef.;* and DG2 F_2 Yir 5815/Marjan *var. pyrocarpa Alef* are in the group with a medium amylose content (20.5; 22.2; 23.0; and 20.0, respectively).

F_8 Mavr/Pak Li *var. bansmatica Koern* with 27.5% of amylose belongs to the high amylose content group.

5. Conclusions

As a result of the assessment of biochemical parameters, promising hybrids of each species and different generations were identified and characterized for use in further breeding work. The heritability of the protein spectrum of 60 kDa was visually characterized according to the data of electrophoretic separation of spare proteins of rice seeds. The amylose content was determined, and the studied hybrids were divided into five groups based on their amylose content. According to the obtained data, promising hybrids will continue to be transferred to the variety. It is planned to derive rice varieties with colored pericarp with different amylose content from these hybrids.

Acknowledgements

This study was funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan. We received targeted financing for project BR18574149.

References

AHMAD, T.M., HAIDER, M.S., RANDHIR, T.O., RANDHIR, R. and AHMAD, S.R., 2023. Spatial analysis of factors influencing bacterial leaf blight in rice production. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83, pp. e264249. https://doi.org/10.1590/1519-6984.264249.

AMAGLIANI, L., O'REGAN, J., KELLY, A.L. and O'MAHONY, J.A., 2016. Chemistry, structure, functionality and applications of rice starch. *Journal of Cereal Science*, vol. 70, pp. 291-300. http://dx.doi.org/10.1016/j.jcs.2016.06.014.

ANUYAHONG, T., CHUSAK, C. and ADISAKWATTANA, S., 2020. Incorporation of anthocyanin-rich riceberry rice in yogurts: effect on physicochemical properties, antioxidant activity and in vitro gastrointestinal digestion. *Lebensmittel-Wissenschaft + Technologie*, vol. 129, pp. 109571. http://dx.doi.org/10.1016/j.lwt.2020.109571.

BHAT, F.M., SOMMANO, S.R., RIAR, C.S., SEESURIYACHAN, P., CHAIYASO, T. and PROM-U-THAI, C., 2020. Status of bioactive compounds from bran of pigmented traditional rice varieties and their scope in production of medicinal food with nutraceutical importance. *Agronomy (Basel)*, vol. 10, no. 11, pp. 1817. http://dx.doi.org/10.3390/agronomy10111817.

BHATTACHARYA, K.R., SOWBHAGYA, C.M. and INDUDHARA SWAMY, Y.M., 1982. Quality problems of rice: a tentative scheme for classification. *Journal of Food Science*, vol. 47, no. 2, pp. 564-569. http://dx.doi.org/10.1111/j.1365-2621.1982.tb10124.x.

BLAGOV, A., OREKHOVA, V., SUKHORUKOV, V., MELNICHENKO, A. and OREKHOV, A., 2024. Mitochondrial dysfunction model in the pathogenesis of inflammatory response development in obesity. *Online Journal of Biological Sciences*, vol. 24, no. 1, pp. 110-120. http://dx.doi.org/10.3844/ojbsci.2024.110.120.

CALINGACION, M., FANG, L., QUIATCHON-BAEZA, L., MUMM, R., RIEDEL, A., HALL, R.D. and FITZGERALD, M., 2015. Delving deeper into technological innovations to understand differences in rice quality. *Rice (New York, N.Y.)*, vol. 8, no. 1, pp. 6. http://dx.doi.org/10.1186/s12284-015-0043-8. PMid:26054242.

CORDERO-LARA, K.I., 2020. Temperate japonica rice (Oryza sativa L.) breeding: history, present and future challenges. *Chilean Journal of Agricultural Research*, vol. 80, no. 2, pp. 303-314. http://dx.doi.org/10.4067/S0718-58392020000200303.

CUSTODIO, M.C., CUEVAS, R.P., YNION, J., LABORTE, A.G., VELASCO, M.L. and DEMONT, M., 2019. Rice quality: how is it defined by consumers, industry, food scientists, and geneticists? *Trends in Food Science & Technology*, vol. 92, pp. 122-137. http://dx.doi.org/10.1016/j.tifs.2019.07.039. PMid:31787805.

GUBAREVA, N.K., GAVRILYUK, I.P. and KONAREV, A.V., 2015. Identifikatsiya sortov selskokhozyaistvennykh kultur po elektroforeticheskim spektram zapasnykh belkov [Identification of crop varieties by electrophoretic spectra of storage proteins]. *Agrarnaya Rossiya*, no. 11, pp. 21-27.

HE, W., WANG, L., LIN, Q.L. and YU, F., 2021. Rice seed storage proteins: biosynthetic pathways and the effects of environmental factors. *Journal of Integrative Plant Biology*, vol. 63, no. 12, pp. 1999-2019. http://dx.doi.org/10.1111/jipb.13176. PMid:34581486.

IQBAL, J., AHMAD, S. and ALI, Q. 2022. A comparative study on the virulence of entomopathogenic fungi against trogoderma granarium (everts) (Coleoptera: Dermestidae) in stored grains rice. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 82, pp. e250778. https://doi.org/10.1590/1519-6984.250778.

JAVED, M.A., ALI, S.W., ASHFAQ, M., TABASSAM, J., ALI, M., IHSANULLAH, M., NAYAB, S.F., KAYA, Y., KHALILI, E., ALI, Q. and YAU, T.E., 2022. Molecular profiling of bacterial blight resistance in Malaysian rice cultivars. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 82, pp. e256189. https://doi.org/10.1590/1519-6984.256189.

JIN, W., LI, N. and HONG, D., 2006. Genetic diversity of seed storage proteins in different ecotype varieties of japonica rice and its application. *Rice Science*, vol. 13, no. 2, pp. 85-92.

KAMRUL, H., TAKASHI, S.T., MONJURUL, A., ROSTOM, A. and CHAYAN, K.S., 2020. Impact of modern rice harvesting practices over traditional ones. *Reviews in Agricultural Science*, vol. 8, pp. 89-108. http://dx.doi.org/10.7831/ras.8.0_89.

KARIMOV, A.Y., 2009. Issledovanie indeksa geneticheskoi skhozhesti i identifikatsiya sortov myagkoi pshenitsy sposobom gliadinovogo markera [A study of the genetic similarity index and identification of bread wheat varieties using the gliadin marker method]. *Sovremennye problemy nauki i obrazovaniya*, no. 6, pp. 13-21.

KERBOUA, K.E., HAIBA, F., BENDJEMIA, F., KERRACHE, M. and BOUMEDIENE, A., 2016. Proposed insights in the pharmaco-dynamic theory of ascorbate pro-oxidant activity. *Online Journal of Biological Sciences*, vol. 16, no. 1, pp. 1-9. http://dx.doi.org/10.3844/ojbsci.2016.1.9.

LAEMMLI, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, vol. 227, no. 5259, pp. 680-685. http://dx.doi.org/10.1038/227680a0. PMid:5432063.

NAIR, K.P., 2019. Chapter four—Utilizing crop wild relatives to combat global warming. In: D.L. SPARKS, editor. *Advances in agronomy*. Cambridge: Academic Press, vol. 153, pp. 175-258.

PAPILLO, V.A., LOCATELLI, M., TRAVAGLIA, F., BORDIGA, M., GARINO, C., ARLORIO, M. and COÏSSON, J.D., 2018. Spray-dried polyphenolic extract from Italian black rice (Oryza sativa L., var. Artemide) as new ingredient for bakery products. *Food Chemistry*, vol. 269, pp. 603-609. http://dx.doi.org/10.1016/j.foodchem.2018.07.059. PMid:30100478.

PENG, B., JIN, K.X., LUO, D.Y., TIAN, X.Y., SUN, Y.F., HUANG, X.H., PANG, R.H., WANG, Q.X., ZHOU, W., YUAN, H.Y., YANG, F., PENG, J., LI, H.-L., SONG, X.-H. and A, X.-X., 2020. The nutritional components of rice are closely related to grain quality traits in rice. *Journal of Biology and Life Science*, vol. 11, no. 2, pp. 239. http://dx.doi.org/10.5296/jbls.v11i2.17809.

SEMESKANDI, M.N., MAZLOOM, P., ARABZADEH, B., MOGHADAM, M.N. and AHMADI, T., 2024. Evaluation of seedling cultivation and irrigation regimes on yield and yield components in Rice Plant. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 84, pp. e266261. https://doi.org/10.1590/1519-6984.266261.

SEREGINA, I.I., TRUKHACHEV, V.I., BELOPUKHOV, S.L., VIGILIANSKII, I.M. and DMITREVSKAIA, I.I., 2022. Yield and quality of dega white lupine grain (Lupinus albus) and Yubileynaya 80 spring wheat (Triticum aestivum L.) depending on the application method of sodium selenite. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 82, pp. e264216. https://doi.org/10.1590/1519-6984.264216.

SHIH, F.F., 2003. An update on the processing of high-protein rice products. *Die Nahrung*, vol. 47, no. 6, pp. 420-424. http://dx.doi.org/10.1002/food.200390093. PMid:14727771.

TAMBUNAN, U.S.F., NASUTION, M.A.F., PARIKESIT, A.A., NOVIARDI, H. and KERAMI, D., 2016. Designing of disulfide cyclic peptide for inhibiting polymerase A and B1 (PA_C-$PB1_N$) in H1N1 virus using molecular simulation approach. *Online Journal of Biological Sciences*, vol. 16, no. 3, pp. 122-129. http://dx.doi.org/10.3844/ojbsci.2016.122.129.

TONG, C., GAO, H.Y., LUO, S.J., LIU, L. and BAO, J.S., 2019. Impact of postharvest operations on rice grain quality: a review. *Comprehensive Reviews in Food Science and Food Safety*, vol. 18, no. 3, pp. 626-640. http://dx.doi.org/10.1111/1541-4337.12439. PMid:33336927.

TUMANIAN, N.G., MUKHINA, ZH.M., ESAULOVA, L.V., PAPULOVA, E.YU., SAVENKO, E. G. and GARKUSHA S. V., 2020. Colored rice varieties of Russian breeding in terms of grain quality for development of functional rice varieties. *E3S Web of Conferences*, vol. 224, pp. 04021. http://dx.doi.org/10.1051/e3sconf/202022404021.

VALARMATHI, S., SENTHIL KUMAR, M., SHARMA, V. and IMRAN, M., 2020. Formulation and evaluation of herbal face cream. *Research Journal of Pharmacy and Technology*, vol. 13, no. 1, pp. 216-218. http://dx.doi.org/10.5958/0974-360X.2020.00043.8.

VIEIRA, C., MARCON, C. and DROSTE, A., 2024. Phytotoxic and cytogenotoxic assessment of glyphosate on Lactuca sativa L. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 84, pp. e257039. https://doi.org/10.1590/1519-6984.257039.

YI, J., QIU, M., LIU, N., TIAN, L., ZHU, X., DECKER, E.A. and MCCLEMENTS, D.J., 2020. Inhibition of lipid and protein oxidation in whey-protein-stabilized emulsions using a natural antioxidant: black rice anthocyanins. *Journal of Agricultural and Food Chemistry*, vol. 68, no. 37, pp. 10149-10156. http://dx.doi.org/10.1021/acs.jafc.0c03978. PMid:32833451.

ZELENSKAYA, O., ZELENSKY, G., OSTAPENKO, N. and TUMANYAN, I., 2018. Genetic resources of rice (Oryza sativa L.) with colored pericarp. *Vavilovskii Zhurnal Genetiki i Selektsii*, vol. 22, no. 3, pp. 296-303. http://dx.doi.org/10.18699/VJ18.363.