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Genetic Regulation Trait "Content of Microelements" in Grains of Russian Rice Varieties

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Abstract: ***Inadequate consumption of trace elements leads to metabolic disorders and the appearance whole range of diseases. First of all, these are diseases of the cardiovascular system, diabetes, and oncological diseases [14], [24]. Black and red grain rice is one of the most promising sources of antioxidants and microelements (from 5 to 20 times more than in white grain varieties). Due to these properties, black rice is recognized as the "Super Food of the 21st Century"[16],[18]. These elements and their compounds provide cancer prevention, reduce the risk of cardiovascular disease, atherosclerosis, type 2 diabetes, increase immunity, improve the synthesis of visual pigments, activate metabolic processes, etc.[26],[29]. It was established that intraspecific diversity by traits observed at the phenotypic level is associated both with structural and regulatory genes [15]. Data on the genetic regulation of the synthesis of substances that increase the nutritional value of grain (micro and macroelements, etc.) will intensify rice breeding in order to create varieties for the production of functional foods. The polymorphism Russian rice varieties was revealed by markers determining the nutritional value of rice, up to eight alleles per locus. Polymorphism was detected in genes linked to the content of signs: iron, zinc, magnesium, manganese, cobalt, calcium, phosphorus, potassium. To increase the efficiency of sampling by feature, four multiplex complexes were formed that control 11 loci that determine the content of micro and macro elements in rice samples. The first controls genes that determine content of Mn, Ca on chromosome 3 and Zn on the eighth chromosome. The second two genes that determine the iron content (on chromosomes 6 and 8) and manganese on the tenth chromosome. The third reveals polymorphism at the loci that determine the content of Zn, P, K on 5, 6, 8 chromosomes. The fourth multiplex complex allows us to assess the variability of two loci on the eighth and fifth chromosome***.

Keywords: rice, stained pericarp, microelements, SSR markers

1. Introduction

Microelements are responsible for the formation of the central nervous and cardiovascular systems, increase the efficiency of the body's immune system, and reduce the number of most common intrauterine abnormalities [17], [24]. Only 4% of people did not show any disorders in mineral metabolism, which are the root cause or indicator of many known diseases [6]. The use of trace elements in clinical medicine is still limited. Thus, in the treatment of certain types of anemia, iron, cobalt, copper, and manganese preparations are used [5], [28], [30]. Some of macro- and microelements contained in food products have antitumor activity: selenium, germanium, potassium, calcium, magnesium, manganese, molybdenum, copper, zinc [27].

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The development of cereal biofortification (especially rice rich in minerals) remains an effective means of combating malnutrition, especially since a significant decrease in the content of trace elements in varieties of vegetables and fruits created in recent years has been revealed [2]. Rice grains are important sources of trace elements such as iron (Fe), zinc (Zn), manganese (Mn), copper (Cu) and selenium (Se). The accumulation of micronutrients in the caryopsis (AMK) refers to traits controlled by quantitative loci (QTL). The range of variation this trait in crushed grains 700 rice samples was : Fe from 0.9 to 9.1 ppm (average value of 2.4); Zn from 5.8 to 29.6 ppm (16.4); Cd 0.002 to 0.054 ppm (0.009); Mn from 3.6 to 22.0 ppm (with an average value of 9.7), Cu from 0.8 to 7.5 ppm (with an average value of 3.2 ppm), Se from 0.01 up to 0.11 ppm (with an average value of 0.04 ppm). Most of the samples of the japonica subspecies have a higher content of Zn and Cu, but lower concentrations of Cd. For the remaining three characters, the distribution of phenotypic values between two subspecies noticeably overlaps, especially in the concentration of Se. The influence of many factors, including various environmental conditions, soils, on the values of this traits has been established. 38 alleles (47.5%) of 80 loci increased the signs of AMK, 42 (52.5%) decreased [21], [33].

Statistical values of the QTL effects for the AMK in polished grains are much lower compared to the QTLs found for the trait in husked rice grains [25], [34]. Donors and sources of increased trace elements in rice samples were identified: Fe more than 20 ppm (Annada, Nagina 22, Svarna, Varsha, Aghonobora), Zn more than 20 ppm (Nagina 22, Honduras, Annada, Lalat, Ratna, Tanu, Svarna, Pitchavari [3]. The genetic mechanism underlying the accumulation of minerals in the caryopsis remains largely unknown. QTL studies were carried out with various populations of rice: recombinant inbred and double haploid lines, groups of varieties contrasting in character (Lu et al., 2008). The use for this purpose of populations contrasting to this traits can significantly reduce the complexity of the study, but allows reveal only loci with significant contributions to the phenotypic manifestation of trait [8].

QTLs that determine trait AMK are grouped in chromosomes 2, 3, 4, 6, 7, and 11. In particular, there are three QTL regions that control the concentration of Cd in rice grains on chromosomes 4, 7, and 11, including one on chromosome 7 was found to be responsible for the formation of a trait in four different works. The gene was identified as OsNramp1; it is responsible for their accumulation in the aleurone layer, and not in the endosperm, which makes up the majority of the ground grain [4], [36]. Other genes identified as loci associated with the accumulation of trace elements were cloned, such as OsVIT and OsNAS for Fe, OsLCT1, and OsHMA3 for Cd, OsNramp5 and OsHMA4 for Mn [18]. Currently, several molecular biologists are attempting to improve the AMK of rice grains using endosperm-specific promoters [36].

In China, 698 collection samples, two subspecies indica and japonica, were studied to identify quantitative trait loci (QTL) associated with the accumulation of Fe, Zn, Cu, Mn, Mg, and Se. In total, 47 QTL regions were found in the genome, including 18 loci and 29 clusters (spanning 62 loci) responsible for formation trait in rice grain. It was found that 10 chromosomal regions associated with formation of trait (when studying contrasting varieties) are located in same regions where QTLs were previously detected when used for this purpose mapping populations [1], [11], [20]. In eight candidate genes in these regions, were identified on chromosomes 1, 4, 6, 7. A total of 192 candidate genes were isolated for further analysis using polymorphism of a million single nucleotide loci (SNPs) [37]. 37 genes (19.3%) showed a reliable association between QTL and trait variation in haplotypes by pairwise comparison. The phenotypic values of the haplotype traits of each candidate gene have been established. Both candidate QTL genes (qFe6-2 and qZn7) and genes that determine the main contribution to the formation of AMK traits (Fe, Zn, and Cd) were identified [32].

In studies where specialized populations were used to map the QTL for these loci, for example, backcrossed inbred lines (BILs) obtained from crosses between donor and elite varieties, the genetic background and genotype-environment interactions were evaluated [19],[22], [23] .

12 loci that determine the iron content in rice samples were identified: three loci are located on the first chromosome: qFe.1 - flanking markers RM259-RM243, qFe1.1 (RM243-RM488), qFe1.2 (RM488-RM490) [13], [21]. The genes associated with the iron content on the second chromosome qFe2-1, qFe.2 have been reported in several studies; they are located in the regions where the markers RM6641, RM53-RM300, and RM 452 are localized [4], [12], [35]. There are also 3 loci located on the twelfth chromosome that define the trait qFe.12, qFe12.1, qFe12.2; they are flanked by markers RM270-RM17, RM17-RM260, RM260-RM7102, respectively [1]. There are also reports of QTL defining trait on 5 (RM574 - RM122), 8 (RM137 - RM325A) and 11 chromosomes (RZ536 - TEL3) [12]. The zinc content in rice samples is determined by six loci on chromosomes 1 (RM34 - RM237), 3 (RM7 - RM517), 5 (RM421), 6 (RZ398 - RM204) and 12 (2 loci) (RM235 - RM17, RM260 - RM7102) [12], [13], [35]. 4 chromosome regions are responsible for the magnesium content: on 3 (in the region where the SSR marker RM5488 is located), 6 (OSR 21), 10 (RM467), 11 (RM332) chromosomes [1], [4], [35]. The manganese content in rice samples is determined by four loci: on 1 (RM243-RM312), 2 (RM6367), 3 (RM227-R1925), 7 RM214 chromosomes [4], [13], [35]. The phosphorus content is controlled by the polymorphism of two chromosome regions on the first and ninth chromosome in the regions where the markers RM 212 and RM 201 are located [4], [10]. Polymorphism of six chromosome regions is associated with copper content: on 2 (RM6378), 3 (R1925 - RM148), 5 (C1447 - RM31), 6 (RM204), 8 (RM201-C472), 11 (RM167) chromosomes [4], [13], [35]. Four loci determined the calcium content on 1 (RM6480), 3 (2 loci) (RM200 - RM227, RM5626 - RM16) and 5 chromosomes (RM598) [4], [7], [13].

2. Materials and methods

As material for research, we used 32 zoned and promising Russian rice varieties from the collection of the All-Russian Rice Research Institute: Khankaysky, Sadko, Primorsky, Liman, Garant, Pavlovsky, Rapan, Novator, Serpentin, Boyarin, Regul, Yantar, Zhemchug, Leader, Khazar, Amethyst, Narcissus, Druzhniy, Sprint, Viola, Dalnevostochniy, Fountain, Kasun, Jupiter, Atlant, Kurchanka, Phakel, Snezhinka, Charm, Anait, Flagman, Izumrud. Among them were varieties of various quality: short-grain, medium-grain, large-grain, long-grain, with various culinary and technological properties. The polymorphism of these varieties was studied using 13 markers distributed over 7 rice chromosomes: 1, 2, 3, 5, 6, 8, 10 (Table 1). We used markers associated with loci that control the content of microelements, nutritional value, culinary characteristics of rice grain.

Rice DNA was isolated from seedlings and leaves using the STAB method in various modifications. The polymerase chain reaction (PCR) and visualization of amplification products were performed according to the methodology of the International Rice Research Institute .

PCR parameters used in this experiment: 5 minutes at 94 ° C - initial denaturation, the following 35 cycles: 1 minute - denaturation at 94 ° C, 1 minute - annealing of primers at 55 ° C, 2 minutes - synthesis at 72 ° C; its last cycle is 7 minutes at 72 ° C. The PCR mixture included 40 ng DNA (2 µl), 1 µl (1 mm) deoxynucleotide triphosphates (dNTPs); 3.7 µl H2O; 1 µl of buffer solution, 0.5 µl (5 µM) of each primer, 1 µl (1.5 units) of Taq polymerase, in a total volume of 10 µl. The amplification products were separated by polyacrylamide gel electrophoresis at a voltage of 100 V.

3. Results

Study of Russian varieties using SSR markers distributed over the rice genome showed polymorphism in many of them (Table 1). In the table we also give other markers located in this chromosomal region, linked to loci that determine the content of trace elements and quality trait in rice samples. They are located no further than 20 cm from the gene of interest, and their polymorphism may also be associated with such trait.

Table 1: Polymorphism of Russian varieties detected by SSR markers located in the chromosome region, which determines the content of microelements and nutritional value of rice samples.

Polymorphic markers for the Russian rice varieties	Chromosome (amount alleles)	Other markers in this chromosome region, in parentheses the location of the marker on chromosome (cM)	Traits defined locus, in this chromosome
RM13	5 (3)	RM437, RM42, RM3777, RM1024, **RM13**, RM435, RM188, RM421 (4; 8; 13; 15; 20; 38; 61, 85)	Quality trait, nourishing value. Fe, Cu, Zn, Ca (Mahender et al., 2016)
RM 25	8 (2)	RM339, RM210; **RM25**; RM5556; RM310, RM3572	Zn, phosphorus, calcium. Fe, Cu (Kaiyang et al, 2008)
RM 30	6 (2)	**RM30**, OSR21, RM162**,**RM62 (204; 178; 156; 118 cM)	Quality trait, nourishing value, culinary properties. Fe, Cu, Zn, Mg (Garcia-Oliveira et al., 2008).
RM 53	2 (3)	RM236, RM424, RM555,**RM53**, RM183, RM44, RM521 (19; 23,4; 31,2; 31,9; 42,4; 44,4;45,8; 73,5)	Fe, protein content, Cu, Mn (Lee et al., 2014)
RM 126	8 (2)	RM38, **RM126**, RM210,RM 80, RM38 (19,9; 21; 21; 22,7; 34,9 cM)	Nutritional value, flavor (James et al., 2007; Zhong et al, 2011).
RM 162	6 (8)	RM30, **RM162,**RM62 (204; 156;118 cM)	Nutritional value, culinary characteristics (Garcia-Oliveira et al., 2008)
RM 227	3 (2)	RM200,**RM227**;RM1925;RM148	Mn, Ca; Cu, Zn, Mg (Kaiyang et al., 2008).
RM 256	6 (1)	RM3; RM241; RM253,**RM256,** RM190, RM204, RM217, RM225, RM241 (2; 4; 6; 6; 10; 14; 20,5; 26,5)	Nutritional value, culinary characteristics (Yun et al., 2014; Yu et al. (2009)
RM 258	10 (3)	RM184; RM271; **RM258**; RM171, RM228; RM229 (26; 37,5; 54; 73; 96,3 ;100)	Ca, nutritional value, culinary characteristic, Mg (Guerinot, Salt, 2014)
RM 259	1 (5)	RM431, RM337, RM490, RM**259,** RM323, RM495, RM753 (0,1; 1,1; 2;5; 6,2;8;16,7 cM)	Fe, nutritional value, culinary characteristics Ca, Zn, Mn, Co, K, P (Garcia-Oliveira et al., 2008) (Zhang et al., 2014)
RM 440	8 (3)	RM3155, RM149**,** RM515	Zn,P,K (Mahender, et al., 2014)
RM 574	5 (3)	RM 574(41-60 cM)	Fe, content of Cu, Zn, Ca (Zhang et al., 2014)
RM 590	10 (2)	RM333	Mn, nutritional value (Mahender et al., 2014)

The markers used in this work are located on seven chromosomes: 1, 2, 3, 5 (two loci), 6 and 8 (three loci), 10 (two loci), on which the maximum number of loci determining the nutritional value of rice is localized. Markers located on the above chromosomes allow controlling polymorphism by a complex of loci associated with the formation of a number of traits that determine the content of micro and macro elements in rice grains, including the accumulation of iron, magnesium, manganese, zinc, cobalt, calcium, phosphorus, and potassium. The studied markers make it possible to control the polymorphism of 3 loci associated with the formation of the trait “iron content” on 1, 2, 5, chromosomes. The distance to the gene of interest is less than 20 cm, the use of 2 flanking markers reduces the likelihood of a false positive conclusion.

To select a flanking marker, in the table we shows a number of markers that are also linked to the signs that determine the nutritional value in this region, the distance from which to the studied marker does not exceed or does not significantly exceed 20 cm. The ability to select a flanking marker allows simultaneously control several quality traits. The maximum number of alleles (five) associated with the formation of the trait “iron content” was noted using the marker RM 259 located on the first chromosome. Markers RM 227 and RM 590 located respectively on the third and tenth chromosomes allow controlling the trait “manganese content”. The polymorphism of two loci, but associated with the zinc content, is controlled by markers RM 25 and RM 440 located on the eighth chromosome. The remaining markers reveal polymorphism at the loci that determine the nutritional value, but in the region of their localization there are genes that determine a number of quality traits, and in each case, research is necessary to identify a microelement with different contents of which associated locus polymorphism.

To reduce the complexity samples evaluating on base of differences in sizes of amplification products and melting temperatures, we formed 4 multiplex complexes, three of which allow us to simultaneously assess polymorphism of some loci (Table 2).

4. Conclusions

Thus, we revealed polymorphism of Russian rice varieties by markers that determine the nutritional value, up to eight alleles per locus. Polymorphism was detected in loci linked with trait content of: iron, zinc, magnesium, manganese, cobalt, calcium, phosphorus, potassium. The presence of several alleles associated with quality traits revealed in the selection material allows efficient selection to increase the nutritional value of studied rice varieties. To increase the efficiency of sampling by feature, four multiplex complexes were formed. It control 11 loci that determine the content of micro and macro elements in Russian rice samples. The first controls the genes that determine the content of Mn, Ca on chromosome 3 and Zn on the eighth chromosome. The second multiplex complexes controls two genes that determine the content of iron (on chromosomes 6 and 8) and manganese on the tenth chromosome. The third helps to identify polymorphism at the loci that determine the content of Zn, P, K and other signs that determine the nutritional value on the 5, 6, 8 chromosomes. The fourth multiplex complex allows us to assess the variability of two loci on the eighth and fifth chromosome.

Table 2: Multiplex complexes for assessing the polymorphism of loci that determine the nutritional value of rice samples

Marker (chromosome)	Melting point, C^0	Amplification product size, b.p.	Locus determined
Multiplex complex 1			
RM 227 (3)	55	106	Mn, Ca
RM 25 (8)	55	146	Zn
RM 126 (8)	55	172	nutritional value
Multiplex complex 2			
RM 590 (10)	55	137	Mn
RM 259 (8)	55	162	Fe
RM 53(6)	55	182	Fe
Multiplex complex 3			
RM 30 (6)	55	105	nutritional value
RM 13 (5)	55	141	nutritional value
RM 440 (8)	55	169	Zn , P, K
Multiplex complex 4			
RM 256 (8)	55	127	nutritional value
RM 574 (5)	55	155	Fe

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