

Research Article

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**The Characteristics of the Russian Varieties of Rice
by the Content of Amylose in Starch**

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ABSTRACT.

The aim of the present study was to evaluate the amylose content in rice, and characteristics of water absorbance of the Russian rice varieties, and to study the variability of the features in new rice varieties. Weather and climate conditions were registered by the sum of effective temperatures in June-September 2014-2017. The materials of the study were rice varieties bred by the All-Russian Rice Research Institute: short and medium size kernels varieties Rapan, Flagman, Kumir, Sonet, Sonata, Privolnyi 4, Rubin, Mars, Ryzhik, Mavr and Gagat that were cultivated on the trial station of ARRI in Krasnodarskiy krai in 2016-2017. The amylose content was identified by the interstate standard GOST 6647-2-2015. The rice was fine milled into the flour that was sieved through the screens with 150-180 μm slots (100-80 mesh). The samples were dispersed in the Potassium hydroxide solution. Iodine solution was added and UV-spectrophotometer at the wavelength of 620 nm was used to identify the optical density of the color complex. The water uptake ratio was identified after the rice boiling in ARK 1 as a volume ratio. There was no influence of weather conditions during the growing year on the studied varieties revealed. The lowest amylose content during the four years (mean value) in low-amylose varieties was observed in Sonata variety (17.3%) and in medium-amylose varieties – in Gagat variety (20.3%).

The limits of the variability of the amylose content in starch were identified for each of the studied varieties. There were differences observed in the water uptake ratios for the rice varieties with similar amylose content. The most stable by amylose content were Sonet, Sonata and, Ryzhik varieties, by the water uptake ratio – Rapan, Flagman, Mavr, Ryzhik, Gagat (CV < 2.0%). The most stable variety by all the features was Rubin. The study of the most important feature “amylose content” and its variability over years allows the authors to predict the culinary value of rice in new varieties. The implementation of the multi-year studies results on this feature in the form of a database “Quality of Rice” in the process of breeding will allow the researchers to optimize the breeding of rice varieties with a high quality of grain suitable for different culinary purposes.

Key words: rice, breeding, features of rice quality, amylose, water uptake ratio, database.

INTRODUCTION:

Rice is a traditional food product in many countries. The breeders in rice-producing countries are constantly breeding new rice varieties with high quality features for the production of the competing rice products for traditional and dietary nutrition and intended for the use of various dishes [20]. The researchers from the Russian breeding center of ARRI primarily focus on the breeding of varieties with various consumer benefits and technological features of quality, including the varieties with short, medium and long kernels, hard kernels with a low and high content of amylose in starch, and with high antioxidants content (color varieties). High technological quality features (low capacity of grain for cracks formation, high grit yield, and content of the wholesome cores in grit), as a rule, they are combined with high consumer benefits [6, 8, 11].

In different countries, the main criterion of food rice varieties is the amylose content in starch. In Philippines, India, Bangladesh, and Iran, high-amylose varieties are more popular; in Japan, Korea, and Egypt – glutinous low-amylose varieties; in China, Malaysia, Myanma, and Nepal - low, medium and high-amylose varieties [20]. The quality feature of grain “amylose content in starch” is the main feature of the variety that determines the culinary benefits, which depends on the genotype and agroclimatic conditions during the vegetation period. Starch is the main dietary non-allergenic source of carbohydrates due to the bound hypoallergenic protein and the most widespread deposited polysaccharide in plants. Amylose and amylopectin are two macromolecule components of the starch granules [10].

Amylose influences on the physicochemical features of starchy parenchyma of the grain. The structure of endosperm is determined by the ratio of long (DP 92-98) and short (DP 25) amylopectin chains, but not medium (DP 43-68) [21]. The reserve starch is synthesized and deposited in amyloplasts that form starch granules (grains). Their size depends on the ratio of amylose and amylopectin. The average length of starch granules (5.9 and 5.5 μm), the range of lengths (4.4 – 8.8 and 3.3 – 8.25 μm), average width (5.7 and 5.2 μm) and its range (4.4 – 8.25 and 3.85 – 8.8 μm) were identified for Jelum and Kokhsar varieties, respectively. The change of morphology of the starch granules can be associated with the changes in the amylose and amylopectin content and its structures that, in their turn, play an important role in the control of the size and form of the starch granules [18]. Pregelatinization of starch grains primarily depends on the content of amylopectin. Amylose acts as an inhibitor of pregelatinization, especially in the presence of lipids. It was revealed that the free associations of the amylose and amylopectin molecules in the native starch granules were responsible for a high water absorbance capacity [10]. Water uptake coefficients and starch water solubility are determined by the interaction of the molecules of water and starch chains in the crystal and amorphous areas and are associated with a different content of amylose and the viscosity structures formed by the negatively charged phosphate groups in the granules of the rice starch [15]. The consistency of boiled rice (stickiness, mealiness) depends not only on the amylose content. Varieties of rice that have

similar content of amylose can have different consistency of the boiled kernels, which can result from the different structure of amylopectins or presence of bigger size of amorphous areas in the starch granules [21]. Dispersing of crystals in starch grains and interaction with long chains are observed during rice boiling, which provides the difference in the starch consistency [13].

Amylose influences the syneresis of starch, which is the main property after the freezing and thawing. Syneresis can differ greatly between the varieties. During the storage, this property enhances, which is associated with the interaction between the leached amylose and amylopectins chains, development of functional zones and retrogradation of starch during its reduction in an ordered structure. The results of the qualitative study of the exploded rice showed a significant positive correlation between the content of protein and amylose, coefficient of elongation and volume expansion [14]. The activity of enzymes that take part in the biosynthesis of starch can also be associated with the amylose content among different starches [10, 16].

The studies on rats showed that amylose in diets improves the digestion during in animals with diarrhea: antidiarrheal effects were better when the food had higher content of amylose [4]. The diet with high content of amylose led to low glycemic index (GI). The viscosity, the identified RVA (during cooling down), can be a useful indicator of the GI in the boiled brown rice [13]. Rice consumption with high content of amylose and resistant starch can reduce the glucose content in blood and the reaction of insulin in comparison with the traditional short kernel low-amylose rice [11]. The evaluation of hypoglycemic properties of 10 Indonesian varieties of rice Pandan Vangi, Royolele, Bengvan Solo, Kenana Bali, Amamo, Tselebes, Chiergan, Batang Pianma, Chisokan and Luci showed that the best (lowest) hypoglycemic activity was observed in Chisokan variety with high amylose content (27.6%) and the lowest digestibility of starch (52.2%) which was ideal dietary food for people with diabetes [13].

The studies on the genetic grounds for the amylose content in rice starch continue. Eight models of regression equations were developed. They contain the data on 15-19 markers with high coefficients ($R^2 = 0.98-0.99$) that correlated with the physicochemical properties of Indonesian rice [9]. Fifty six markers of polymorphism that involved eleven chromosomes of rice were registered with the average number of 3.02 alleles per locus during the evaluation of genetic variants in rice mutants by means of SSR markers associated with the amylose content [24]. Dispersion of amylose content equal to 59.3% was assigned to the gene *Wx* identified by the marker RM190, while 56.1% and 24.6% dispersion in the amylose content and the consistency of gel corresponded with the polymorphism of the *Wx* gene identified by the 484 / W2R- ACC I. Besides, the presence of markers SSR and CAPS can explain 72.4% of dispersion in the amylose content [25].

The specialists from International Center for Tropical Agriculture (CIAT) and the project RiceCAP conduct the genome sequencing of the elite germ plasm which is widely used in rice cultivation in Latin America and the USA. Based on the fact that general markers, developed on the basis of the changes in the global gem plasma, can become ineffective and considering the importance of high content of amylose in the determination of grain quality (for Latin America and USA), the researchers studied the gene *GBSSI* that is associated with the amylose content. Apart from the known earlier three markers SNP, four new markers (*Waxy-4*, *Waxy-5*, *Waxy-6*, *Waxy-7*) were developed in this gene by means of genotyping of the population. It was shown that these markers could be used for tracking the alleles that provide high amylose content in rice [17]. In Russia, new varieties of rice were bred with different culinary beneficial properties determined by the content of amylose in starch. The study on the amylose content is conducted already at this stage of the breeding process of low, medium and high-amylose varieties. However, the issues of the correlation between the parameters of culinary properties of the

varieties, including the water absorbance, and their variability, which reduces the effectiveness of the targeted breeding process, are still not solved.

The aim of the present work is to evaluate the quality properties of Russian rice varieties: content of starch amylose, water uptake ratio and their variability when cultivated in agroclimatic conditions of Krasnodarskiy Krai in 2014-2017.

MATERIALS AND METHODS.

The material of the study was short and medium kernel size rice varieties bred in ARRI: Rapan, Flagman, Kumir, Sonet, Sonata, Privolnyi 4, Rubin, Mars, Ryzhik, Mavr and Gagat cultivated in the trial plot of ARRI in Krasnodarskiy Krai in 2016-2017. The trials were set in quadruple replication, the variants location was randomized, the area of each trial plot was 20 m². The varieties Rapan and Flagman were taken as the standard. The varieties were cultivated on black, slightly alkalized, heavy loamy, suitable for rice soil. The plough layer contained 2.9% of humus (according to Tyurin I.V.), 4.3 mg/100g [1] of easily hydrolyzed nitrogen, 0.32% of nitrogen, 4.1 mg/100g of mobile phosphorus and mobile potassium 21.9 mg/100g (according to Kirsanov A.T.). The soil pH was 7. The crop was drilled in rows, the seed rate was 7 mln of viable kernels per hectare. NPK ratio was 120:50:30. The agrotechnical procedures during the trials agreed with the recommendations of ARRI. The irrigation type was shortened flooding [9].

Rice kernels were husked by the husking machine "Satake" (Japan), polished by the polisher LUR 1M. The content of amylose was identified by the GOST 6647-1-2015 [1]. The rice was fine milled and sieved through the screens with the 150-180 µm slots (100-80 mesh). The samples were dispersed in the Potassium hydroxide solution. Iodine solution was added and UV-spectrophotometer at the wavelength of 620 nm was used to identify the optical density of the color complex. Weight fraction of the amylose in the sample was identified by the calibration curve that was drawn using the probes of rice with the known weight fraction of amylose (standard probes). The water uptake ratio was identified after the rice boiling in ARK 1 as a volume ratio. Weather-climatic conditions were evaluated by the sum of effective temperatures in June-September 2014-2017 (Table 1).

Mathematical and statistical processing of the data was performed according to the methods of Dospekhov using the software package Microsoft Excel [3].

RESULTS AND DISCUSSION.

The conditions of the vegetation for rice in 2017 and 2016 were worse than in 2015. At the end of the 1st-3rd decade of August, the sum of effective temperatures was significantly higher, in the 3rd decade of August (end of the vegetation period), the sum of effective air temperatures at the level of perennial (1363 °C) was only in 2015, in 2014, 2015-2017, it was significantly higher (Table 1).

Table 1. The sum of effective (higher than 10°C) air temperatures in April-September 2013-2016, °C

Month	Decade	Year				Average perennial sum of effective air temperatures, °C
		2014	2015	2016	2017	
June	I	484	360	404	513	345
	II	593	489	530	620	449
	III	709	610	691	725	562
July	I	846	758	831	820	687
	II	1009	875	990	950	819
	III	1187	1051	1144	1120	971
August	I	1363	1232	1324	1301	1108
	II	1537	1299	1484	1470	1235
	III	1690	1363	1677	1625	1363
September	I	1844	1500	1807	1740	1456
	II	1934	1602	1904	1845	1530
	III	1983	1738	1942	1916	1586

In Table 2, the results of the study on the content of amylose and water uptake ratio are presented (eleven rice varieties bred in ARRI and cultivated in Krasnodarskiy Krai in 2014-2017).

All the rice varieties in the study were low (Rapan, Flagman, Sonet, Sonata, Mars, Ryzhik, Privolnyi 4) and medium-amylose (Kumir, Rubin, Mavr, Gagat). The content of amylose in them ranged from 17.3-18.9% and 20.3-22.4%, respectively. The varieties Rubin, Mark, Ryzhik, Mavr and Gagat had color pericarp of kernels, Rubin, Mark, Ryzhik – red pericarp, Mavr and Gagat had black pericarp (Table 2).

Table 2. The content of amylose and water uptake ratio in the rice varieties bred in the ARRI, yield 2014-2017

Year		Variety	Amylose content, %	Water uptake ratio
2014		Rapan	16.9	4.82
		Flagman	18.7	4.80
		Kumir	22.4	4.81
		Sonet	19.2	4.99
		Privolnyi 4	20.5	4.99
		Sonata	17.5	5.31
		Rubin	20.9	4.16
		Mars	18.0	4.80
		Ryzgik	18.8	4.76
		Mavr	22.7	4.92
		Gagat	22.2	4.90
2015		Rapan	17.5	4.71
		Flagman	18.3	4.78
		Kumir	22.0	4.75
		Sonet	19.0	4.82
		Privolnyi 4	18.0	4.83
		Sonata	17.5	5.01
		Rubin	22.2	5.11
		Mars	18.0	4.70
		Ryzgik	19.3	4.68
		Mavr	22.2	4.84
		Gagat	19.5	4.84
2016		Rapan	17.9	4.89
		Flagman	18.0	4.86
		Kumir	23.0	5.01
		Sonet	19.5	5.35
		Privolnyi 4	18.9	5.21
		Sonata	17.0	5.30
		Rubin	20.5	4.76
		Mars	18.5	4.80
		Ryzgik	18.9	4.81
		Mavr	22.0	5.02
		Gagat	19.2	4.99
2017		Rapan	17.8	4.87
		Flagman	17.6	4.90
		Kumir	21.8	5.01
		Sonet	19.8	5.23
		Privolnyi 4	18.6	5.20
		Sonata	17.3	5.30
		Rubin	20.4	4.60
		Mars	18.9	4.90
		Ryzgik	18.5	4.81

	Mavr	21.0	5.00
	Gagat	20.1	5.01
LSD ₀₅		0,13	0.082

Color rice varieties have increased nutritional value due to the high content of antioxidants, including anthocyanins. These varieties have increased demand on the market and, presently, they are cultivated in the Krasnodarskiy Krai. Physicochemical characteristics of color rice varieties, that are not traditional for Russia and are on demand by the people with healthy eating habits, are understudied. Their major part, bred in Russia, was classified as medium-amylose, while the majority of white kernel varieties bred in Russia are low-amylose.

Each variety has the limits of variation of the content of starch amylose. Over the four years, the content of amylose in the Rapan varied from 16.9% in 2014 to 17.9% in 2016; in the Flagman variety - from 17.6 in 2017 to 18.0 % in 2016; in the Sonet variety – from 19.0% in 2015 to 19.8 % in 2017; in the Privolnyi 4 variety – from 18.0 in 2015 to 20.5 % in 2014; in the Sonata variety - from 17.0 in 2016 to 17.5% in 2014 and 2015; in red kernel varieties: Mars variety – 18.0 in 2014 and 2015 to 18.9% in 2017, Ryzhik variety – from 18.5 in 2017 to 18.9% in 2016. In medium-amylose varieties, the amylose content varied in the Kumir variety from 21.8% in 2017 to 23.0% in 2016; in red kernel variety Rubin – from 20.4 in 2017 to 22.0 in 2015; in black kernel variety Mavr – from 21.0% in 2017 to 22.7% in 2014; variety Gagat – from 19.2% in 2016 to 22.2% in 2014 and 2015. The intensive accumulation of amylose and maximum values were observed in 2014 in the Privolnyi 4, Sonata, Mavr and Gagat varieties, in 2016 – in Rapan, Flagman, Kumir, Ryzhik, Rubin varieties, in 2016 – in Rapan, Flagman, Kumir, Ryzhik, Rubin, in 2017 – in Sonet, Mars varieties.

The influence of the year conditions on the trial results in the test group was not observed. The lowest content of amylose over the four years (average value) in low-amylose varieties was identified in Sonata variety (17.3%), in medium-amylose – in Gagat variety (20.3%). The

majority of color varieties will provide mealiness in terms of consistency when boiled.

Water uptake ratio in medium-amylose varieties was higher than in low-amylose, except for Sonata (5.01-5.30) and Kumir (4.75) in 2015; Privolnyi 4 and Sonet (5.21, 5.35, respectively) in 2016; Privolnyi 4 and Sonet (5.20, 5.23, respectively) in 2017. The difference in the water uptake ratio between the varieties with similar content of amylose can result from the prevalence of amylopectin with different length of the chains and different ratio of amorphous and crystal parts of the starch parenchyma, presence of phosphates in the starch grains, lipids, nitrogenous matters that mask or inflate the amylose content.

Based on the fact that the variation of the feature is weak, if the variation coefficient does not exceed 33.3%, the variations of the amylose content and water uptake ratio are considered to be weak (Table 3). The coefficient of variability of amylose content was higher in Mavr (3.25%), Rubin (3.95%), Privolnyi 4 (5.62%), Gagat (6.68%) than in the majority of the other varieties; the water uptake coefficient was higher in Privolnyi 4 (3.61%), Sonet (4.67%), Rubin (8.46%). The most stable varieties by the feature “amylose content” were Sonet, Sonata, and Ryzhik; by the feature “water uptake ratio” – Rapan, Flagman, Mavr, Ryzhik, and Gagat (CV < 2.0%). The most stable variety by the both features was Rubin.

Table 3. Mean values and variability of the amylose content and water uptake ratio in the rice varieties bred by the ARRI, yield 2014-2017

Variety	Amylose content		Water uptake ratio	
	CV	Mean	CV	Mean
Rapan	2.57	17.5	1.67	4.82
Flagman	2.56	18.2	1.14	4.84
Kumir	2.24	22.4	2.76	4.89
Sonet	1.81	19.4	4.67	5.10
Privolnyi 4	5.62	19.0	3.61	5.06
Sonata	1.36	17.3	2.81	5.23

Rubin	3.95	21.0	8.46	4.67
Mars	2.44	18.6	1.87	4.80
Ryzgik	1.75	18.9	1.29	4.76
Mavr	3.25	22.0	1.67	4.95
Gagat	6.68	20.3	1.61	4.94

Note. CV – variability, Mean – mean value

At the next stage of the study, the authors calculated the Spearman's correlation criterion that allowed them to estimate the statistical significance, intensity and the direction of the correlations between the features "amylose content" and "water uptake ratio" (Table 4).

Table 4. Spearman correlation criterion for the features "amylose content" and "water uptake ratio"

Variety	Spearman coefficient	P	Correlation
Rapan	0.800	P<0.05	high
Flagman	-0.800		high
Kumir	0.150		low
Sonet	0.800		high
Privolnyi 4	0.400		moderate
Sonata	0.100		low
Rubin	0.400		moderate
Mars	0.850		high
Ryzgik	-0.550		visible
Mavr	-0.600		visible
Gagat	0.000		low

R the varieties Flagman, Ryzhik, and Mavr, the correlation was the opposite, unlike for the majority of the studied varieties. It was high in the Rapan, Flagman, Sonet, and Mars varieties and it was low for the Kumir, Sonata, and Gagat varieties. The difference was statistically significant. The significant difference in the correlation between the main features of quality indicated the inconsistency of the cause-effect relations in the expression of these main quality features that characterize the nutritious and culinary benefits of rice.

CONCLUSION.

The results of the present study showed insignificant (variation coefficient < 10%) variability of the features of amylose content and water uptake ratio in the weather conditions in 2014-2017 (CV 1.36%).

New information technologies, like databases, that allow the researchers to optimize the conduction of fundamental and applied studies is widely implemented in the breeding process of the highly productive and competitive varieties of rice [2, 5]. An information resource based on the computer technologies and the catalogue of the fund "Database on the Oryza S.L. rice samples" were created in the ARRI [4, 8]. Along with this, the authors developed and created the database "Rice quality" (the grant №13-04-96550 and №16-07-230000) [6, 7]. One of the main features of rice, that is included into the database, that can be manipulated and altered is "amylose content" during the trials and evaluation of the initial material at the stages of the breeding process. This feature was formulated in the data domain of the infological model of the database "Quality of rice".

The study of the main feature of the quality of rice "amylose content in starch", its variability under different weather conditions allows the researchers to predict culinary benefits of rice in new varieties. The use of the perennial study results on this feature in the form of a database will allow the researchers to optimize the processes of breeding of high-quality rice intended for different culinary purposes.

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