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FEATURES OF RICE (*Oryza sativa* L.) VARIETIES FOR ORGANIC FARMING IN CONNECTION WITH MARKER ASSISTED BREEDING

(review)

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Abstract

Organic agriculture is actively developing worldwide with a 30 % annual increase (S.Y. Dhurai et al., 2014). Today, the market for organic products reaches more than $200 billion a year. Products grown by organic farming technologies cost 20 % and sometimes 100 % higher. However, decrease in crop yields in organic farming largely eliminates the cost advantage (G.N. Fadkin et al., 2015). The use of specialized varieties should increase the profitability of organic farming (V. Seufert et al., 2012). However, there is still no clear separation in generating breeding material for these technologies. Purpose of this work is to review characteristics that must be selected when creating rice varieties for organic farming and effective working methods. Variety for this technology should possess a number of characteristics, i.e. high adaptability to biotic and abiotic stresses, competitiveness of the genotype, efficiency of mineral nutrition and photosynthesis (T. Vanaja et al., 2013). Note, all of these traits are complex and largely interconnected. So, the competitiveness of the genotype is ensured by a number of features, i.e., high growth rate; effective tillering; morphotype with minimal shading in dense crops; high efficiency of photosynthesis for the full use of solar energy; high root absorption (E.T. van Bueren et al., 2011; J.K. Goncharova et al., 2018). Increasing specific adaptability to a complex of stresses requires more effort and does not guarantee a result due to a significant decrease in the effect of individual genes resulted from intralocus and intergenic interactions. In nature, a complex of factors acts on the plant, which depreciates specific adaptability. Specific resistance to pathogens, as a rule, is overcome in a very short time (A.H. Bruggen, 1995). The great promise of increasing the overall adaptability of plant due to non-specific adaptability is shown. The most polymorphic loci of the Russian rice varieties for non-specific adaptability associated with the efficiency of genetic systems providing the growth rate, photosynthesis, mineral nutrition are summarized (L. Huang et al., 2016). Intensive growth, high photosynthetic activity and the effectiveness of mineral nutrition increase the vitality, allow plants to pass stress sensitive phases as quickly as possible, which reduces the likelihood of damage caused by extreme temperatures or other factors that reduce viability, including during organic farming. In Russian rice varieties, Microsatellite markers RM154, RM600, RM550, RM347, RM240, RM154, and RM509 are associated with loci for the efficiency of photosynthesis, RM261, RM6314, RM126, RM463, RM405, RM509, RM242 are associated with loci for mineral nutrition, RM463, RM245, RM242, RM3276, RM5508, RM574, RM542 are associated with salt resistance, and RM261, RM405, RM463, RM242, RM6314 are linked to loci for seedling growth rates. The markers identified by us are located in the same chromosome regions as the genes that determine the germination energy, drought resistance, tolerance to low temperatures, the morphotype and size of the root system, the ratio of the aboveground to the underground part of the plant, the stability of cell membranes under stress conditions, and the photosynthetic potential of the variety (G.A. Manjunatha et al., 2017; J. Ali et al., 2018).

Keywords: rice, adaptability, abiotic stresses, drought, salinization, non-specific resistance, mineral nutrition, organic farming

Mineral fertilizers and pesticides allowed a significant increase in crop yields during a certain period of use. However, over time, negative consequences (soil erosion, environmental problems, and an increase in people morbidity) also arose [1-3], thence, organic farming systems for crop cultivation [4-6] with the use of organic fertilizers, biologicals and biological plant protection methods [7-9] are topical. Organic farms confirm the possibility to obtain stable yields, especially when using crops capable of nitrogen fixation [10-12]. Initially, the rejection of mineral fertilizers often leads to a decrease in yield [13-15]. It can be especially significant (up to 60%) in the first year of application of environmentally friendly technologies [16-18]. Soil fertility is restored in the next 3-4 years, during which the yield approaches the original (19-21). At this time, the availability of nutrients of organic fertilizers for plants increases due to humus formation [22-24].

The advantage of organic farming systems (OFS) is the provision of sustainable crop yields under stressful conditions (drought, salinity, temperatures outside the variety's response range) [25-27]. The use of OFS for intensive varieties is often not reasonable, since their yield decreases by more than 30% if the level of mineral nutrition is low [28-30]. This necessitates to develop a novel approach to breeding varieties for OFS technologies.

In this review we consider the characteristics for which breeding is necessary when creating rice varieties for organic farming, and the appropriate methodological approaches and breeding technologies.

Characterization of varieties for OFS. The traits that a variety for OFS should have are still debated [31-33]; however, they undoubtedly include high adaptability under biotic and abiotic stresses, genotype competitiveness, effective mineral nutrition and photosynthesis [34-36). All these traits are complex and largely interrelated. Thus, the high efficiency of mineral nutrition and photosynthesis ensures high adaptability to all stress factors and the competitiveness of the genotype [37-39].

Genotype competitiveness. The high competitiveness of the genotype is one of the main traits that allows the variety to be used in organic farming. The components of the competitiveness of rice plants include a high growth rate, effective tillering, a morphotype that provides minimal shading in dense sowing, high efficiency of photosynthesis, which allows the most complete use of solar energy even with shading, formation of a root system with high absorbing capacity [40-42].

The adaptability to stress can be influenced by increasing either the specific adaptability to each stress, or non-specific adaptability, which simultaneously enhances resistance to various stressors. Changing specific adaptability requires great efforts and does not guarantee the result, since all traits are polygenic, therefore, both intralocus and intergenic gene interactions can largely neutralize the effects of individual genes [43-45]. In nature, a plant is influenced by a complex of factors, which devalues one-way adaptability. Specific resistance to pathogens, as a rule, is overcome by them in a very short time. Therefore, from our point of view, the second strategy is more promising when an increase in general adaptability (non-specific resistance) occurs due to the functioning of several genetic systems that provide effective photosynthesis and mineral nutrition, a high rate of growth and development, and resistance to salinity.

Microsatellite markers associated with loci that determine plant photosynthesis efficiency of Russian rice varieties. Molecular markers linked to gene loci (quantitative traits loci, QTL), affecting the efficiency of photosynthesis and other traits in rice varieties of different geographic origin, is shown at http://www.gramene.org. However, their applicability for assessing Russian breeding material must be confirmed. It is necessary to confirm reliable separation of contrasting groups with the use of these markers [46, 47].

The study of allelic polymorphism of intragenic SSR markers and markers linked to genes that determine the photosynthetic potential of Russian rice cultivars revealed polymorphic loci (Fig. 1) [47].

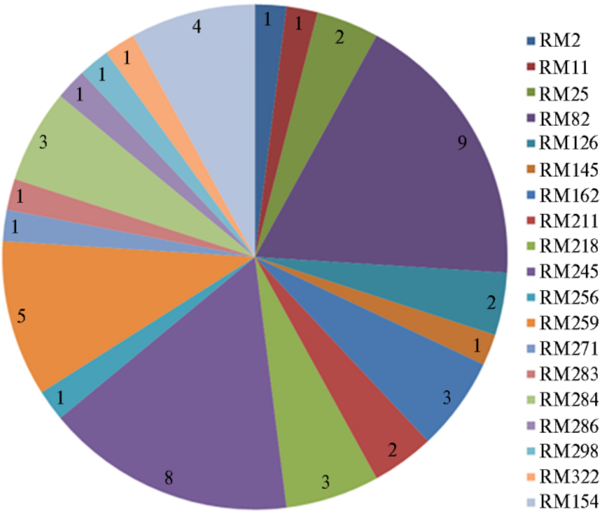

Fig. 1. Allelic polymorphism of markers (the number of alleles is indicated) **associated with photosynthesis efficiency of Russian rice varieties [47].**

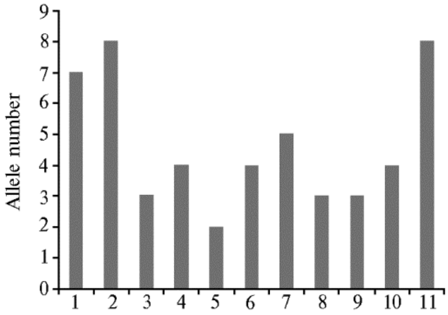

Fig. 2. Microsatellite markers linked to loci for photosynthesis efficiency of Russian rice varieties: 1 — RM600, 2 — RM5508, 3 — RM509, 4 — RM5361, 5 — RM347, 6 — RM154, 7 — RM240, 8 — RM162, 9 — RM574, 10 — RM5707, 11 — RM245 [47].

Analysis of the revealed polymorphism showed that some of the markers variable in amplification products can separate groups of varieties with different efficiency of photosynthesis at $p \leq 0.05$. Three of them (RM154, RM600, RM5508) linked to loci that determine the carotenoid level, two markers are linked to loci controlling specific surface density (RM347, RM240) and chlorophyll a content (RM154, RM509). Figure 2 shows intragenic markers with the maximum number of alleles which are associated with photosynthetic efficiency and divide Russian rice varieties into contrasting groups according to the trait [47].

These markers can be involved in marker-assisted selection (MAS) for the traits that determine the efficiency of photosynthesis in Russian rice varieties. The importance of the genetic system which determines the photosynthetic potential of the variety, has been shown by many researchers [48-51]. It is noted that varieties for OFS, as a rule, use light more efficiently. They have a long period of photosynthesis and a high chlorophyll content, especially in the upper leaves. The photosynthetic function of plant organs is ensured by the optimal architectonics of crops. In particular, key traits are plant height, resistance to lodging, and the presence of long and wide erectoid leaves. Note, the plant height for OFS significantly exceeds that in varieties for intensive farming technologies, in some works, up to 119 cm, that is, 20-30 cm higher compared to plant height in traditional agriculture) [44, 46].

Mineral nutrition response markers and chromosome regions. The potential for using genotypic differences in ability to assimilate minerals is enormous, since there is a 20-fold differences between the extreme manifestations of the trait [52-54]. The problem is that high-yielding varieties, as a rule, are not adapted to the lack of mineral nutrients. The mechanism of adaptation of rice plants to such a deficiency differs in different genotypes and includes an increase in root size, an intensification of absorption, and an increase in the internal efficiency of fertilizer use. However, there are reports that in most of the studied genotypes the latter indicator varies slightly [55-57].

Nutrition response of Russian rice varieties is poorly studied. Our studies have shown high yield variability in rice varieties at different levels of nitrogen fertilizers. Rapan variety shows the greatest variability of the trait ($Cv = 33.4\%$), whereas Vodopad variety is the most stable ($Cv = 15.7\%$). Without mineral fertilizers, the varieties reduced the yield by 49.05% on average with 55.93% reduction (variation from 53.33 to 58.71%) in varieties Rapan, Yubileyny 85, and Nautilus of intensive type. The grain yield of Vodopad variety decreased by 32%. In other words, the specialized “organic” varieties can additionally provide for more than 20 c/ha of rice grain and a 1.5-fold increase in OFS profitability [16].

Investigation of dose-dependent response to nitrogen fertilization revealed Vodopad variety to be in the lead without fertilization, 61 c/ha vs. an average yield of 48.2 c/ha. The yield of Nautilus (46.2 c/ha), Yubileiny 85 (43.1 c ha) and Rapan (40.8 c/ha) was lower than the average value of 48.2 c/ha. The change in the yield upon application of fertilizers was determined by the coefficient of linear regression of its relationship with the doses of applied nitrogen fertilizers [16].

On average, a 1 kg/ha nitrogen fertilizer provided 23 kg/ha increase in rice yield. The gain decreased with an increase in the dose of the fertilizer, from 32 kg/ha for N_0-N_{91} to 21 kg/ha for N_{92}-N_{137} and 15 kg/ha for N_{138}-N_{184} (Fig. 3). The revealed differences of Russian varieties allow breeding for increased nitrogen efficiency under different cultivation technologies [58].

Our previous works showed that the development of root system largely determines adaptability of rice plants to a deficient mineral nutrition, however, the question of which molecular markers can reliably group domestic samples contrasting in the manifestation of this trait during different phases of vegetation has not been studied [56].

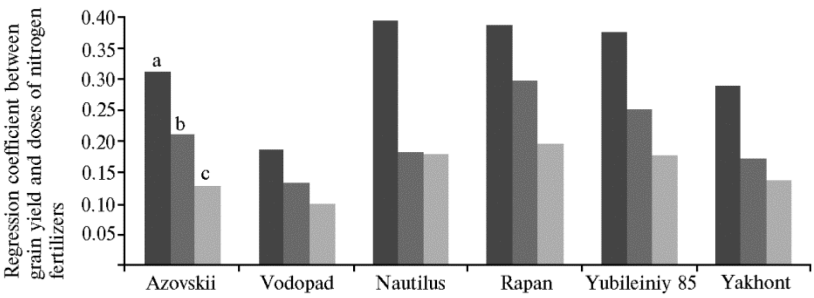

Fig. 3. Grain yields of Russian rice varieties depending on N-fertilizer doses (per active ingredient): a — 0-91 kg/ha, b — 92-137 kg/ha, c — 138-184 kg/ha (test plot of FSC of the Federal Rice Research Center rice, Krasnodar, 2017-2018) [16].

In a study of Russian rice varieties with microstellite markers, it was shown that the size of the root system is determined by genes in 14 loci [30], and these genes are not the same at different stages of plant development. Seven SSR markers, RM261, RM6314, RM126, RM463, RM405, RM509, and RM242 differentiated the samples by the efficiency of mineral nutrition during germination. None of the markers reliably divided the samples by the efficiency of mineral nutrition at the tillering stage. The markers RM245, RM284, RM574, RM258, RM227, RM509, RM3428, RM440, and RM154 showed a high probability of association with the trait. Ten SSR markers, RM284, RM335, RM245, RM600, RM53, RM542, RM261, RM5361, RM5371, and RM6314 discriminated domestic rice varieties contrasting in adaptability to a lack of mineral nutrition during ripening (Table 1).

1. SSR markers discriminating Russian rice varieties with contrast adaptability to deficient mineral nutrition

Gene	Traits	SSR marker	Chromosome	References
TRN7-1	Root number, 65 days after planting	RM542	7	[59]
LFSNS	Leaf structure, the chlorophyll content in the second leaf at tasseling vs. day 30 of growth	RM 245	9	[60]
qTRN1-2	Root number, 65 days after planting	RM600	1	[59]
OSAD-JCAP	Osmotic regulation	RM284	8	[61]
rdgf4 LFSNS	Leaf senescence, the chlorophyll content in the second leaf at tasseling vs. day 30 of growth	RM335	4	[62]
AQEI046-LFSNS	Leaf senescence, the chlorophyll content in the second leaf at tasseling vs. day 30 of growth	RM53	2	[62]
qTRN4-1	Root number 85 days after planting	RM261	4	[59]
NCN	Efficiency of mineral nutrient utilization	RM5371	6	[63]
NCN	Efficiency of mineral nutrient utilization	RM5361	5	[63]
NCN	Efficiency of mineral nutrient utilization	RM6314	4	[30, 64]
OSADJCAP	Osmotic regulation	RM126	8	[61]
qRTT9-1	Root thickness 65 days after planting	RM242	9	[59]
qPHT12-1	Plant height	RM463	12	[59]
qFRP-12	Grain number per panicle			
NCN	Efficiency of mineral nutrient utilization	RM509	5	[63, 65]
qRTV5-1	Root volume, 65 days after planting	RM289	5	[59]
qPL-5	Panicle length	RM405	5	[66]
qYI-5	Spikelets per panicle			
NCN	Efficiency of mineral nutrient utilization	RM3155	8	[30, 64]

Note. NCN means that no common name of the gene is accepted.

Previously, both specific and nonspecific genes were mapped that enhance the responsiveness of rice plants to mineral nutrition; SSR markers were also located in the regions of localization of these genes [67-69]. The loci for the effectiveness of mineral nutrition in Russian rice varieties are defined in the scholar publications as being responsible for the number, volume and thickness of roots 65 days after planting, for the capability of osmotic regulation, and for leaf senescence assessed by the ratio of chlorophyll content in the second leaf at tasseling and after 30-day growth. Since there is a close correlation between the sizes of the aboveground and underground parts of plants and the pleiotropic influence of many genes is known, the loci that determine plant height, panicle length, and the grain number per panicle were also attributed to those that increase the efficiency of mineral nutrient utilization [70-72].

SSR markers of salt tolerance of Russian rice varieties. Table 2 shows SSR markers reliably grouping Russian rice varieties by different salt tolerance during flowering. Analysis of genes previously mapped in the regions of localization of these markers [72] showed that only two markers, RM25 and RM240 are associated with specific genes for salinity resistance enabling osmotic regulation. Other markers are linked to nonspecific loci that increase the viability and resistance to a number of stressors [72-75].

Markers enabling reliable discrimination of domestic rice varieties with contrast adaptability to salinity as assessed by changes in the length of the embryonic root have not been established. The obtained result is anticipated, since the adaptability to salinity is determined by polygenes or gene clusters. The studied samples carry sets of genes that, acting together, hide the effect of one or another locus. However, this did not prevent the identification of loci most likely associated with the trait in question in seedlings. Identification was allowed due to an increase in the sensitivity threshold of the method by applying a significance level of $p \leq 0.09$ [76]. At the accepted level of significance, a numerous of loci were identified that determine the change in the root length during salinization in the early growing season. In most cases, the varieties are also separated due to nonspecific genes that increase resistance to salinity (76).

2. SSR markers associated with salt tolerance of Russian rice varieties [72]

SSR marker	Chromosome	Amplicon, bp (primer melting temperature, °C)	Traits
		Flowering	
RM574	5	155 (55)	Root system features
RM245	9	150 (55)	Photosynthesis efficiency during growth and maturation
RM240	2	132 (55)	Osmotic regulation, length of growing period
RM53	2	182 (55)	Leaf senescence, length of growing period
RM25	8	146 (55)	Length of growing period, osmotic regulation, leaf senescence,
RM590	10	137 (55)	Grain fracture
RM24	1	192(55)	Ratio of root number to stem number, length of growing period, total leaf parea
RM5361	5	138 (55)	No data available
		Seedlings (as per root length)	
RM574	5	155 (55)	Root volume, root thickness
RM245	9	150 (55)	Photosynthetic potential, length of growing period
RM542	7	113 (55)	Root volume, the angle of rice stem inclination, plant height
RM463	12	192 (55)	Plant height
RM242	9	225 (55)	Root length and thickness, cold tolerance, in vitro culturing, germination vigor, cell-membrane stability, plant height
RM3276	4	163 (50)	Salt tolerance
RM5508	7	177 (50)	Salt tolerance
		Seedlings (as per stem length)	
RM574*	5	155(55)	Root system features
RM154*	2	183 (61)	Photosynthetic potential
RM141	6	136 (55)	Photosynthetic potential
RM82	7	186 (55)	Photosynthetic potential, resistance to low temperatures
RM286	11	110 (55)	Length of growing period, photosynthetic potential
RM227	3	106 (55)	Leaf features, germination vigor, length of growing period, root dry weight
RM24	1	192 (55)	Ratio of root number to stem number, length of growing period, leaf features
RM542	7	113 (55)	Root weight, plant height
RM126	8	171 (55)	Osmotic regulation, length of growing period, Leaf senescence

* Markers reliably discriminating samples at $p \leq 0.05$.

Markers RM463, RM245, RM242, RM3276, RM5508, RM574, RM542 are linked to nonspecific loci that determine adaptability of seedlings to salinity. Markers RM463, RM242 are associated with genes encoding the photosynthetic potential. The RM242 is mapped in the chromosomal region where genes are localized that determine adaptability to stress due to higher cell-membrane tolerance and the growth rate. Markers RM574, RM542, and RM242 flank loci that determine root length, thickness, volume, and efficiency, thus allowing better rice plant responsiveness to mineral nutrition [77-79].

Microsatellite markers associated with growth rate of seedlings. According to our data [76], most loci which determine growth rate in Russian rice gene pool, are monomorphic, since for a long time, a high level of water was used to control weeds. The rapid appearance of plant above the water level provides even sprouts and further high productivity. Nevertheless, statistical analysis revealed polymorphic regions determining the growth rate of domestic varieties. The data of http://www.gramene.org shows that genes associated with root system formation and germination vigor had already been found in the identified loci (Table 3).

3. SSR markers for clustering Russian rice varieties based on growth rate parameters [76]

SSR marker	Chromosome	Amplicons, bp	Traits
		Seeding stem length	
RM289	5	108	Stem and leaf size, growth vigor
		Root length	
RM242	9	225	Tolerance to low temperatures, root size and activity, ratio of root length to size and number of tillers and leaves, differentiation of explants, tolerance to membrane stressors, germination rate
RM126	4	125	Adaptiveness to deficit irrigation, tolerance to low temperatures, root size, the ratio of the root length to tiller length
		Seedling weight	
RM405	5	110	Stem length, panicle length, leaf size
RM261	4	125	Drought tolerance, tolerance to low temperatures, stem length, root length, panicle length, leaf size
RM242	9	225	In vitro culturing, root size, root activity and length compared to stem and panicle activity and length, tolerance to low temperatures, germination rate, tolerance to membrane stressors
RM463	12	192	Size of the aboveground organs
RM6314	4	169	No data available

The markers identified by us are mapped in the chromosomal regions bearing genes that determine the germination vigor, drought tolerance, tolerance to low temperatures, the root morphotype and size, the weight proportion of the aboveground to the underground parts of the plant, and cell-membrane stability under stress conditions [30, 56, 76]. Stem formation, according to our data, was determined by loci linked to the RM289 marker. In RM289 is mapped in the region that contains genes affecting the germination rate, plant height, differentiation of explants, the relative weight of roots, and the rate of seedling appearance [39, 40]. Differences in clustering varieties contrast in the growth rates of seedlings were significant ($p \leq 0.05$) for markers RM261, RM405, RM463, RM242, and RM6314 located on chromosomes 4, 5, 9, and 12, respectively (see Table 3).

We found a relationship between the growth rate of seedlings and several loci that determine adaptability to abiotic stresses [76]. Intensive growth, high photosynthetic activity and the efficiency of mineral nutrient utilization increase plant viability and allow the plant to pass through stress-sensitive phases as quickly as possible, which reduces the likelihood of damage by extreme temperatures or other factors reducing vitality, including those in organic farming [80-83]. Selection for an increase in nonspecific adaptability is the most promising in creating varieties with sustainable productivity for organic agriculture [84, 85].

Thus, rice varieties for organic farming should have a high adaptability to biotic and abiotic stresses, genotype competitiveness, efficiency of mineral nutrient utilization and photosynthesis. The high competitiveness of the genotype is a complex trait that includes high growth rate, effective tillering, a morphotype that provides minimal shading in a dense crop, and the root system with a high absorbing capacity. To achieve sustainable productivity in organic farming, varieties with non-specific adaptability are the most promising. The non-specific adaptability is ensured by several genetic systems that control photosynthesis, the efficiency of mineral nutrition, the high rates of growth and development, and the resistance to salinity. In Russian rice varieties, microsatellite markers RM154, RM600, RM550, RM347, RM240, RM154, and RM509 are associated with loci for the efficiency of photosynthesis. Markers RM261, RM6314, RM126, RM463, RM405, RM509, and

RM242 differentiate rice samples by the effectiveness of mineral nutrition during germination, RM463, RM245, RM242, RM3276, RM5508, RM574, RM542 reliably discriminate by salt tolerance. Polymorphism by markers RM261, RM405, RM463, RM242 and RM6314 are associated with seedling growth rate. The listed genetic markers can be involved in breeding rice varieties for organic farming.

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