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MOLECULAR MARKERS ASSOCIATED WITH HIGH EARLY GROWTH RATE OF RUSSIAN RICE (*Oryza sativa* L.) VARIETIES

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Abstract

The high rate during early growth is expected to be the main basis of next "green revolution". Many researches take into account that this trait is mainly responsible for physiological advantage of heterotic hybrids in crops. The rapid development of the root system provides an advantage in absorption of mineral substances and the photosynthetic apparatus formation. Heterotic hybrids can quickly overpass the phase sensitive to stresses thereby improving the adaptability to different stress factors. Significant differences between varieties and subspecies in the growth rate of seedlings are widely used for effective breeding. The growth rate is a plant-height-related trait. Heritability of seedling height, weight, and the length of embryo root is very high (87-90 %). This paper is the first report on loci that determine the growth rate during germination period in rice (*Oryza sativa* L.) varieties bred in Russia. The aim of our work was to reveal effective SSR markers for chromosome loci and regions which are involved in the control of traits responsible for high rate of early growth in the Russian rice (*Oryza sativa* L.) gene pool. Seeds of 32 varieties bred in Russia and hybrids were surface-sterilized for 40 min with a 20 % sodium hypochlorite solution (95.2 % active Cl; OOO Grinfild RUS, Russia). Thirty grains of each sample were germinated (in duplicate) for 7 days at 28-29 °C in a thermostat. Morphophysiological parameters of 20 seedlings of each variety or hybrid, i.e. the weight, the size of coleoptile and the embryonic root formation, were measures. As per the finding of this study, the loci that determine the seedling weight are located on four chromosomes near the markers RM261, RM405, RM463, RM242, RM6314, RM289, and RM126. The embryonic root growth rate is determined by two chromosomal regions on chromosomes 4 and 9, the seedling height — by only one locus located on the chromosome 5 near the marker RM289. One can simplify identification of chromosomal regions that determine target trait values in a set of unknown varieties or hybrids. Bulk breeding methods allows markers with the maximum phenotypic effect to be identified with no need to create a population recombinant inbred lines (RILs) or double haploid lines (DH) for mapping. In particular, exploiting cultivars which are contrast in the traits of interest is much more informative. Such phenotypic analysis reveals the most important loci for the entire group of varieties tested. Next, the markers can be found which are closely linked to the genes that determine the trait in the identified chromosomal region (http://www.gramene.org). Markers that are enough polymorphic to ensure reliable separation of varieties into contrasting groups may be further used in breeding work. Not always genes, differentiating varieties by a certain trait in one region of the world will be effective for another region. Therefore, such preliminary assessment is obligatory. Thus, in this study using original technique to simplifies identification of the loci of interest we have identified 8 chromosomal regions associated with early growth rate in Russian rice varieties.

Keywords: *Oryza sativa* L., rice, molecular markers, genetics, growth rate, early stages of ontogenesis, seedlings, weight, height, embryonic roots

One of the important agronomic features of rice intended for direct

seeding is the high growth rate of the shoots, which ensures the plants' advantage both in case of flooding and in case of moisture deficiency. It has been found out that the growth rate can be the indicator of high productivity and presence of heterosis effect [1, 2]. However, in different populations the unequal results of mapping the genes responsible for the growth rate have been obtained.

The considerable polymorphism between varieties and between subspec[[3]. A high growth rate is related to the genes determining the plant's height [4]. The heritability of the seedling height, weight and the length of the embryo root is 87-90% [5]. The traits characterizing the seedling are mutually related that is evidenced by the high correlation between them [6], but their genetics are mostly unstudied [7, 8]. The seedling height under different growing conditions is controlled by several genes, however, the correlation between the data obtained at contrasting temperatures has been noted to be low [9]. The field germination and growth rates of the varieties of *indica* subspecies in cold conditions are low, therefore the identification of the genes influencing on the seedling growth rate at low temperatures is important for the selection of varieties of this subspecies [10]. The relation of the genes determining the chlorosis of rice seedlings caused by cold with the loci responsible for growth rate has been shown. The loci which determine the seedling hormonal status including those involved in the biosynthesis of gibberellin participate in this process. The increased chlorophyll content also contributes to the increase in seedlings' growth rate by accelerating the formation and accumulation of carbohydrates [11]. A high concentration of sucrose and glucose, as well as the amylase activity in germinating seeds are the prerequisites for the rapid growth of seedlings [12, 13]. Highly productive forms in most cases have a longer and branched root system at the initial stages of plant development [14, 15]. Five-day-old rice seedlings show maximum differences in this trait [16].

The rate of formation of the seedlings' organs is determined by the effectiveness of photosynthesis and mineral nutrition at the early stages of development. In addition, the improvement of young plant nutrition also increases its resistance to biotic and abiotic stresses [17, 18].

A high growth rate in seedlings of hybrids with heterosis effect is due to gene overexpression. Note that overexpression of the genes determining the cells division rate, replication beginning, transcription and translation is deemed as one of the possible mechanisms which ensure a heterotic effect. When investigating almost 14 thousand genes, an overexpression was characteristic for more than 15%, wherein the expression of 9% of genes was 2 times higher than that of parents. An increased expression has been noted for regulatory and structural genes. A hormonal regulation, carbohydrate metabolism and mitochondrial activity are involved in this process [19, 20]. The obtained data make it possible to assume that during the hybridization, the starting advantage of F_1 hybrids may be conditioned by the decrease in the methylation effects which increase the expression of heterosis promoting loci. Heterotic hybrids differ from their parental forms with a number of epigenetic changes of the genome including the methylation or removal of this effect. The optimal course of biochemical reactions caused by changing the functional state of genes, and cascade reactions in polygenic structures are responsible for the heterotic response of the hybrid genome [21].

High rate of seedling metabolism and growth also ensures yield stability of varieties due to the rapid passage of the phases which are most sensitive to stresses. Such traits are useful also in terms of the formation of even sprouts on flooded and unlevelled checks, because the high speed of the seedling passage through the water layer ensures the rice plant viability [22]. The genetics of these traits had been studied during seed germination for 5 days from under the water

layer (20 cm) in the dark. The hybrids between the samples of the *indica* subspecies or intersubspecies hybrids were used in the work. When creating highly productive rice varieties, the control of traits participating in the obtaining of even sprouts can be ensured by the stacking of several positive QTLs (quantitative trait loci) which contribution to the trait formation and to an individual plant phenotype is insignificant.

The gene pool of the *japonica* subspecies has not been used for a long time for the identification of the genes responsible for the resistance to germination in an oxygenless medium and for mapping QTLs which determine the formation of these traits [23]. For the first time such experiments were performed by Chinese scientists. Wang et al. [24] have studied 247 recombinant inbred lines (RILs) which were derived from the hybrid of two forms of the *japonica* subspecies — Xiushui 79 variety and C Bao restorer line, as well as 98 backcross inbred lines (BILs) obtained from crossing Nipponbare (*japonica*)/Kasalath (*indica*)//Nip-ponbare (*japonica*). Two relevant QTLs have been found on the 2nd and 7th chromosomes in the RIL population. The positive *qSAT-1-R* allele obtained from C Bao variety determined 8.7% of the phenotypic dispersion by the coleoptile length. The *qSAT-2-R* allele (the amplification product size 140 bps) was closely linked to the RM525 SSR marker to which 9.8% of the phenotypic dispersion is related. Its positive allele also belonged to C Bao variety. The *qSAT-7-R* locus (the amplification product size 250 bps) was closely linked to the RM418 SSR marker [24]. Six QTLs on the coleoptile length in an oxygenless medium were found in the BIL population on the 2nd, 3rd, 5th, 8th, 9th and 12th chromosomes. These QTLs conditioned from 5.8 to 16.2% of the phenotypic manifestation of the trait. The Nipponbare variety carries the positive alleles of the *qSAT-2-B, qSAT-3-B* and *qSAT-9-B* genes. The Kasalath sample contains the positive alleles of the *qSAT-5-B, qSAT-8-B* and *qSAT-12-B* loci. In the Kasalath sample, the coleoptile was more than 11 mm longer on average than in the Nipponbare variety, in which the coleoptile length was 5.5 mm. The trait value in Kasalath/Nipponbare hybrids is 6.4±1.3 mm, the variation coefficient is 20.2%. One QTL related to the seedling stem length was located between RM525 and RM2127. This *qCL-2-R* locus determines 5.2% of phenotypic variability. Its positive allele belongs to Xiushui 79. The QTLs related to the seedling stem length have been found on four other chromosomes, but their contribution did not exceed 10%. The *qCL-1-B* locus hybrids were obtained from the parent form of Nipponbare [24].

In other works with using recombinant inbred lines (RILs) for the molecular marking of populations, five QTLs which determine the coleoptile length under the stress (lack of oxygen) have been found. They are located on the 1st, 2nd, 5th, 5th and 7th chromosomes. The positive alleles of the *qAG-1, qAG-G2* and *qAG-7* genes which increase the rate of germination in oxygenless medium (anoxic growth, AG) are carried by the Kinmaze variety. The *qAG-5a* and *qAG-5b* loci have been inherited from the DV85 sample. The RILs have been obtained by crossing Kinmaze (*japonica*) and DV85 (*indica*). When phenotyping the trait for the subsequent statistical analysis, the average values of the coleoptile length were used [25-27].

From the point of view of the research technique, it is necessary to take into account that the germination time increases as the temperature decreases [11]. If seeds have been subjected to preliminary soaking (priming) for 24 or 48 hours at 20 °C and for 12 hours at 30 °C the germination time decreases. Thus seeds soaking for 12 hours at 30 °C reduce the germination time by almost 18 hours compared to the control. A shorter germination time is also characteristic of samples subjected to preliminary soaking followed by drying. In addition,

priming increases the uniformity in the growth rate of samples [28].

In the present work, we for the first time identified the loci which determine the growth rate during the germination period of domestic rice varieties.

Our goal was to find the loci and chromosomal regions which determine the rapid coming up and development of rice seedlings, as well as the study of the genetics of these traits.

Techniques. The experiments were carried out in 2013-2019. The collection samples (32 varieties of Russian selection) and hybrids of rice (*Oryza sativa* L.) had been studied using the generally accepted methods [16]. The hybridization between the varieties of domestic selection with contrast traits was carried out by the twell method (Borlaug's method) according to the full diallel scheme [29].

The seeds were treated with the 20% sterilizing solution of Belizna, a household detergent sodium hypochlorite with the active chlorine content of 95.2% (Greenfield RUS LLC, Russia) for 40 minutes. 30 seeds from each sample (in 2 replicates) were grown in the thermostat during 7 days at 28-29 °C. The weight of seedling, sizes of coleoptile and embryo root of 20 plants of each cultivar or hybrid were measured.

DNAs were isolated from the etiolated seedlings and leaves using the STAB method in various modifications. The polymorphism was investigated with 60 neutral or linked to seedlings' high growth rate SSR markers distributed over 12 chromosomes of rice. When marking the samples, the standard conditions of polymerase chain reaction (PCR) for the reaction mixture volume of 10 μl [30] and the following amplification mode were used: 5 min at 94°C (initial denaturation); 1 min at 94 °C (denaturation), 1 min at 55 °C (annealing of primers), 2 min at 72 °C (synthesis) (35 cycles); 7 min at 72 °C (Multiback System DNA amplifier, Bio-Rad, USA). The PCR mixture included 40 ng DNA (2 μl), 1 μl of dNTPs (1 mM), 3.7 μl H_2O, 1 μl buffer solution, 0.3 μl $MgCl_2$; 1 μl of primers, 1 μl of Taq DNA polymerase (Evrogen CJSC, Russia). The PCR and visualization of amplification products were performed according to the technique of the International Rice Research Institute, Philippines [9]. The amplification products were separated by electrophoresis in polyacrylamide gel at 100V in the electrophoretic chamber designed for 204 samples (C.B.S. Scientific, USA).

The analysis of variance has been made basing on the relative size of the amplification product for the relevant marker. The amplification product size of a sample with its minimum value has been taken as 1 (one), the rest alleles were designated in accordance with the increase in the size of the amplification product. The means (M) and their standard errors (±SEM) for the morphophysiological traits have been calculated. The relationship between the data on genotyping and phenotyping of a trait have been analyzed after the statistical processing which has been performed using the Statistica 6.0 software (StatSoft, Inc., USA) with the analysis of variance and cluster analysis [31]. The validity of differences has been evaluated according to the Fisher's F-test and p significance level.

Results. The markers we used are listed in Table 1.

The most of the hybrids were characterized by heterosis in terms of growth rate, therefore in the studied population the high growth rate was the dominant trait. The high growth rate ensured the high productivity of hybrids that is evidenced by the value of the correlation coefficient $r > 0.9$ between the traits which determine the productivity (total weight of grains per plant, weight of the main panicle, weight of the side panicles, number of filled spikelets) and which are related to the seedlings' growth rate. The data analysis by the Hayman's method showed that a trait value is determined by polygenes, as well as by the non-directedness of dominance, i.e. by the existence of both dominant and

recessive genes increasing the trait in the studied varieties [16, 32).

1. Distribution over the chromosomes of rice (*Oryza sativa* L.) of the neutral SSR markers and ones linked to the traits determining the seedlings' size and formation rate

Chromosome	Number of markers	Markers
1	5	RM104, RM259, RM600, RM5638, RM24
2	7	RM53, RM154, RM240, RM318, RM322, RM2770, RM5707
3	3	RM227, RM347, RM218
4	9	RM124, RM127, RM140, RM255, RM261, RM317, RM335, RM3276, RM6314
5	9	RM13, RM30, RM289, RM405, RM440, RM509, RM574, RM5361, RM6024
6	6	RM141, RM162, RM276, RM588, RM5371, RM6811
7	5	RM82, RM542, RM5508, RM7110, RM11
8	6	RM25, RM126, RM256, RM284, RM3155, RM8243
9	4	RM242, RM245, RM444, RM7048
10	2	RM258, RM590
11	2	RM286, RM3428
12	2	RM463, RM6410

N o t e. The information is available on the website http://www.gramene.org [32].

Characterization of the seedlings' polymorphism has made it possible to divide the samples into the groups which significantly ($p < 0.05$) differ in morphophysiological traits (Table 2).

2. Polymorphism of Russian rice (*Oryza sativa* L.) varieties by morphophysiological traits ($M \pm$SEM, lab tests, 2013-2015)

Variety	Seedling weight (SW), g	Group by SW	Embryo root length (ERL), cm	Group by ERL	Coleoptile length (CL), cm	Group by CL
Liman	1.08 ± 0.0038^{e}	5	$0.9+0.0009^{g}$	2	$5.5+0.018^{k}$	1
Garant	$1.11+0.0021^{e}$	5	$0.6+0.0013^{i}$	4	$3.8+0.190^{m}$	3
Pavlovskiy	$1.12+0.0011^{d}$	4	$0.8+0.0008^{h}$	3	$0.7+0.092^{o}$	5
Rapan	$1.13+0.0007^{d}$	4	$0.5+0.0010^{j}$	5	$2.5+0.057^{n}$	4
Snezhinka	$1.14+0.0018^{d}$	4	$1.0+0.0013^{g}$	2	$4.0+0.126^{l}$	2
Primorskiy	$1.14+0.0045^{d}$	4	$1.0+0.0020^{g}$	2	$2.2+0.200^{n}$	4
Regul	$1.25+0.0031^{d}$	4	$0.8+0.0011^{h}$	3	$2.6+0.067^{n}$	4
Atlant	$1.25+0.0022^{d}$	4	$0.8+0.0019^{h}$	3	$3.3+0.155^{m}$	3
Lider	$1.26+0.0033^{c}$	3	$0.7+0.0006^{h}$	3	$2.9+0.094^{n}$	4
Khazar	$1.27+0.0041^{c}$	3	$0.8+0.0015^{h}$	3	$3.9+0.066^{m}$	3
Novator	$1.27+0.0049^{c}$	3	$0.7+0.0020^{i}$	4	$2.5+0.137^{n}$	4
Izumrud	$1.29+0.0050^{c}$	3	$0.9+0.0008^{g}$	2	$3.0+0.093^{m}$	3
Kurchanka	$1.33+0.0015^{c}$	3	$1.0+0.0012^{g}$	2	$5.1+0.095^{k}$	1
Serpantin	$1.39+0.0017^{b}$	2	$1.0+0.0009^{f}$	1	$4.2+0.124^{l}$	2
Ametist	$1.47+0.0044^{b}$	2	$1.1+0.0007^{f}$	1	$5.2+0.178^{k}$	1
Yantar	$1.54+0.0029^{a}$	1	$1.2+0.0009^{f}$	1	$3.6+0.134^{m}$	3

N o t e. The analysis has been made on the samples which are the most contrasting by the trait. For each trait the differences between the values marked with different letters are statistically significant at $p < 0.05$.

We have done marking in the groups contrasting by the trait for the analysis of allelic diversity in loci. When investigating the Russian gene pool, most of the markers, which, according to other authors, are related to seedling growth rate, turned out to be monomorphic or their polymorphism was not related to the trait formation (Fig. 1).

In order to identify the chromosomal regions which determine the seedling traits in domestic rice varieties, the variance analysis of the relationship between the seedling size and weight with the allelic diversity of loci has been made. As the result, 10 microsatellite markers the polymorphism of which has the valid relationship ($p < 0.05$) with the changing of seedling size have been identified (Table 3).

We have also analyzed (Table 4) the available information on the relationship of the markers we identified with the loci responsible for any traits in rice plants (http://www.gramene.org). Previously, the genes associated with the root formation and germination energy have already been found in these chro-

mosomal loci. In the region of markers localization, the genes which determine the germination energy, resistance to drought, tolerance to low temperatures, root number, ratio of the root-to-stem length, relative weight of the roots and stability of the cell membranes under stress are located. According to our data, the stem formation was determined by loci located in the region of RM289 marker. In the zone of its localization, there are genes responsible for the germination rate and plant height [33]. The differences of the varieties' clusters with the contrasting growth rates of seedling were valid for the *RM261, RM405, RM463, RM242* and *RM6314* markers located on the 4th, 5th, 12th, 9th and 4th chromosomes respectively (see Table 4). In the region where these markers are located, the loci related with the tolerance to moisture deficiency, number of roots, resistance to low temperatures, length of the root system and main panicle, differentiation of explants, relative root weight, rate of seedling formation and stability of the membrane complex of the sample have been described [33].

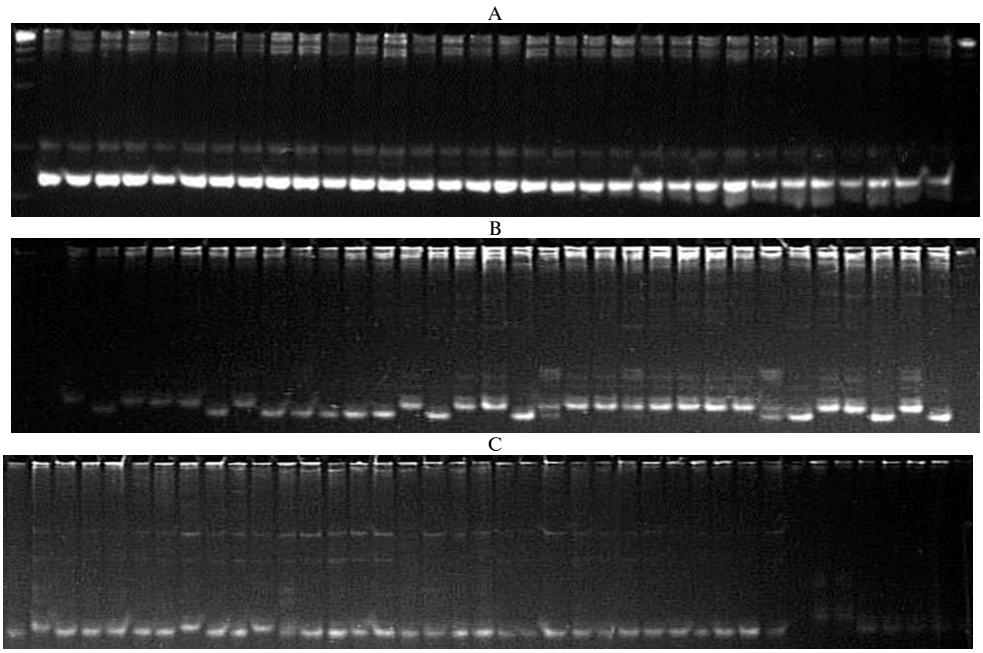

Fig. 1. Molecular marking of Russian rice (*Oryza sativa* L.) varieties using SSR markers: A — RM7048 (monomorphic marker), B — RM213 (polymorphism is not related to the trait formation in Russian rice varieties), C — RM261 (polymorphism is related to the trait formation in Russian rice varieties). From the left to right: molecular weight marker, varieties Khankayskiy, Sadko, Primorskiy, Liman, Garant, Pavlovskiy, Rapan, Novator, Serpantin, Boyarin, Regul, Yantar, Zhemchug, Lider, Khazar, Ametist, Nartsiss, Druzhnyy, Sprint, Viola, Dalnevostochnyy, Fontan, Kasun, Yupiter, Atlant, Kurchanka, Fakel, Snezhinka, Anait, Flagman, Izumrud, Nartsiss varieties, molecular weight marker (DNA length marker, 100 + 50 bps, Diaem, Russia).

3. Analysis of the validity of differences in seedling growth rate in Russian rice (*Oryza sativa* L.) varieties using SSR markers

SSR marker	SS Effect (intergroup sum of squares)	SS Error (intragroup sum of squares)	Fisher's *F*-test	Significance level p
		Embryo root length		
RM126	1.971	1.472	3.702	0.042
RM509	0.952	0.804	3.271	0.050
RM242	2.202	0.802	7.563	0.001
RM463	2.441	2.003	3.351	0.050
RM289	0.173	2.271	0.212	0.939
		Seedling weight		
RM405	11.723	8.031	4.011	0.031

Table 3 continued

RM261	3.641	0.801	12.501	0.000
RM6314	3.604	0.832	11.892	0.000
RM242	2.201	0.803	7.563	0.001
RM463	3.940	0.504	21.661	0.000
RM289	0.603	1.831	0.912	0.499
RM3155	4.641	4.802	2.661	0.091
		Seedling height		
RM6410	1.043	2.401	1.191	0.370
RM463	0.743	3.702	0.553	0.700
RM289	1.641	0.801	5.632	0.011

4. Microsatellite markers for clusterization of Russian rice (*Oryza sativa* L.) varieties basing on the traits determining the growth rate

SSR marker	Chromosome	Tm, °C	Amplicons, bps	Genes in the region of the marker localization [33]
		Clusters for the embryo stem length		
RM289	5	55	108	Size of the aerial part, germination energy
		Clusters for the embryo root length		
RM242	9	55	225	Tolerance to low temperatures, activity and size of the root system (length, number of roots and their thickness, root weight) and its length relative to the size of the aerial part (weight of the stem, its length, number of shoots and leaves), differentiation of explants, membrane stability at stress, germination rate
RM126	4	55	125	Adaptability to the lack of irrigation water, tolerance to low temperatures, size of the root system and its length relative to the size of the aerial part
		Clusters for seedling weight		
RM405	5	55	110	Size of the aboveground generative and vegetative organs
RM261	4	55	125	Adaptability to the lack of irrigation water, tolerance to low temperatures, size of the root system, size of the aboveground generative and vegetative organs
RM242	9	55	225	Differentiation of explants, size of the root system, its activity and length relative to the size of the aerial part, tolerance to low temperatures, germination rate, membrane stability under stress, size of the aboveground generative and vegetative organs relative to underground part
RM463	12	55	192	Size of the aboveground organs
RM6314	4	50	169	No information

We have found the relationship between the seedling growth rates and many loci determining the adaptability to abiotic stresses. This result was expected, because the high rate of formation of the root and photosynthetic systems determines a lower dependence of a seedling on external factors through increasing its adaptability. The rapid passage by seedlings of the phases in which young plants are most sensitive to stress also reduces the likelihood of their damage by extreme temperatures or other factors reducing the viability [16].

The effectiveness of using the identified markers for clusterization of varieties was different, most of the markers allowed us to select only the most contrasting groups. For example, *RM242, RM463* and *RM6314* markers could only be used to identify the samples of the 1st group with the most intense manifestation of the trait. *RM126* and *RM242* allowed valid identification of two groups of samples, with the maximum and minimum value of the trait (Fig. 2).

The result of the researches can be considered natural, since the studied

traits are inherited polygenically. The loci with the multidirectional effects which compensate the effect of each other do not provide identification of the function of certain genes determining a trait. The chromosomal regions localized for the first time, which are related to the rapid formation of seedlings, subsequently will allow us to develop the marker-assisted selection techniques for Russian samples.

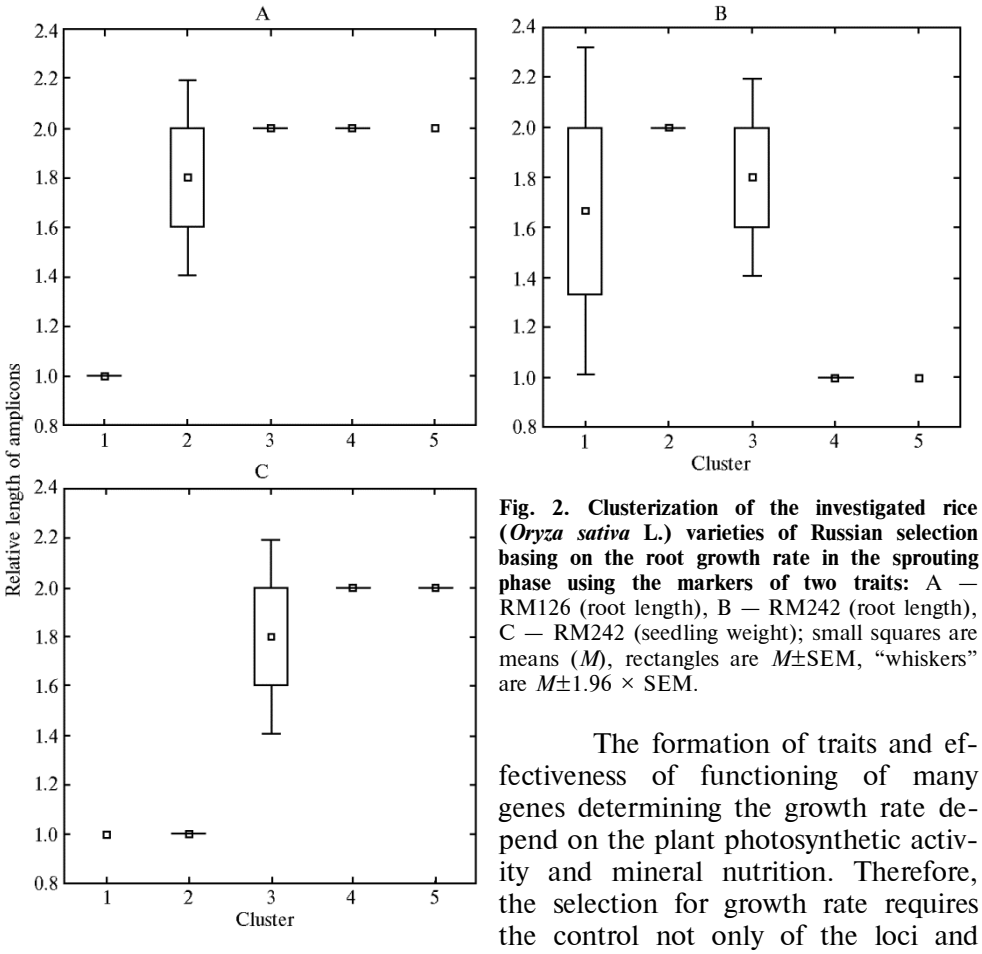

Fig. 2. Clusterization of the investigated rice (*Oryza sativa* L.) varieties of Russian selection basing on the root growth rate in the sprouting phase using the markers of two traits: A — RM126 (root length), B — RM242 (root length), C — RM242 (seedling weight); small squares are means (*M*), rectangles are *M*±SEM, "whiskers" are *M*±1.96 × SEM.

The formation of traits and effectiveness of functioning of many genes determining the growth rate depend on the plant photosynthetic activity and mineral nutrition. Therefore, the selection for growth rate requires the control not only of the loci and genes which are directly related to the seedling growth rate, but also of the complexes which determine the effectiveness of photosynthesis, mineral nutrition, activity of a number of enzymes and adaptability to stresses.

Due to the small sample size and the number of markers, we could identify only the loci with the maximum phenotypic effect. We have not found any markers to reliably separate the groups of varieties by the total and relative content of chlorophyll at the significance level recognized in biological studies ($p \leq 0.05$). The decrease of the significance level to $p \leq 0.09$ increased the sensitivity threshold of the method and nevertheless allows us to identify several markers which may be related to the said traits. The information on the genes localization collected on the website http://www.gramene.org makes it possible not only to use in the work well-known markers, but also to verify the obtained data, because the earlier found relationship of the identified chromosomal regions with the investigated traits has confirmed our results indirectly.

The RM245, RM162, RM154 and RM240 markers, which we identified as informative, are intragenic for the traits determining the effectiveness of photosynthesis [38]. The separation of the trait into its components made it possible

to establish that RM509 marker is related with the content of chlorophyll-a. We have not find in the literature any data on the association of the RM5361, RM5707, RM5508, RM347, RM509, RM600 and RM574 markers located on the 5th, 2nd, 7th, 3rd, 5th and 1st chromosomes and related to the separation of domestic varieties into groups with different photosynthesis efficiencies and with any traits of photosynthesis. The markers with four-digit numbers are relatively new, they were used in experimental works rarely. Perhaps, in the region of these markers there are genes which are specific for the Russian gene pool.

The RM574 and RM600 markers are known to be related to the effectiveness of mineral nutrition, which largely determines the traits we study, i.e. growth rate and photosynthesis [33]. With the deficiency of phosphorus, potassium and magnesium, the photochemical and dark reactions of photosynthesis are disturbed. It has been shown that the application of nitrogen fertilizers enhances the plant photosynthetic activity [16]. Many compounds functioning as electrons carriers contain iron (cytochromes, ferredoxin) or copper (plastocyanin). It is natural that when lack of these elements, the photosynthesis intensity decreases [33, 38].

Our results testify about the possibility of simplified identification of chromosomal regions which determine the trait parameters when investigating unknown samples. At the stage when thousands of genes are localized, a bulk breeding can be used to identify the markers related to the maximum desired phenotypic effect. For this, there is no need to create and perform phenotyping of populations as for recombinant inbred lines (RILs) or dihaploid lines (DH) that requires several years of work. Much more information can be obtained by using the varieties which are contrasting by the trait. This makes it possible within short time to determine the loci which are the most important for the trait formation in the whole investigated group of varieties. Such information will make it possible to put the markers closely linked to the genes determining the trait in the identified chromosome region. In further work, just these markers can be used if the study of their polymorphism will confirm the possibility of reliable separation of varieties into contrasting groups. This preliminary work is necessary because the genes which differentiate varieties by any trait in one region of the world are not always effective for other region, in which the trait can be determined by absolutely different loci.

The analysis of other authors' papers showed that the loci related to the seedlings formation are localized on all rice chromosomes, and they are different in different samples. The dihaploid and recombinant or backcross inbred lines, hybrids of the second and third generations (from 80 to 2800 samples) have been used for marking [24, 34, 35]. The outcomes of the researches also were different. An increase in population volume does not guarantee the increase in marking efficiency. For example, when sampling amount of 2810 samples in the F_2 population, the authors identified one locus, while in the population of 191 RILs, 15 loci have been identified [36, 37]. It is very important to select maximally contrasting samples as parent forms. In our work, the loci on the 4th, 5th, 9th and 12th chromosomes have been identified. The locus found on the 9th chromosome participated in the formation of traits responsible for the seedling weight and coleoptile length, two loci were found on the 5th chromosome, three loci on the 4th, and one — on the 12th chromosome. The information about *qCTS-9* and *qCTS-12* loci having the significant phenotypic effect (from 5.5 to 22.4%) found on the 9th and 12th chromosomes have been previously reported by Chinese and Indian scientists who worked mainly with populations based on interspecies hybrids [34, 35]. From one to three loci influencing the traits formation have also been found on the 5th and 4th chromosomes of the *indica* subspecies [25, 37]. The most often identified loci found on the 1st, 2nd, 8th chro-

mosomes were not related to polymorphism on the studied trait in the population of domestic rice samples, for which many loci linked to growth parameters were monomorphic that is explained by long-term task-oriented selection [33].

So, for the first time for the Russian rice gene pool, we have identified eight chromosomal regions related to the growth rate, and the technique which simplifies their identification when investigating unknown samples has been proposed. In order to detect the markers with the maximum phenotypic effect, a bulk breeding and varieties contrasting by a trait can be used. This will reveal the markers closely linked to the genes determining a trait in the identified chromosome region (http://www.gramene.org). If the study of polymorphism will confirm the possibility of reliable separation of varieties into contrasting groups using such markers, they can be used in the future work. As the result of our researches, it has been established that the weight of Russian varieties' seedlings is determined by the loci located in the regions of location of the RM261, RM6314, RM405, RM242 and RM463 markers on the 4th, 5th, 9th and 12th rice chromosomes respectively. Two chromosome regions on the 4th and 9th chromosomes (RM126, RM242) determines the embryo root length, the locus on the 5th chromosome located in the RM289 marker region determines the seedling height. The technique developed in these researches is applicable for mapping the loci of quantitative traits of other crops.

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