Revealing the component composition of the multilinear millet variety Quartet during long-term seed production

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Abstract. Reducing the pesticide load on natural and agroecosystems in the production of agricultural products in crop production is the most pressing problem of the XXI century. Use of disease-resistant varieties of agricultural crops, which makes it possible to completely exclude the use of some of the pesticides required in the technological cycle, is an ecologically and economically significant factor in modern production. Particularly important in the cultivation of disease-resistant varieties is to reduce the probability of the pathogen developing new virulent properties that overcome this resistance of the variety with a long-term existence in production. These conditions are met by multilinear varieties that are heterogeneous in nature. As an object of analysis, we took the Quartet, multilinear in terms of smut resistance, common millet variety, which has been in production since 2001. The variety, with more than 20 years of cultivation in Russia, neighboring countries, as well as Germany and Switzerland, has shown not only excellent yielding qualities, but also actual resistance to local populations of the pathogen. The authors analyzed the difference from the original composition of the component composition of the variety after many years of production, when reseeding with its own seeds, in the conditions of one farm. On the farm, the obligatory method of treating millet seeds against smut was excluded from the cultivation technology.

1. Introduction
One of the main provisions of modern biological economy is the strengthening of natural mechanisms of regulation of plants and animals within the agroecosystem.

Earlier, during the cultivation of local varieties-populations, many diseases of cultivated plants were of a limited local character and did not cause massive epiphytoses. This can be clearly seen on the example of the interaction of the obligate parasite of smut and millet plant. Indeed, until the XIX century, millet was almost the main human food throughout Eurasia. The cultivated local varieties were practically not affected by smut. Local millet in commercial crops is mainly a variegated mixture of varieties. These varieties differ sharply from each other in the shape and color of the panicle, in the size, shape and color of the grain, in the growing season (the difference in maturation of various varieties reached 2...3 weeks), the degree of shedding, etc. [1]. The disease occurred, but its level was scanty, in the list of the main diseases of millet, it was assigned a secondary role [2,3]. Mass epiphytoses of smut diseases on millet (the level of damage up to 80%) arose in the middle of the second half of the XX century, with the introduction of more productive varieties from other regions and the establishment of selection [4]. New varieties began to displace local varieties of the population, depleting the genetic balanced polymorphism for smut resistance [5].

Study of the individual characteristics of different races and populations of smut Sporisorium...
destruens (Schlecht) Yanky, also genes of resistance of millet to them (Sp), was most fully carried out in the Research Institute of Agriculture of the South-East. 17 races of smut were identified for virulence, more than 10 pathotypes and 7 genes of millet resistance to it were identified [6]. The races are widespread - 1, 2, 5, 8, and 9. The rest of the pathotypes (3, 4A, 6, 7, 7A, 10, 11 and 12) are of local importance [7].

For the first time in the history of millet breeding in VNIIZBK in 1998, a multilinear variety of common millet Quartet was created, included in the State Register of the Russian Federation since 2001 in the Central Chernozem region and since 2002 in the Central regions and consisting of 4 lines - analogues with effective dominant genes for race-specific smut resistance $Sp_1, Sp_2, Sp_3$ and $Sp_4$ [5].

The purpose of our experiment was to identify a change in natural conditions of long-term production - a redistribution of the original genetic components (lines) of a multilinear variety against the background of the lack of chemical protection against disease, laid down in the basic ratio of 3:3:3:1.

2. Materials and methods
The multilinear millet variety Quartet has been continuously cultivated in the Streletskskoye experimental farm in the Oryol region since 1998 for production and seed purposes, when replanted with its own seeds, without dressing before sowing. Other varieties of millet are not used on the farm. According to the State Sorting Commission of the Russian Federation, in some years for the period 2001-2019, manifestation of smut affection of crops of different varieties of millet at the variety plots of the Oryol region reached 4%. In the Streletskskoye experimental farm for 22 years no smut infestation of industrial crops of the Quartet variety was found during annual testing. Visual examination in 2016-2020 also did not reveal manifestation of smut in the production crops of the farm.

Experimental infectious plots of 2018-2020 were located on the selection crop rotation of the Federal State Budgetary Scientific Institution FNTs ZBK. The area of each plot (width 1.65 m, length 2 m, 4 rows, row spacing 0.45 m) was 3.3 sq. m. The soil is dark gray forest, medium loamy, medium cultivated, mircorelief of the site is leveled. According to the main physical and chemical parameters, this soil is typical for the natural and economic zone. The arable and meter-long soil layer is characterized by high retention capacity (118…345 mm). Possible reserves of moisture available to plants in the soil layer 0…35 cm correspond to 88 mm, and in the meter layer 262 mm. The maximum gyroscopic moisture content is 6.8...7.5% of the soil mass, the moisture content of stable wilting is 9.6...13.3%. Sowing was carried out in mid-May, with a cassette seeder, 200 seeds per row, based on the rate of 2.7 million plants per hectare, with a germination rate of 90%. As a control for smut, plots with differentiator lines were laid (Sp0…Sp7).

In 2018, the seeds obtained from the Streletskskoye experimental farm varieties of millet Quartet (harvest 2015) were used to determine the degree of infestation by pure races of smut (1, 2, 3, 4, 6a, 8, and 12) and their mixture (1+6A+8), at maximum infestation 2%. The experiment also included millet varieties completely unstable to smut (Sp0) with seeds of various sizes (M1000 = 7,5…9,6 g). The races of smut reproduced in 2016 were obtained from the Agricultural Research Institute of the South-East from the head of the millet breeding laboratory Tikhonov N.P.

In 2018, 250 panicles of mature plants were selected and threshed directly from the production planting of the millet variety Quartet of the Streletskskoye experimental farm. The yield of each plant was sown in 2019 in separate rows in the plot to identify the proportion of the original lines of the cultivar with different resistance genes, with a maximum infestation of 2% by different races (1, 2, 6A and 8), smut - 60 cultivars, and by race 1 - 240 specimen.

In 2020, according to the results of 2019, against an infectious background of 5 races of smut (1, 2, 3, 6A and 8), a refinement experiment was laid to identify the resistance of the offspring of panicles selected in 2018 from the production planting of the Streletskskoye experimental farm millet varieties Quartet on 100 plots.

The identification of cultivars of common millet was carried out according to the resistance and susceptibility of Sp genes to smut races (according to the methods of Tikhonov N.P., 1991, 2006,
Table 1. Identification of smut resistance genes in cultivars of common millet.

| Race 1 | Race 2 | Race 6A | Race 8 | Race 3 | Assumed resistance genes |
|--------|--------|---------|--------|--------|---------------------------|
| S      | S      | S       | S      | S      | Sp 0                       |
| R      | S      | Rdw     | R      | S      | Sp 1                       |
| R      | R      | R       | S      | S      | Sp 2                       |
| S      | R      | R       | R      | R      | Sp 3                       |
| R      | R      | S       | R      | S      | Sp 4                       |
| Rdw    | S      | S       | Rdw    | S      | Sp 5a                      |
| R      | S      | S       | R      | S      | Sp 5b                      |
| S      | S      | S       | R      | S      | Sp 6a                      |
| Sdw    | Sdw    | S       | R      | S      | Sp 6b                      |
| Rdw    | Rdw    | S       | Rdw    | S      | Sp 7                       |

Notes: R and S - stability and susceptibility; dw – dwarfism.

3. Results

According to the results of the analysis, it was revealed that for the period 2001-2018 the original genetic polymorphism of the multilinear cultivar is preserved (genes Sp1, Sp2, Sp3, Sp4). The formation of separate lines with the properties of smut resistance, which are not typical for initially clean lines, was also noted. The main analysis indicators for the ratio of genetic components for head smut resistance are presented in the table 2.

Table 2. Results of identification of the genetic components of the millet variety Quartet on an infectious background in the Federal Research Center ZBK, 2019.

| Trial | Number of variety samples | Sp1 | Sp2 | Sp3 | Sp4 | Indefinitely / culling * |
|-------|---------------------------|-----|-----|-----|-----|--------------------------|
| Infectious background at 2% spore load by different races (1, 2, 6A, 8) smut, pcs. | 52 | 16 | 17 | 5 | 6 | 8 |
| Proportion | 100% | 30.8% | 32.7% | 9.6% | 11.5% | 15.4% |
| Infectious background at 2% spore load of one race (race 1) smut, pcs. | 232 | x | x | 32 | x | 13 |
| Proportion | 100% | - | - | 14.6% | - | x |

*) The reaction of millet cultivars to different races of smut did not match the identification key, or the cultivar was culled for technical reasons.

As a result of comparing the proportion of surviving plants, there is a general decrease in plant survival (average 10%) in the case of their genetic resistance against a severe infectious background of the pathogen. There is a greater number (diseased and healthy) surviving harvesting plants in an unstable genotype (table 3) with linear correlation coefficient $r = 0.7$. This effect is especially noted on races 2, 6A, 8, and insignificant on race 1 of smut (picture 1).
Table 3. Average survival rates and damage to millet plants against the background of 2% infestation by different races of smut, 2019.

| Resistance gene | Number of samples, pcs. | Race 1 | Race 2 | Race 6A | Race 8 |
|----------------|-------------------------|--------|--------|---------|--------|
|                | Survived, pcs / sample | Survived, pcs / sample | % damage | Survived, pcs / sample | % damage | Survived, pcs / sample | % damage |
| Sp1            | 16                      | 21.2   | 0      | 28.9    | 48.3   | 10.7   | 0                    |
| Sp2            | 17                      | 21.1   | 0      | 24.6    |        | 20.3   | 0                    |
| Sp3            | 5                       | 16.5   | 10.7   | 20.4    | 17.7   | 16.5   | 0                    |
| Sp4            | 6                       | 16.3   | 0      | 20.5    | 0      | 22.8   | 49.9                 |
| Indefinitely   | 8                       | 14.5   | 4.7    | 18.8    | 13.3   | 12.9   | 5.5                  |

Figure 1. Average degree of damage to the identified individual components of millet Quartet with resistance genes Sp1…Sp4 on a hard infectious smut background (2%), 2019.

In 2020, the genotypes that gave an uncertain result for the identification of the resistance gene were again analyzed against an infectious background by races 1, 2, 3, 6A, 8 smut. The offspring of plants No.72 and No.167 confirmed the absence of the resistance gene (Sp0). Selection No.135 confirmed resistance to 1, 2, 6A and 8 races of smut, but was unstable to 3 races. Selection No.134 contained the gene Sp2. Отбор No.3 2 was not affected by 1, 3, 8 races, but was unstable to 2 and 6A. Offsprings of plant No.107 confirmed instability to 1, 2, 3, 6A races and resistance to 8 race.

It was noted that the visual degree of damage to different genotypes varied greatly - from single diseased plants to 80% damage against the background of one smut race, with a stable 60...90% damage to the control with Sp0.

4. Summary and discussion

For more than 20 years in production, when replanting with its seeds, the Quartet variety retained the
originally established genetic polymorphism of the multilinear variety (genes $Sp1$, $Sp2$, $Sp3$, $Sp4$) in ratio $31:32:10:12$ and $15\%$ of component with altered resistance genes, i.e. almost in the original basic ratio of clean lines $3:3:3:1$ with minor changes. In the process of long-term cultivation, when a multilinear millet variety is sown with its own seeds, the built-in complex resistance to smut remains at a sufficient level for a long existence of the variety in production.

It can be assumed that the regression of the line with the $Sp3$ gene from a level of $30\%$ to a level of $10\%$ indicates the existence in natural conditions of the $1$ smut race [8], which causes outbreaks of the disease (2% damage) in the Oryol region over the years.

As a result of interaction within a heterogeneous variety between neighboring flowering plants, processes are taking place that create components that are unstable and stable on other principles than those originally embedded in the baseline genome. This is also indirectly evidenced by the manifestation of different levels of damage of different genotypes, with the same identified resistance gene, on the same smut race and located on neighboring plots. The revealed effect of reducing survival rate of resistant component of multilinear cultivar against background of pathogen effect requires additional verification.

Summing up the results of more than 20 years of existence of the multilinear millet variety Quartet in production, we can conclude that heterogeneous varieties are more adaptable to changing conditions due to changing component ratios and the emergence of new protective properties not previously inherent in these components. The multilinear variety limits the spread of the pathogen to a negligible level in a natural way, without any pesticide treatments and does not reduce its yield and quality indicators.

5. References
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