RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
56 (1) 89-100, 2024
http://doi.org/10.54910/sabrao2024.56.1.8
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

IMPROVING COLOURED RICE GRAIN QUALITY THROUGH ACCELERATED BREEDING

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SUMMARY

The presented study sought to phenotype the rice cultivars procured from the Unique Scientific Installation (USI) - Collection of Federal Scientific Rice Centre, Krasnodar, Russian Federation, as sources of valuable grain quality traits, as well as BC_2 populations with the selection of the best genotypes for quality traits in developing the red grain rice cultivars with high nutritional properties. The studied rice plant material, grown in the artificial climate chambers of FSBSI - Federal Scientific Rice Centre, had the following conditions: temperature – 28–30 °C during the day (12 h) and 24 °C at night (12 h); illumination – 30,000 lux, and humidity = 70%. Rice determination and phenotyping for grain quality traits commenced on high-tech certified equipment per GOSTs. The experiment results had the parental genotypes used in the backcrossing program assigned to the group of medium-grain cultivars, except for the long-grain cultivars, i.e., Svetlana and Gagat. The 1000-grain weight ranged from 23.0 to 27.4 g, filminess (17.8% to 19.9%), and total milling yield (65.0% to 70.4%). Low fracturing appeared in rice cultivars, viz., Rubin, Alliance, Kurazh, Gagat, and VNIIR10163, while a high vitreosity emerged in rice cultivars Veles, Svetlana, VNIIR10163, and Khaw-sri-nin. BC_2 plant populations' evaluation depended on the technological quality traits. The effective heterosis was evident for most grain quality traits. Rice genotypes selected based on lower fracturing and higher grain size will undergo further breeding work to develop the high-yielding rice genotypes with desirable grain quality traits.

Keywords: Rice, cultivars, backcrosses, rice breeding, technological grain quality traits, grain vitreosity, grain fracturing

Communicating Editor: Dr. Himmah Rustiami

Manuscript received: August 15, 2023; Accepted: November 15, 2023.

Citation: Mukhina ZhM, Tumanyan NG, Garkusha SV, Papulova EYu, Chukhir NP, Chukhir IN, Gnenniy EYu, Esaulova LV, Malyuchenko EA, Vakhrusheva NI (2024). Improving coloured rice grain quality through accelerated breeding. *SABRAO J. Breed. Genet.* 56(1): 89-100. http://doi.org/10.54910/sabrao2024.56.1.8.

Key findings: In the accelerated breeding program of rice cultivars, using the BC_2 populations helped identify the promising rice genotypes with lower fractures and a larger grain size than the parental genotypes. The best-isolated rice samples with lower grain fracturing distinctly included BC_2 Gagat/Svetlana//Svetlana, Dig.2327/Veles//Veles, and Dig.2327/Alliance//Alliance. Noting also the heterotic effects based on the grain size occurred in BC_2 populations Mavr/Svetlana//Svetlana and Mavr/Kurazh//Kurazh. These hybrid combinations and their parental genotypes are deployable in an accelerated breeding of rice cultivars.

INTRODUCTION

Rice may not serve as the staple food in Russia; however, its wide use with other cereals makes it occupy a special place in the country's traditional, baby, and dietary nutrition. The rice grain mainly makes up the production of milled rice. The nutritional value of milled rice, like other food products, must satisfy humans' prime nutrients, i.e., proteins, carbohydrates, fats, vitamins, macro-, and microelements. Rice eating and culinary quality (ECQ) is critical to its economic value in the market and consumers' consumption. Therefore, the grain quality of the newly developed rice genotypes is imperative in rice breeding programs. In most Asian countries, rice is the chief source of energy, protein, and trace elements (Junfei *et al*., 2015). Colored rice, being rich with anthocyanins and phenolic compounds, is also vital in human nutrition due to its antioxidant properties, reducing reactive free radicals that damage cells and contribute to the oxidative damage to lipids and low-density lipoproteins (Lao *et al*., 2018; Prasad *et al*., 2019).

Cereal breeding to enhance the nutritional values, including antioxidant content as nutritive factors, gained recognition as relevant and accomplished worldwide (Polonsky *et al*., 2018). In Russia, most breeding focused on white and colored-grain rice cultivars (with colored-grain pericarps) with high antioxidant contents (Tumanyan *et al*., 2016; Garkusha *et al*., 2022). Thus, determining the high nutritional value of milled rice cultivars by the different breeding approaches relied on the involvement of the broadest range of germplasm, with an integrated approach requiring efficient phenotypic evaluation for grain quality traits to improve the selection, which is time-consuming, costly, and has low productivity.

Breeders attribute breeding innovation to molecular markers associated with specific target traits. In a germplasm collection, genetic diversity assessment can use microsatellite DNA loci (SSR) and association-mapping methods (Park *et al*., 2019). Currently, large-scale genotyping by sequencing has successful application in crop plants, especially in rice. Thus, using a panel of markers genotyped a large segregating F_2 population of rice obtained by crossing the Nipponbare variety (*Oryza sativa* ssp. japonica cv.) with African wild rice (*O. longistaminata*) (Furuta *et al*., 2017). Their results further identified 8,154 informative SNP markers when analyzing 1,081 F_2 plants. Quantitative trait loci (QTLs) responsible for the yield trait 'number of panicles' were localized on chromosomes 1, 3, 4, and 8. Different breeding programs also introduced genes of interest into the rice genome. The breeding potential of the *Pigm* gene associated with rice resistance to Magnaporthe oryzae attained assessment, introducing the *Pigm* gene in two rice cultivars using backcrossing and marker-assisted selection (MAS) (Feng *et al*., 2022).

In practical breeding schemes, the genomic approach for crop plants is prevalent but very expensive. However, its use is on a conveyor basis by various companies, i.e., 'Limagrain' for developing modern sunflower hybrids, 'Euralis Semans' for developing breeding forms of sorghum crops (grain, sugar sorghum, sorghum-Sudanese hybrids), and Syngenta (sunflower, corn, vegetable, and other crops). The genomic screening involvement and developing breeding materials determine the production success of numerous companies and underlie the promotion to foreign markets. The high cost of the technology is limiting the use of these technologies. Creating Russian cultivars of different crops, including rice, often progress through conventional and classical breeding methods.

Various food products' development uses rice cultivar grains created from ancient times and in the modern breeding process. However, the relevance of breeding new rice cultivars is dependent on the desire of the consumers and producers to determine grain cultivation with high technological traits and healthy nutrition parameters (Han *et al*., 2004; Koutroubas *et al*., 2004; Addison *et al*., 2015; Odoom *et al*., 2021). Rice eating and cooking (ECQ) qualities are crucial to its economic values in the market and imperative in rice breeding programs. Remarkable advancements have occurred in ECQ genetic research worldwide, which raises the prospects of further increasing ECQ in rice. Marker-assisted breeding has identified rare alleles in diverse rice cultivars conferring superior ECQ traits (Li *et al*., 2020; Sreenivasulu *et al*., 2022). Most cultivated rice cultivars with relevant flavor attributes are allelic variants of badh2-E2 and badh2-E7. The developed functional molecular markers SNP, SNP_badh2-E2, and SNP_badh2-E7 can benefit in improving the genetic makeup of aromatic rice using biotechnological approaches (Li *et al*., 2020).

Colored rice varieties are rich in amino acids, phenolic compounds, and antioxidants (Tyagi *et al.*, 2022). The selection of colored rice varieties progresses globally due to their high nutritional value. In Korea (Dong-A University), 16 informative pairs of primers with high PIC achieved their selection, which were then used to assess the genetic diversity in the collection (Jae-Ryoung *et al.*, 2019). Solving the problems of creating colored rice varieties of Russia has reached the development of systems of molecular markers to control the target loci inclusion in the genotype of the variation. The production of rice varieties with colored pericarp has links with the inclusion in the genotype of the created varieties of genes and loci that determine drought resistance due to insufficient irrigation water in most rice-growing regions (Goncharova *et al.*, 2021).

Significant genes and QTLs regulate the starch composition by manipulating amylose content and thermal and adhesive properties. Many QTLs' recognition for ECQ properties included protein content and aroma parameters (Mukhina *et al*., 2022). During the grain-filling phase, starch accumulates in the rice endosperm, mainly synthesized by four different enzymes, i.e., ADP glucose pyrophosphorylase (AGPase), starch synthase (SSs), starch branching enzymes (SBEs), and starch degrading enzymes (DBEs). Post-translational adaptation of these enzymes through phosphorylation, alternative splicing, and allosteric variations is vital in managing the starch content and amylose-amylopectin composition (Adegoke *et al*., 2021). At the molecular level, variations in gene expressions are generally acceptable sources of phenotypic diversity in crop plants and livestock. Crossing parental genotypes to obtain cultivars with high grain quality traits and nutritional values has an initial complement with selecting donor parents with valuable quality traits. The prevailing study aimed to phenotype the rice cultivars obtained from the USI - Collection of Federal Scientific Rice Centre, Krasnodar, Russian Federation, as donors of valuable traits for grain quality to select the best ones for subsequent large-scale genotyping by sequencing (GBS), for developing rice cultivars with high nutritional properties.

MATERIALS AND METHODS

Plant material

Grain-quality traits evaluation ensued on a set of rice cultivars with genetic diversity, procured from the USI - Collection of Genetic Resources of Rice, Vegetable, and Melon Crops, Federal Scientific Rice Centre, Krasnodar, Russian Federation, to restock the collection of rice genetic sources with target traits comprised of Russian and foreign breeding germplasm. After screening and selection, the promising parental types as effective sources of target traits

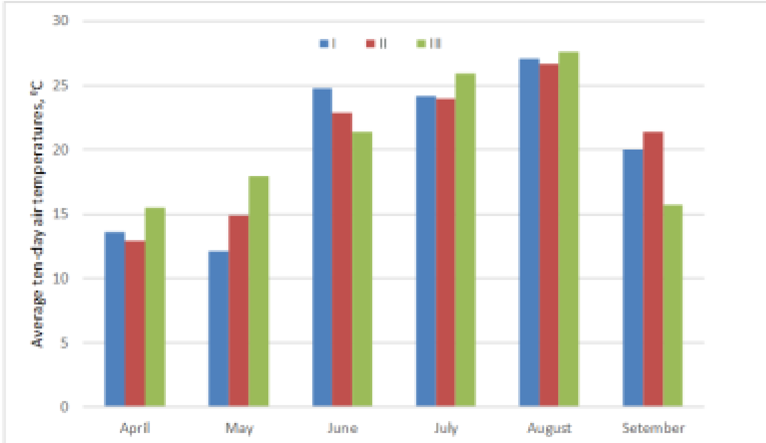

Figure 1. Average 10-day air temperatures in April-September 2022.

became samples for hybridization programs to increase the nutritional value of milled rice. The breeding program grew the experimental material for obtaining backcrosses and segregating populations in artificial climate chambers (ACC) of the FSBSI - Federal Scientific Rice Centre.

Study site and meteorological conditions

The cultivation and screening of rice parental genotypes commenced under the small-plot experiment on an optimal agricultural background. The grain harvesting of rice cultivars proceeded manually in the phase of full ripeness. Growing the studied BC_2 plant material transpired in artificial climate chambers of the FSBSI - Federal Scientific Rice Centre, Krasnodar. Field studies in 2021 occurred at the experimental irrigated plot of FSBSI - Federal Scientific Rice Centre, Krasnodar region, Russian Federation. Rice genotypes studied with different grain colors and shapes came from 18 countries.

The weather conditions for growing rice genotypes have characteristics of the values of the average 10-day air temperature in April–September 2022 (Figure 1). In August, during the grain filling period, the average air temperature in the first 10 days was 27.1 °C, in 20 days - 26.7 °C, and at 30 days - 27.6 °C, which was lower by 1 °C, higher by 2.8 °C, and 1 .5 °C, respectively, than the long-term average.

Experimental material (BC_2) growth in artificial climate chambers (ACC) of the FSBSI - Federal Scientific Rice Centre had the following conditions: temperature – 28 °C to 30 °C during the day (12 h), 24 °C at night (12 h), illumination – 30,000 lux, and humidity - 70%. The volume of vessels is 12 liters, with 10 plants in each pot, and two to four containers for each genotype.

Research methodology

In the breeding program to obtain backcrosses and segregate populations, hybridization started in artificial climate chambers (ACC) of the FSBSI - Federal Scientific Rice Centre, under controlled conditions of temperature and humidity. The entire process of hybridization continued by the Twell method (Figure 2) (Chukhir *et al*., 2019).

Phenotyping of parental genotypes, promising rice samples, and BC_2 samples for grain quality traits ran on certified equipment according to GOSTs and from instructions for scientific instruments. Determination of the 1000-grain weight followed the GOST 10842-89 "Grains of cereals and legumes and oilseeds," using the ELVIZ-2 moisture analyzer, the ASESh-8-2 air-thermal measuring unit, the SLY-C automatic seed counter, and electronic

Figure 2. Panicle castration and pollination with Twell method.

laboratory scales Cas CUW-420H. Filminess detection relied on the GOST 10843-76 (on a peeling-grinding unit). Determination of rice grain vitreosity and fracturing emerged as per GOST 10987-76 - in transmitted light using the transilluminoscopes DSZ-3 and DSZ-2M. Grain shape and its linear dimensions' recognition ran on a scanner (image analysis system LA 2400, WinFOLIA) using the computer program Seedling, Canada.

Statistical analysis

The statistical data processing and analysis employed the Microsoft Excel program.

RESULTS AND DISCUSSION

Rice parental genotypes for the hybridization and backcrossing program came from the USI - Collection of Genetic Resources of Rice, Vegetable and Melon Crops, Federal Scientific Rice Centre, Krasnodar, Russian Federation. The BC_2 populations' phenotyping continued for grain quality traits (grain vitreosity and fracturing, size, and shape), using high-tech research methods. Colored rice, being rich with anthocyanins and phenolic compounds, is also vital in human nutrition due to its antioxidant properties (Lao *et al*., 2018; Prasad *et al*., 2019).

Rice cultivars with desirable grain quality traits became parental genotypes, which also differed for technological quality traits (Tables 1 and 2). According to the grain shape, all rice cultivars' assignment depended on the shape, such as a group with oval shape (Rubin, Veles, Alliance, Red Blastonik, and Dihaploid Heibar), elongated (Mavr, Kurazh, Gagat, and Dig. lo-2327-10), and long grain (Svetlana and Khaw-sri-nin). The 1000-grain weight ranged from 23.0 to 27.4 g. For duration, contrasting the rice samples also included early ripening (95 days) to late ripening (145 days). According to the color of the pericarp, the rice parental genotypes comprised six red, four white, and two purple-colored cultivars. Breeding mostly focus on the white and colored-grain rice cultivars and with high antioxidant contents (Tumanyan *et al*., 2016; Garkusha *et al*., 2022).

The rice cultivars' filminess ranged from 17.8% to 19.9%. The rice cultivars, i.e., Rubin, Alliance, Kurazh, Gagat, and VNIIR10163, have low grain fracturing (up to 12%). However, the highest grain vitreosity (more than 90%) was evident in Veles, Svetlana, VNIIR10163, and Khaw-sri-nin. The total milling yield varied from 65.0% (cultivar Dihaploid Heibar) to 70.4% (cultivar Alliance). Hybridization continued to obtain the 15 cross combinations of rice samples contrasting for the studied grain quality traits (Figure 1) to develop mapping populations, generating the F_1, F_2, and F_3 populations from these cross combinations. Backcrossing ensued for getting BC_2 and BC_3 populations. The obtained BC_1 and BC_2 populations are available in Table 3. The evaluation results of BC_2 samples for grain

Table 1. Technological grain quality traits of rice parental forms and yield, 2022 (artificial climate chamber).

Variety	Country of origin	Grain shape	Color of pericarp	Duration, days
Rubin	Russia	Oval	red	115
Veles	Russia	Oval	white	123
Alliance	Russia	Oval	white	118
Mavr	Russia	Prolonged	purple	118
Svetlana	Russia	Long	white	112
Kurazh	Russia	Prolonged	white	118
Gagat	Russia	Prolonged	purple	123
Red Blastonik	Russia	Oval	red	115
Dihaploid Heibar	Russia	Oval	red	105
Dig. lo-2327-10	Russia	Prolonged	red	100
VNIIR 10163	Russia	Oval	red	95
Khaw-sri-nin	Thailand	Long	red	128

Table 2. Technological grain quality traits of rice parental forms and yield, 2022 (artificial climate chamber).

Cultivars	1000-grain weight (g)	Filminess, (%)	Vitreosity, (%)	Fracturing, (%)	Total milling yield (%)	l/b
Rubin	25.0	18.1	82	11	68.2	2.2
Veles	26.8	17.8	91	21	69.3	2.5
Alliance	26.3	17.9	88	7	70.4	2.6
Mavr	24.1	18.7	76	24	68.2	2.9
Svetlana	23.0	18.7	92	17	67.9	3.7
Kurazh	23.9	18.5	89	9	68.5	3.0
Gagat	26.4	19.9	75	3	65.2	3.4
Red Blastonik	26.3	19.2	87	21	69.2	2.7
Dihaploid Heibar	23.8	19.1	89	18	65.0	2.8
Dihaploid k.2327	26.5	18.7	90	17	68.3	3.0
VNIIR 101	27.4	17.9	92	12	68.8	2.6
Khaw-sri-nin	26.5	18.4	93	13	68.2	2.7

Table 3. Obtaining hybrid generations (artificial climate chamber).

BC_1 populations	Number of caryopses (#)	BC_2 populations	Number of caryopses (#)
Rubin/Veles	18	Rubin/Veles//Veles	23
Rubin/Alliance	70	Rubin/Alliance//Alliance	21
Mavr/Svetlana	120	Mavr/Svetlana//Svetlana	32
Mavr/Kurazh	108	Mavr/Kurazh//Kurazh	25
Gagat/Svetlana	20	Gagat/Svetlana//Svetlana	8
Red-Blastonik/Veles	80	Red-Blastonik/Veles//Veles	20
Red-Blastonik/Alliance	120	Red-Blastonik/Alliance//Alliance	34
Dih. Heibar/Veles	70	Dih. Heibar/Veles//Veles	79
Dih. Heibar/Alliance	70	Dih. Heibar/Alliance//Alliance	32
Dih. 2327/Veles	152	Dih. 2327/Veles//Veles	43
Dih. 2327/Alliance	67	Dih. 2327/Alliance//Alliance	30
VNIIR10163/Veles	83	VNIIR 10163/Veles//Veles	21
VNIIR 10163/Alliance	108	VNIIR 10163/Alliance//Alliance	41
Khaw-sri-nin/Svetlana	1	Khaw-sri-nin/Svetlana//Svetlana	5
Khaw-srinin /Kurazh	4	Khaw-sri-nin /Kurazh//Kurazh	3

Table 4. Grain quality traits of BC_2 samples: Grain size, form, and filminess.

No.	Sample, BC_2	1000-grain weight (g)	Grain form, l/b Range (%)	Number (#)	Filminess (%) Range (%)	Filminess (%) samples (#)
1	Rubin/Veles//Veles	18.9-20.3	2.5-2.9	12	18.2-18.6	8
		22.4-25.1	2.5	8	21.0-23.2	12
2	Rubin/Alliance//Alliance	22.3-23.7	2.7-2.9	3	18.6-18.8	3
		20.6-21.4	3.0-3.2	2	17.5-17.9	2
3	Mavr/Svetlana//Svetlana	21.5-21.9	3.0-3.1	35	17.0-17.3	35
		25.5-26.3	3.5-3.6	15	17.0-17.3	15
4	Mavr/Kurazh//Kurazh	22.4-26.2	2.7-2.9	10	17.1-17.5	10
		26.7-27.9	3.1-3.4	8	17.2-17.4	8
		21.0-21.2	3.4-3.5	13	17.0-17.4	13
5	Gagat/Svetlana//Svetlana	20.6-23.0	3.0-3.2	10	19.0-19.2	10
		20.6-23.0	3.4-3.8	18	19.1-19.3	18
6	Red-Blastonik/Veles//Veles	26.2-26.3	2.2-2.5	1	19.0-19.7	1
		22.6-26.7	2.6-2.7	5	19.1-19.2	5
7	Red-Blastonik/Alliance//Alliance	20.8-24.0	2.4-2.6	17	18.2-18.5	17
		21.0-24.0	2.7-2.9	20	18.2-18.6	20
8	Dih. Heibar/Veles//Veles	21.9-25.3	2.4-2.6	5	17.5-17.9	5
		22.1-25.1	2.7-2.8	2	17.4-17.8	2
9	Dih. Heibar/Alliance//Alliance	19.2-22.3	2.5-2.8	18	18.1-18.3	18
		18.7-22.8	2.9-3.2	3	18.2-18.4	3
10	Dih. 2327/Veles//Veles	21.7-25.5	2.5-3.0	61	18.5-19.0	46
		20.5-25.6	3.1-3.6	21	19.0-19.3	36
11	Dih. 2327/Alliance//Alliance	21.0-23.7	2.7-3.2	2	20.0-21.0	5
		21.9-25.1	3.3-3.5	5	20.0-21.0	2
12	VNIIR 10163/Veles//Veles	26.2-28.8	2.5-2.7	2	16.8-17.0	2
13	VNIIR 10163/Alliance//Alliance	21.4-26.8	2.4-2.7	18	19.2-19.6	18
		22.3-25.3	2.8-3.2	21	19.4-19.7	21
14	Khaw-sri-nin/Svetlana//Svetlana	26.3-29.4	2.7-3.0	3	19.5-19.7	3
15	Khaw-sri-nin /Kurazh//Kurazh	25.8-28.3	2.6-3.0	2	18.2-18.8	2
	Total			340		340

technological and quality traits appear in Tables 4 and 5. Rice resistance to Magnaporthe oryzae attained assessment, introducing the *Pigm* gene in rice cultivars using backcrossing (Feng *et al*., 2022).

In BC_2 populations, long-grain rice genotypes' identification with a 1000-grain weight of 20.6 to 21.4 g emerged for Ruby/Alliance//Alliance, 21.5–21.9 and 25.5–26.3 g for Mavr/Svetlana//Svetlana, 26.7–27.9 and 21.0–21.2 g - Mavr/Kurazh//Kurazh, 20.6-23.0 g - Gagat/Svetlana//Svetlana, 18.7-–22.8 g - Dig. Heibar/Alliance//Alliance, 20.5–25.6 g - Dig. 2327/Veles//Veles, 21.9–25.1 g - Dig. 2327/Alliance//Alliance, 26.2–29.1 g - Khaw-sri-nin/Svetlana//Svetlana, and 26.1–28.4 g Khaw-sri-nin/Kurazh//Kurazh (Table 2). The grain filminess of these rice genotypes ranged from 17.0% to 17.9% in Rubin/Alliance//Alliance, Mavr/Svetlana//Svetlana, and Mavr/Kurazh//Kurazh; 18.2%–18.4% in Dig. Heibar/Alliance//Alliance; 18.3%–19.0% in Khaw-sri-nin/Kurazh//Kurazh; and 19.0%–19.9% in Gagat/Svetlana//Svetlana, Dig. 2327/Veles//Veles, Dig. 2327/Alliance//Alliance, and Khaw-sri-nin/Svetlana//Svetlana. Medium-rice grain genotypes in the BC_2 populations had medium-grain sizes (18.9 to 28.8 g). Low grain fracturing (up to 12%) was prominent in the populations Rubin/Veles//Veles, Mavr/Svetlana//Svetlana, Gagat/Svetlana//Svetlana, Red-Blastonik/Alliance//Alliance, Dig. Heibar/Alliance//Alliance, Dig. 2327/Veles// Veles, Dig. 2327/Alliance//Alliance, and VNIIR 10163/Alliance//Alliance (Table 3). The grain vitreosity of these rice genotypes ranged from 48% to 92%.

Table 5. Grain quality traits of BC_2 samples: Vitreosity, fracturing.

No.	Sample, BC_2	Vitreosity, Range (%)	Fracturing, Range (%)	Samples (#)
1	Rubin/Veles//Veles	80-90	3-5	5
		48-53	5-10	6
		48-53	13-22	9
2	Rubin/Alliance//Alliance	70-80	12-15	5
3	Mavr/Svetlana//Svetlana	48-60	1-7	50
4	Mavr/Kurazh//Kurazh	60-68	22-28	31
5	Gagat/Svetlana//Svetlana	62-68	0-3	5
		62-68	4-10	23
6	Red-Blastonik/Veles//Veles	50-63	8-15	6
7	Red-Blastonik/Alliance//Alliance	75-84	5-8	8
		85-91	10-15	29
8	Dih. Heibar/Veles//Veles	78-84	13-18	7
9	Dih. Heibar/Alliance//Alliance	63-70	2-10	21
10	Dih. 2327/Veles//Veles	57-60	38-41	33
		57-60	2-8	41
		62-71	2-8	8
11	Dih. 2327/Alliance//Alliance	89-92	2-6	7
12	VNIIR10163/Veles//Veles	76-82	12-14	2
13	VNIIR10163/Alliance//Alliance	62-65	8-10	26
		62-65	0-2	13
14	Khaw- sri-nin /Svetlana//Svetlana	72-81	17-20	3
15	Khaw- sri-nin /Kurazh//Kurazh	69-79	17-19	2
	Total			340

Accelerating the breeding process used marker-assisted backcrossing in the segregating populations, with the best rice genotypes selected according to the technological grain traits. The priority indicator for sampling was the trait of grain fracturing (Table 4). The BC_2 samples Gagat/Svetlana//Svetlana, Dig.2327/Veles// Veles, and Dig.2327/Alliance//Alliance manifested minimum fracturing. The value of this trait in sample 5.1.2 was 0%; in samples 5.1.4 and 10.3.8, the said value was 3%; and in sample 11.3.9, the value was 2%. The following rice samples characteristic of the highest 1000-grain weight were Mavr/Svetlana//Svetlana (3.1.9 to 26.6 g), Mavr/Kurazh//Kurazh.1.8 (27.4 g), 4.1.12 (27.9 g), and Dig .2327/Veles//Veles 10.6.1 (26.5 g). The least filminess (17.4%) resulted in the Red-Blastonik/Veles//Veles sample 6.2.4 population, with the highest (22.2%) in the Mavr/Svetlana//Svetlana sample 3.1.12 population.

Grain vitreosity for all the rice combinations ranged from 50% to 90%. Out of these 26 best samples, 16 genotypes gained assignment with the group of medium grains, their l/b ranged from 2.5 (populations Rubin/Veles//Veles sample 1.1.2, Red-Blastonik/Alns//Alliance sample 7.1.6, Dig. Heibar/Veles//Veles sample 7.1.6, and Dig. Heibar/Veles//Veles sample 8.3.12) to 3.0 (populations Mavr/Svetlana//Svetlana sample 3.3.3, Mavr/Kurazh//Kurazh sample 4.1.8, Gagat/Svetlana/ /Svetlana sample 5.1.4, Digit Heibar/Alliance//Alliance sample 9.1.14, and Digit 2327/Veles//Veles samples 10.4.8 and 10.6.1). Eight samples, viz., populations Mavr/Svetlana//Svetlana samples 3.1.12 (l/b 3.5) and 3.1.9 (l/b 3.6), Mavr/Kurazh//Kurazh sample 4.1.12 and Dig. 2327/Veles//Veles samples 10.5.2 and 10.6.2 (l/b 3.1), Gagat/Svetlana//Svetlana sample 5.1.2 (l/b 3.8), Khaw-sri-nin/Svetlana//Svetlana sample 14.1.7 (l/b 3.2), and Khaw-sri-nin /Kurazh//Kurazh (l/b 3.3) attained the long-grain group assignment (Table 6).

Table 6. Technological quality traits of best samples.

No.	BC_2	Sample	1000-grain weight (g)	Filminess, (%)	Vitreosity, (%)	Fracturing, (%)	Pericarp color	l/b
1	Rubin/Veles//Veles	1.1.2	25.1	20.1	65	10	red	2,5
		1.2.1	20.3	18.4	53	5	red	2,9
2	Mavr/Svetlana//Svetlana	3.1.9	26.6	21.2	55	8	purp	3,6
		3.1.12	24.7	22.2	50	5	purp	3,5
		3.3.3	26.3	21.5	57	11	purp	3,0
3	Mavr/Kurazh//Kurazh	4.1.8	27.4	17.0	63	25	red	3,0
		4.1.12	27.9	17.8	65	27	red	3,1
4	Gagat/Svetlana//Svetlana	5.1.2	22.8	20.0	64	0	purp	3,8
		5.1.4	23.0	19.8	65	3	purp	3,0
5	Red-Blastonik/Veles//Veles	6.2.4	21.4	17.4	50	11	red	2,6
6	Red-Blastonik/Alliance// Alliance	7.1.6	20.8	18.0	78	6	red	2,5
		7.4.12	24.4	18.2	80	12	red	2,7
7	Dih. Heibar/Veles//Veles	8.2.7	24.5	17.8	83	15	red	2,8
		8.3.12	25.3	18.1	81	16	red	2,5
8		9.1.14	22.0	18.5	70	8	red	3,0
9	Dih. Heibar/Alliance// Alliance Альянс	9.1.15	21.7	18.2	68	5	red	2,9
10	Dih. 2327/Veles//Veles	10.3.1	25.5	19.1	65	10	red	2,8
		10.3.8	24.6	19.0	57	3	red	2,8
		10.4.8	24.9	18.9	65	12	red	3,0
		10.5.2	23.7	19.0	64	5	red	3,1
		10.6.1	26.5	1.,9	72	18	red	3,0
		10.6.2	25.6	18.9	70	15	red	3,1
11	Dih. 2327/Alliance// Alliance	11.3.9	21.0	20.0	90	2	red	2,7
12	VNIIR 10163/Alliance// Alliance Альянс	13.3.7	25.3	18.6	87	12	red	2,9
13	Khaw- sri-nin/ Svetlana//Svetlana	14.1.7	21.5	18.3	90	8	purp	3,2
14	Khaw-sri-nin/ Kurazh//Kurazh	15.1.3	19.8	18.6	89	10	purp	3,3

The results about the grain size of rice parental genotypes and BC_2 populations with a heterotic effect appear in Figure 3. The 1000-grain weight in most samples was lower than the parental genotypes and revealed negative heterotic effects. However, heterosis for 1000-grain weight was apparent in the BC_2 populations Mavr/Svetlana//Svetlana (samples 3.1.9, 3.1.12, and 3.3.3) and Mavr/Kurazh//Kurazh (samples 4.1.8 and 4.1.12). The population of Dig. Heibar/Veles//Veles has the rice samples 8.3.12 and 8.2.7 showing heterosis with one of the parental forms. Marker-assisted breeding has identified rare alleles in diverse rice cultivars conferring superior ECQ traits (Li *et al*., 2020; Sreenivasulu *et al*., 2022).

The rice parental genotypes and their BC_2 populations with heterotic effects for grain fracturing are available in Figure 4. The heterotic effects of grain fracturing were evident in all the rice BC_2 populations except for the Mavr/Kurazh//Kurazh population. In the BC_2 populations of VNIIR 10163/Alliance/Alliance (13.3.7) and Khaw-sri-nin /Kurazh//Kurazh (15.1.3), the heterosis related to one of the parental forms. Thus, germplasm screening is a tool for fast-forming pre-breeding rice resources in the direction of breeding for grain quality (Tumanyan *et al*., 2016; 2022; Mulyaningsih *et al*., 2023). The acceleration of the breeding scheme was possible using artificial climate chambers.

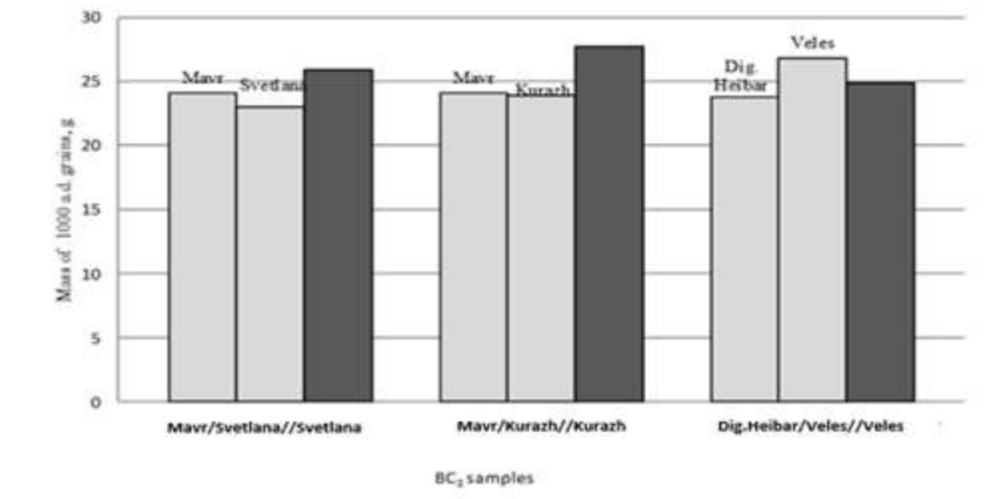

Figure 3. Grain size of parental forms and BC_2 with heterosis effect.

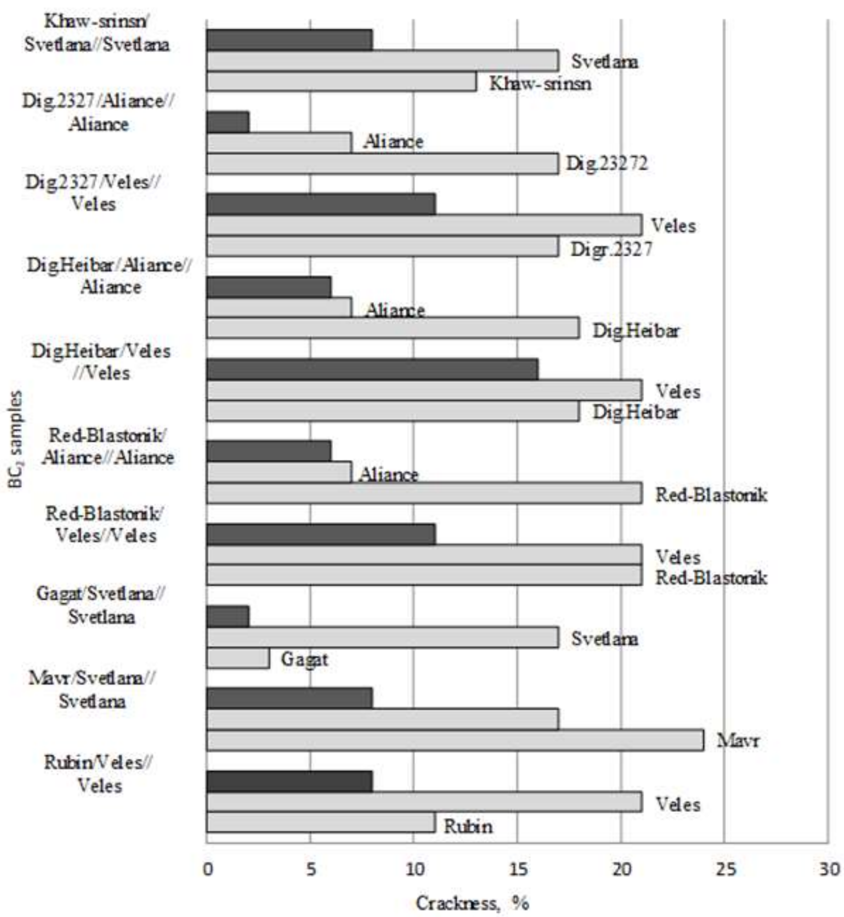

Figure 4. Fracturing of parental forms and BC_2 with heterosis effect.

CONCLUSIONS

In accelerated breeding, selecting 12 rice cultivars with high-quality traits served as parental forms. Obtaining the second-generation backcrosses helped study the grain quality traits of the samples with the colored pericarp of the segregating population. Study results on the quality of backcrosses revealed the heterotic effects for most of the rice grain-quality traits. In most samples, the grain size was smaller than the parental forms. However, isolating several samples found bigger by 1000-grain weight in the populations was possible. The BC_2 samples also differed in grain fracturing values. Samples of BC_2 population, i.e., Gagat/Svetlana//Svetlana, Dig.2327/ Veles//Veles, and Dig.2327/Alliance//Alliance were distinct with minimum cracks with an index of 0%–3%. Many rice samples with the highest 1000-grain weight and heterosis were characteristics of Mavr/Svetlana//Svetlana – 26.6 g, Mavr/Kurazh//Kurazh - 27.4 – 27.9 g, and Dig. 2327/Veles//Veles – 26.5 g. The smallest filminess (17.4%) was visible in the Red Blastonic/Veles//Veles population. Samples with lower fracturing and higher grain size will proceed in further breeding work to maintain the best indicators of the grain quality traits in new rice cultivars.

ACKNOWLEDGMENTS

The work received a support grant from the Russian Science Foundation, KNF No. 22-16-20015, "Innovative breeding technology for developing rice cultivars with high nutritional quality of grain."

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