Locus determining 1000 grains weight at high temperatures (Russian rice varieties)

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Abstract. In Russia, increase in average air temperatures was noted in comparison with long-term data. Earlier it was shown that increase in average temperatures leads to a decrease in the productivity of plants for certain characteristics up to 90% and a yield of up to ten percent with an increase of 1 °C. High temperatures above 30 °C reduce the grain content, the total number and number of spikelets, weight grains from a plant, the mass of a thousand grains. A chromosomal region was established that reliably (at significance level 0.05) contributing to the formation trait mass of 1000 grains in domestic rice varieties in the region of localization of the RM 509 marker. A decrease significance level to 0.09 made it possible to identify another chromosome region with a possible localization of genes, determining the mass of 1000 grains at high temperatures. It is located in region of localization RM 13 marker. Four chromosomal regions have been identified that significantly contribute to the redistribution of plastic substances between grain and chaff under stress in domestic rice varieties on the first, fourth, ninth chromosomes (two loci) in the regions of localization of markers RM140, RM 261, RM 245, RM242 respectively.

1. Introduction
Recently, climate change has affected many regions of the world, including Russia, where increase in average air temperatures was also noted compared to long-term data. Forecasts predicting a further increase in temperature up to 5 °C by the end of the twenty-first century are not comforting either [1-2].

Earlier it was shown that an increase in average temperatures leads to a decrease of productivity plants for individual characteristics up to 90% and a yield of more than ten percent with an increase of 1-2 °C. High temperatures above 30 °C reduce grain content, total number, and number of spikelets completed, the mass of grain per plant, the mass of a thousand grains. The analysis of the yield of many agricultural crops in recent years has shown its decline by 5-10% due to exposure high temperatures and drought. Temperatures over 30–35 °C during the tillering and flowering phases reduce grain content of rice panicle; in some varieties empty grain is noted under stress more than 80% [3-5].

The total number of spikelets and productive stems, weight of 1000 grains, also decreases. Non-cracking of anthers and sterility of pollen are one of the mechanisms leading to empty grain in plants.
5-6]. The graininess of the panicle also depends on the flowering time; earlier flowering during the day or shortening of the growing season allows avoiding exposure to critical temperatures during fertilization [7-8]. The mass of 1000 grains is the most constant characteristic for crops, however, it is also subject to change under stress. Thus, in rice, it at 35 °C in the flowering phase decreased on average by 20% when studying 30 varieties, the variation of the trait ranged from 2 to 47%, and intravarietal variability of trait was also noted. High temperature tolerance loci have been mapped on many chromosomes in rice. On chromosomes 1 and 6, there are two regions that influence the formation of the trait. On chromosome 4, there is one qHTSF4.1 locus, which increases the grain size of the panicle by 15%. QTL loci with less significant phenotypic manifestation were also found on other rice chromosomes [9-10].

However, breeding of domestic rice varieties for adaptability to high temperatures has not been previously carried out, and the polymorphism of the loci determining the trait has not been studied either.

Research goal. Identification of loci that determine the mass of 1000 grains in domestic rice varieties when exposed to high temperatures.

2. Materials and Methods
Phenotyping of 23 domestic rice varieties (Table 1) by trait was carried out from 2013 to 2016. Plants of the control and experimental variants were grown on a vegetation plot with optimal doses of mineral nutrition (N120P60K60). Before flowering, vessels with plants (10 per vessel) were brought into the chambers. Sample of 20 plants of the variety per variant of the experiment. To isolate DNA from rice seedlings and leaves, the STAB method with modifications was used. Polymerase chain reaction (PCR) and analysis of the resulting amplification product were performed according to the method of the International Rice Institute. In the experiment, the following PCR parameters were used: initial denaturation for five minutes at 94 °C, thirty-five cycles: 60 sec. - denaturation 94 °C, 60 sec. - annealing of primers at 55 °C, 120 sec - synthesis 72 °C; elongation - 420 sec. 72 °C. PCR reaction volume - 10 μl: DNA - 2 μl, 1μl (1 mM) deoxynucleotide triphosphates; 3.7 μl H2O; 1 μl - PCR buffer solution, 0.5 μl - each primer, 1 μl - Taq - polymerase. Electrophoresis was carried out using a polyacrylamide gel at a voltage 100 V. Data processing was carried out in the Statistica 10 program; the method of group selection was used for marking [13]. The 42 SSR markers under study were distributed across all 12 rice chromosomes.

3. Results
The carried out phenotyping made it possible to divide varieties into groups with different resistance to stress (table 1). Rice cultivars were labeled using forty-two molecular SSR markers, both neutral and adaptive.

| Table 1 | Influence of air temperature on the trait "Weight of 1000 grains" in rice varieties. |
Variety | Weight of 1000 grains, g | Variability, %
--- | --- | ---
VNIIR 8242 | 12.3 | 22.5* | 45.2
Amethyst | 18 | 24.1* | 25.4
Atlant | 20.8 | 21.7 | 4.5
Boyarin | 21.4 | 23.6 | 9.5
Guarantee | 15.8 | 22.9* | 31
Druzhnyy | 18.5 | 20.6 | 8.2
Zhemchug | 14.8 | 21.9* | 32.2
Izumrud | 19.3 | 22.9* | 15.5
Kasun | 19 | 23.2* | 18.1
Kurchanka | 22.7 | 23.1 | 1.9
Lyman | 16.7 | 22.7* | 26.4
Narcissus | 15 | 28.8* | 47.9
Novator | 15.2 | 21.6* | 29.4
Pavlovsky | 25.6 | 26.7 | 4.2
Regulus | 18.9 | 23.3* | 19.1
Sadko | 20 | 23.0* | 13
Serpentine | 18.4 | 21.3* | 13.8
Snezhinka | 18.8 | 23.6* | 20.6
Sprint | 17.9 | 22.9* | 21.7
Strelets | 15.1 | 22.2* | 31.9
Fakel | 21.7 | 24.5* | 11.5
Fontan | 21.1 | 23.9* | 11.7
Khazar | 13.8 | 20.9* | 30.7
Khankayskiy | 18.9 | 25.1* | 24.9
Jupiter | 16.7 | 20.1* | 16.6
theaverage | 18.2 | 23.0* | 20.6
max | 25.6 | 28.8* | 47.9
min | 12.3 | 20.6* | 1.9

* - statistically significant at P <0.05

Analysis of variance made it possible to establish a relationship between stress resistance and variability of varieties for individual SSR markers (table 2).

**Table 2.** Analysis of variance of the relationship between stress resistance for the studied trait and polymorphism of rice varieties for SSR markers.
Since the gene plasma in the study is genetically heterogeneous, the work could identify the loci with the most significant contribution to the phenotype. The influence of the genotype of the variety on the manifestation of the trait was not taken into account.

But, even with such assumptions, we identified a chromosomal region that reliably (at a significance level of 0.05) contributes to the formation of a trait in domestic rice varieties in the area of localization of the RM 509 marker (Figure 1).
Figure 1. Division of rice varieties into contrasting stress resistance groups using SSR markers. Y-axis is relative size of amplification products. 1 - 2 groups of varieties resistant and not resistant to stress on trait “weight of 1000 grains.”

A decrease in the significance level to 0.09 made it possible to isolate one more chromosome region with a possible localization of genes that determine the weight of 1000 grains of domestic rice varieties at high temperatures. It is located in the region of localization RM 13 marker. Earlier, qRTT5-1, AQFW115-SPKNB, qFER-5, qYI-5 genes were localized in this region, which respectively determine the thickness of the root system (root thickness), the number of spikelets (spikelet number), their fertility (spikelet fertility), grain yield. All of the above signs are to one degree or another associated with the formation of the trait the mass of 1000 grains, which indirectly confirms the possibility of participation of the identified region in the formation of trait.

Another group of genes associated with the formation of mass of 1000 grains under stress are genes affecting the redistribution of plastic substances between the chaff and the forming caryopses; a significant effect on trait has been proven for 4 loci located in the regions of localization of markers RM245, RM140, RM261, RM242 (Table 3, Figure 2).
Figure 2. Division rice varieties into contrasting to stress resistance groups using SSR markers. Y-axis is relative size of amplification products. 1 - 2 groups of varieties resistant and not resistant to stress on trait "chaff mass".

Table 3 Analysis of variance of the relationship between stress resistance and polymorphism of rice varieties by SSR markers on trait "chaff mass".

|       | Sum.kv. | St.Sv. | Wed. kv. | Sum.kv. | St.sv. | Wed.  | F     | p    |
|-------|---------|--------|----------|---------|--------|-------|-------|------|
| RM245 | 8.86    | 1.00   | 8.86     | 37.91   | 20.00  | 1.90  | 4.67  | 0.043|
| RM140 | 1.05    | 1.00   | 1.05     | 2.22    | 20.00  | 0.11  | 9.45  | 0.006|
| RM261 | 1.05    | 1.00   | 1.05     | 4.22    | 20.00  | 0.21  | 4.98  | 0.037|
| RM242 | 0.72    | 1.00   | 0.72     | 3.15    | 20.00  | 0.16  | 4.57  | 0.045|
| RM6811| 2.74    | 1.00   | 2.74     | 17.08   | 20.00  | 0.85  | 3.21  | 0.088|
| RM289 | 0.50    | 1.00   | 0.50     | 2.77    | 20.00  | 0.14  | 3.64  | 0.071|

4. Conclusions
1. A chromosomal region has been established that reliably (at a significance level 0.05) contributes to the formation of trait 1000-grain mass in domestic rice varieties in the area of localization of the RM 509 marker.

2. Decrease in the significance level to 0.09 made it possible to isolate one more chromosome region with a possible localization of genes that determine the mass of 1000 grains of domestic rice varieties at high temperatures. It is located in the region of localization of the RM 13 marker. Earlier, the qRTT5-1, AQFW115-SPKNB, qFER-5, qYI-5 genes were localized in this region, which respectively determine the thickness of the root system (root thickness), the number of spikelets (spikelet number), their fertility (spikelet fertility), grain yield.

Four chromosome regions were identified that reliably (at a significance level of 0.05) contribute to the redistribution of plastic substances between grain and chaff under stress in domestic rice varieties on the first, fourth, ninth chromosomes (two loci) in the regions of localization of markers RM140, RM 261, RM 245, RM242 respectively.

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