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Address: PO Box 463, office 456, SFSCA RAS Building, Krasnoobsk, Novosibirsk District,
Novosibirsk Region, 630501, Russia. Tel/fax: +7-383-348-37-62
e-mail: sibvestnik@sfsca.ru, vestnik.nsk@ngs.ru; www. sibvest.elpub.ru

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SIBIRSKII VESTNIK SEL'SKOKHOZYAISTVENNOI NAUKI

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Тел./факс (383)348-37-62 e-mail: sibvestnik@sfsca.ru, vestnik.nsk@ngs.ru; https://sibvest.elpub.ru/jour

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ВЛИЯНИЕ СИСТЕМ ОСНОВНОЙ ОБРАБОТКИ НА ИЗМЕНЕНИЕ АГРЕГАТНОГО СОСТАВА ТЕМНО-СЕРОЙ ЛЕСНОЙ ПОЧВЫ В СЕВЕРНОМ ЗАУРАЛЬЕ

(✉)**Перфильев Н.В., Вьюшина О.А.**
Научно-исследовательский институт сельского хозяйства Северного Зауралья – филиал Тюменского научного центра Сибирского отделения Российской академии наук
Тюмень, Россия
(✉)e-mail: p.nikolay52@yandex.ru

В длительном стационарном опыте (1988–2019 гг.) изучено воздействие отвальной, безотвальной, комбинированной, дифференцированной, поверхностной, плоскорезной систем основной обработки на изменение агрегатного состава темно-серой лесной почвы. Исследования проходили в северной лесостепи Северного Зауралья (Тюменская область). За 30-летний период использования в пашне почва слоя 0–20 см сохраняла структурное состояние, не уступающее исходному по большинству исследуемых вариантов обработки. С увеличением глубины профиля почвы до слоя 0–30 см за счет повышения доли глыбистой фракции в нижнем слое 10–30 см происходило снижение содержания агрономически ценной структуры в сравнении со слоем 0–20 см, а также с исходным состоянием по большинству исследуемых систем обработки. Самое высокое содержание агрономически ценной структуры в слое почвы 0–20 см было по отвальной, плоскорезной и дифференцированной системам обработки (72,8–77,8%). За 30-летний период содержание агрономически ценной структуры (10–0,25 мм) в слое почвы 0–20 см увеличилось по данным обработкам на 6,12–13,45%, коэффициент структурности – на 21,9–60,3%. По остальным системам обработки содержание данной фракции (67,5–69,8%) и коэффициент структурности (2,07–2,31) были близкими исходному состоянию – 68,6% и 2,19 соответственно. Средневзвешенный диаметр агрономически ценных агрегатов увеличился от 2,71 мм при исходном состоянии до 3,00–3,29 мм (7,7–21,2%) за счет существенного увеличения доли этих агрегатов в слое 0–10 см. В целом по профилю почвы 0–30 см самые высокие показатели структурного состояния оставались по отвальной, плоскорезной и дифференцированной системам обработки. Безотвальная, комбинированная и поверхностная обработки снижали в сравнении с отвальной системой содержание агрономически ценной структуры на 9,7–15,9%, вели к снижению коэффициента структурности на 0,99–1,39 ед.

Ключевые слова: структура почвы, система основной обработки, коэффициент структурности, средневзвешенный диаметр агрегатов

EFFECT OF TILLAGE SYSTEMS ON CHANGES IN THE AGGREGATE COMPOSITION OF DARK GRAY FOREST SOILS IN THE NORTHERN TRANS-URALS

(✉)**Perfilyev N.V., Vyushina O.A.**
Scientific Research Institute of Agriculture for Northern Trans-Ural Region - Branch of Tyumen Scientific Centre of Siberian Branch of the Russian Academy of Sciences
Tyumen, Russia
(✉)e-mail: p.nikolay52@yandex.ru

In a long-term stationary experiment (1988-2019) the impact of mouldboard, non-mouldboard, combined, differentiated, surface and sweep-blade tillage systems on the change in the aggregate

composition of dark gray forest soils was studied. The research took place in the northern forest-steppe of the Northern Trans-Ural (Tyumen Region). Over a 30-year period of use in arable land, the soil of 0-20 cm layer retained its structural condition not inferior to the initial one for most of the studied variants of cultivation. With increasing the depth of the soil profile up to 0-30 cm layer due to an increase in the proportion of clumpy fraction in the lower layer 10-30 cm there was a decrease in the content of agronomically valuable structure compared to the layer 0-20 cm, as well as with the initial state on most of the studied systems of cultivation. The highest content of agronomically valuable structure in the soil layer 0-20 cm was on the mouldboard, sweep-blade and differentiated tillage systems (72.8-77.8%). Over the 30-year period, the agronomically valuable structure content (10-0.25 mm) in the soil layer 0-20 cm increased by 6.12-13.45% in these treatments, the structure coefficient by 21.9-60.3%. For the other treatment systems, the content of this fraction (67.5-69.8%) and the structure coefficient (2.07-2.31) were close to the initial condition - 68.6% and 2.19, respectively. The average weighted diameter of agronomically valuable aggregates increased from 2.71 mm in the initial condition to 3.00-3.29 mm (7.7-21.2%) due to a significant increase in the proportion of these aggregates in the 0-10 cm layer. In general, the highest indicators of the structural condition of the soil profile of 0-30 cm remained on the mouldboard, sweep-blade and differentiated systems of cultivation. Non-mouldboard, combined and surface tillage reduced the content of agronomically valuable structure by 9.7-15.9% compared with the mouldboard system, and led to a decrease in the coefficient of structure by 0.99-1.39 units.

Keywords: soil structure, tillage system, structure coefficient, average weighted diameter of aggregates

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INTRODUCTION

In the process of active agricultural use of arable land for many years the state of soil fertility can change both positively and negatively. It depends on the conditions of arable land use, the degree of compliance with basic scientifically sound parameters of zonal farming systems, concerning its main elements.

Taking into account the importance of all components of these elements, tillage, especially the basic one, takes an important place in the formation of soil fertility. Tillage is the main tool for regulating agrophysical properties of soil, on which its biological activity, nutrient regime, phytosanitary state of crops, and fertility depend [1].

One of the important indicators of the agrophysical properties of soil is its structure, i.e. the ability of the soil to break up into separate aggregates (lumps), which vary in shape and size [2, 3]. In turn, changes in the aggregate composition lead to changes in the physical properties of the soil [4, 5]. A decrease in the content of agronomically valuable aggregates and an increase in lumpiness lead to a decrease in crop productivity [6].

Currently, there are many techniques and methods of tillage of varying degrees of inten-

sity, as well as conflicting opinions on which of them most effectively influence the improvement of its agrophysical properties. Some scientists believe that, when minimized, tillage improves soil structure [7-10], while others are supporters of conventional tillage technology [11, 12]. Researchers face the task of overcoming these contradictions [13]. In this regard, the development of tillage systems that ensure the formation and sustainable maintenance of optimal parameters of soil agrophysical properties, including its structural condition, is an urgent task of modern agriculture [14].

The purpose of the study is to determine the effect of different tillage systems on the structural and aggregate composition of dark gray forest soils during their long-term use in the northern forest-steppe conditions of the Tyumen region.

MATERIAL AND METHODS

The effect of different systems of main tillage with elements of minimization under long-term application (completion of the 6th rotation of crop rotation) on the structural-aggregate state of the dark gray forest heavy loamy soil was studied. The experiment was laid in the experimental field of the Research Institute of Agriculture of the Northern Trans-Ural Region, branch of the Tyumen Scientific Center of the Siberian Branch of the Russian Academy of Sciences (57006°, 39 "N; 65025°, 22 "E), altitude - 101 m above sea level. The experiment was carried out in 1988-2019 in a cereal fallow rotation of complete fallow - winter rye - spring wheat - leguminous plants - spring barley, deployed in time and space. Agrochemical characteristics of the soil: humus content 4.2-5.0%, pH of the salt extract - 6.0-6.4, depth of the humus horizon - 25-27 cm. The sum of the absorbed bases in the arable layer is 18,6-25,6 mg-eq./100 g of soil. The content of mobile nutrients in the 0-20 cm layer before sowing: $N\text{-}NO_3$ - 4.26-6.57 mg/kg, P_2O_5 - 12.5-15.6 mg/100 g, K_2O - 15.7-17.2 mg/100 g.

The experiment scheme included six variants of the main tillage system:

– mouldboard - plowing to a depth of 20-22 cm;

– non- mouldboard - subsurface loosening at 20-22 cm;

– combined - alternation of plowing and subsurface loosening at 20-22 cm;

– differentiated - in fallow and after winter rye, cultivation at 12-14 cm, plowing at 20-22 cm for legumes, discing at 10-12 cm for barley and after it;

– flat-cut - annual cultivation with Smaragd-6 cultivator at 12-14 cm;

– surface - annually disking at 10-12 cm.

Experimental variants were laid on fertilized background at the rate of $N_{40}P_{40}K_{40}$ kg a.i. per 1 ha of crop rotation area. In spring, the conventional pre-sowing treatment and seeding with SZP-3,6 seeder was carried out. Treatment against weeds was carried out by general background herbicides. Crop residues were shredded during harvesting and left in the field for incorporation into the soil during tillage. Soil sampling for analysis was conducted at the beginning of the experiment (1988) and at the end of the 6th rotation (2019) of the crop rotation in 6-fold replications along layers 0-10, 10-20, and 20-30 cm. Aggregate soil composition was determined by the method of N.I. Savvinov, the coefficient of structure was calculated in accordance with the existing recommendations. Mathematical processing of the data was carried out according to B.A. Dospekhov and O.D. Sorokin [1-3]. The weighted average diameter (WAD) of the aggregates was determined by the formula

$$WAD = d_1xP_1 + d_2xP_2 + d_nxP_n/100,$$

where d_1, d_2, d_n – average diameter of the aggregate fraction, mm; P_1, P_2, P_n – content of the appropriate fraction, %.

[1] *Vadyunina A.F., Korchagina Z.A.* Methods for studying the physical properties of soils. Moscow: Agropromizdat. 1986. 416 p.
[2] *Dospekhov B.A*. Methodology of field experiment; ed. 4-th revised and supplemented. Moscow: Kolos, 1979. 416 p.
[3] *Sorokin O.D.* Applied statistics on the computer. Krasnoobsk: SUE EBCA SB RAAS, 2004. 162 p.

RESULTS AND DISCUSSION

Our results of determining the soil structure showed that the initial condition of the 0-30 cm soil layer of the experimental plot was generally quite satisfactory (see Table 1).

The content of agronomically valuable fraction of the aggregates (10.00-0.25 mm) was 68.0%, i.e., slightly below the excellent score (80%) according to the evaluation criterion of S.I. Dolgov and P.U. Bakhtin, which is explained by the specifics of the mechanical composition of heavy loamy soil - their great connectivity, significant content of physical clay in such soil [4,5]. While the aggregates of clay fraction more than 0,25 mm (5,11%) were slightly present in the total structure of the microstructure, more than a quarter (26,8%) were the aggregates of clay fraction more than 10 mm, which, as a result of soil moistening, freezing and thawing, can break down and thereby replenish the share of the agronomically valuable structure (10,00-0,25 mm) [15]. At the same time, the structure coefficient (Cstr) in the 0-30 cm layer was 2.13, which characterizes the structural condition as excellent (see Table 1)[6] [2].

The use of dark gray forest soils in the arable farming during the cultivation of cereals in a cereal fallow rotation for six rotations with the use of different systems of main tillage has led to changes in the structural condition of the soil, which consists in quantitative redistribution in comparison with the initial state of the fractional composition of the structure on the soil horizons.

Табл. 1. Влияние систем основной обработки почвы на структурное состояние слоев почвы 0–20 и 0–30 см по завершении 6-й ротации зернопарового севооборота (1988–2019 гг.)

Table 1. Effect of tillage systems on the structural condition of 0–20 and 0–30 cm layer at the end of the 6th rotation of grain and fallow crop rotation, (1988-2019).

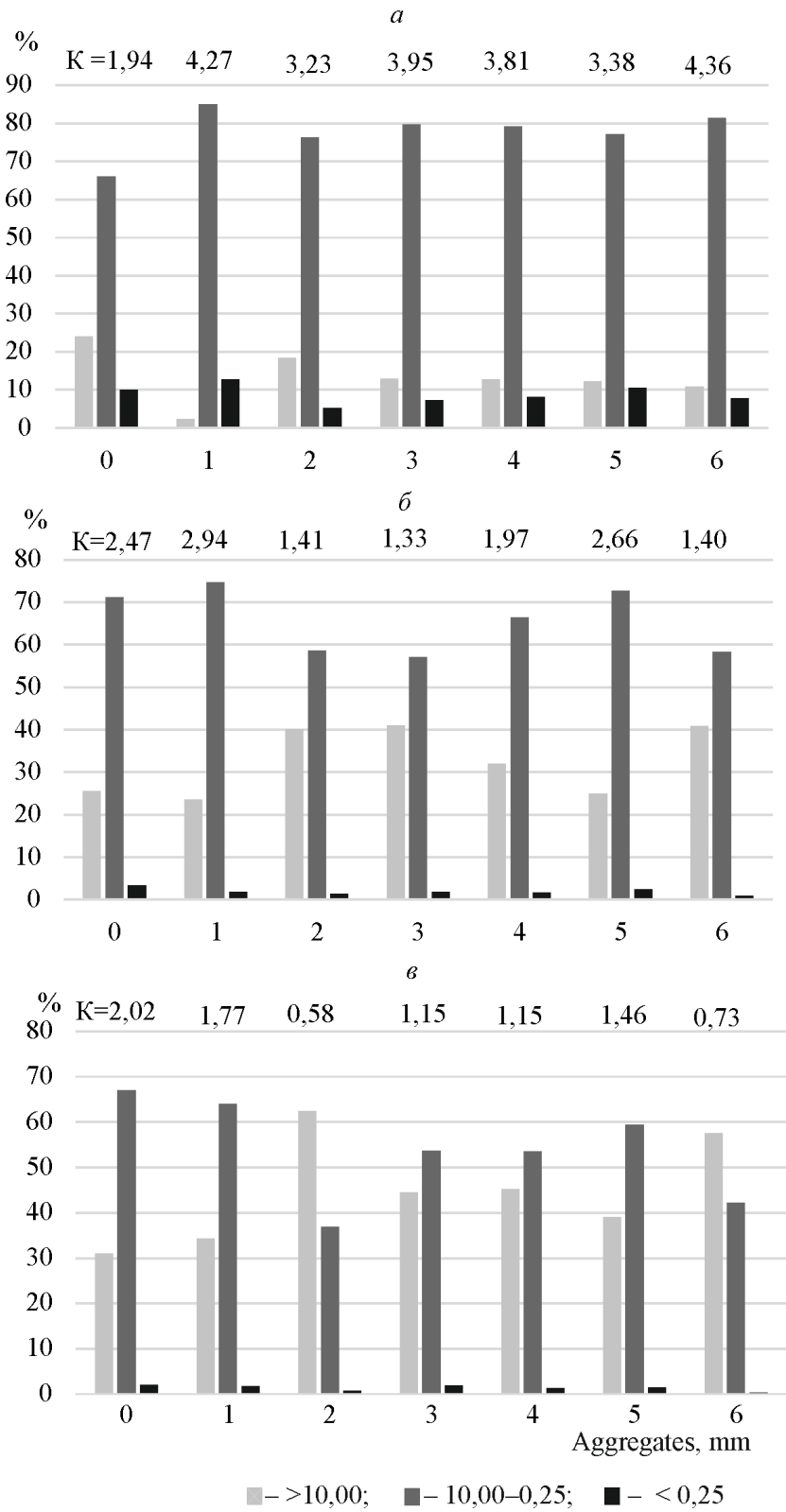
Processing number	Soil layer, cm	Aggregate content (%) size (mm)			Structure coefficient	Weighted average diameter of the aggregates, mm	
		> 10,0	10,00–0,25	< 0,25		Total aggregates	10,00–0,25
Initial condition (1988)							
0	0–20	24,7	68,6	6,7	2,19	5,13	2,71
	0–30	26,9	68,0	5,1	2,13	5,53	2,88
On completion of the 6th rotation (2019)							
1	0–20	15,4	77,8	6,8	3,51	4,26	2,70
	0–30	21,7	73,2	5,1	2,73	5,00	2,81
2	0–20	29,2	67,5	3,3	2,07	6,22	3,29
	0–30	40,3	57,3	2,4	1,34	6,91	2,88
3	0–20	27,0	68,5	4,5	2,17	5,63	2,92
	0–30	32,8	63,5	3,7	1,74	6,20	2,90
4	0–20	22,4	72,8	4,8	2,67	5,38	3,13
	0–30	30,0	66,4	3,6	1,97	6,02	3,01
5	0–20	18,6	74,9	6,5	2,99	4,88	3,00
	0–30	25,4	69,8	4,8	2,30	5,63	3,07
6	0–20	25,8	69,8	4,4	2,31	5,70	3,10
	0–30	36,4	60,6	3,0	1,54	6,49	2,84
LSD_{05}	0–20	6,9	5,1	1,8	0,62	1,10	0,58
	0–30	7,3	6,4	2,2	0,73	1,36	0,45

Note. Here and in Table 2: 0 - initial condition; 1 - mouldboard; 2 - non-mouldboard; 3 - combined; 4 - differentiated; 5 - surface; 6 - sweep blade.

[4] *Karetin L.N.* Soils of the Tyumen Region. Novosibirsk: Nauka, 1990. 258 p.

[5] *Safonov A.F., Stratonovich M.V.* Practicum on Farming with Soil Science. Moscow: Agropromizdat, 1990. 208 p.

[6] *Medvedev V.V.* Soil structure (methods, genesis, classification, evolution, geography, monitoring, protection). Kharkov, 2008. 406 p.



Содержание фракций агрегатов (%) по горизонтам почвы. Коэффициент структурности в начале ротации севооборота (исходное 1988 г.) и по завершении 6-й ротации севооборота (2019 г.).

Примечание. 0 – исходное состояние; 1 – отвальная обработка; 2 – безотвальная; 3 – комбинированная; 4 – дифференцированная; 5 – поверхностная; 6 – плоскорезная. Слой почвы, см: *а* – 0–10; *б* – 10–20; *в* – 20–30

Aggregate fraction content (%), by soil horizon. Structure coefficient at the beginning of crop rotation (initial 1988) and at the end of the 6th rotation of the crop rotation (2019).

Note. 0 - initial; 1 - mouldboard; 2 - no-till; 3 - combined; 4 - differentiated; 5 - surface; 6 - sweep blade. Soil layer, cm: *а* - 0-10; *б* - 10-20; *в* - 20-30

There was a decrease in the lump fraction (more than 10 mm) in the layer 0-10 cm from the initial value of 24.0% to 7.3-18.4%, in relative values by 23.3-69.5%. On the contrary, in the layers 10-20 and 20-30 cm the content of the lump fraction increased in all the systems of tillage except the mouldboard one: in the layer 10-20 cm - from 25,5 to 32,0-40,8%, 20-30 cm - from 31,0 to 34,3-57,5%, in absolute values in the layer 10-20 cm by 6,5-15,3%, 20-30 cm - by 3,3-26,5% respectively (see the figure).

In general, in the layer 0-20 cm due to the marked redistribution across the soil layers, the content of the lump fraction did not have such significant differences from the initial state in this layer. The content of the aggregates >10 mm in the layer 0-20 cm at the beginning of the research was 24.7%, after 30 years - 15.4-29.5%. In the soil layer 0-30 cm the initial content of these aggregates was 26.8%, at the end of the study - 21.7-40.3%. Lumpiness in the layer 0-30 cm did not increase only by the mouldboard system of cultivation (21.7%).

The increase in lumpiness in the layer 0-30 cm by 22.3-50.0% in comparison with the initial state was noted for the variants of no-till, combined, surface systems of cultivation. It occurred mainly by increasing the fraction of the aggregates >10 mm in the lower layer 20-30 cm from 31.1% in the initial condition to 57.5-62.4%, in relative values of 85.0-100%.

Microstructure (fractions less than 0.25 mm) in the overall structure was not predominant. Its maximum content was noted in the layer 0-10 cm - 7,80-11,65%, 10-30 cm - 0,91-1,86% (see the figure), in general for soil layers 0-20 and 0-30 cm - respectively 3,30-6,74 and 3,00-5,10% (see Table 1). In this regard, the content of agronomically valuable fraction in the main layer, especially in the layer 10-30 cm, was due to the presence and value of the lumpy fraction.

Reduction of lumpiness led to an increase in the content of agronomically valuable fraction of the structure and, conversely, an increase in lumpiness led to a decrease in the content of agronomically valuable aggregates.

Generalizing the changes of the structural condition of the soil for the 30-year period of using the arable land by the content of the agronomically valuable fraction 10,00-0,25 mm on the layer 0-20 cm on the whole the following can be said. Marked changes of structural composition of the soil in the layer 0-10 cm in favor of sustainable improvement by 15,6-22,8 %, keeping of similar indicators, especially on the mouldboard and flat tillage systems, or some decrease of it on other studied variants of tillage in the layer 10-20 cm in comparison with the initial condition contributed to the fact that the soil of the layer 0-20 cm kept the structural condition, not worse than the initial condition on most of the studied variants of soil treatment (see table 1).

At the beginning of the experiment the initial coefficient of structure was 2.19, at the end of the research - 2.07-3.51. At the same time, the content of agronomically valuable structure in the 0-20 cm layer by the mouldboard, flat-cut and differentiated tillage systems increased by 4,20-9,23% in absolute values, which is 6,12-13,45% in relation to the initial value of 68,6%. The structure coefficient of 2,67-3,51 for them was higher than the initial one by 0,48-1,32, or 21,9-60,3%. By non-mouldboard, combined, and surface tillage systems, the content of these structural components was almost equal (67.5-69.8%, Cstr = 2.07-2.31) to the initial level.

In the 0-20 cm layer, the highest content of agronomically valuable structure was observed for the mouldboard - 77.8%, flat-cut - 74.9% and differentiated - 72.8% of the tillage systems with a coefficient of structure of 2.67-3.51. The rest of the systems were inferior to the mouldboard system in terms of agronomically valuable aggregates content 8.0-10.4%, in terms of Cstr - 0.60-1.44 units.

The average weighted diameter of the aggregates in the layer 0-20 cm, as a rule, for all variants of the main tillage, except for mouldboard and flat-cut tillage, was 4.26-4.88 mm. The given indicator during the investigated period increased in comparison with the initial one from 5,13 to 5,38-6,22 mm, i.e. by 0,25-1,09 mm (4,9-21,2%) mainly due to the increase of the content of lumps >10 mm in the layer 10-20 cm (see table 2).

The most significant increase in the size of the aggregates compared with the initial condi-

tion was for non-mouldboard and surface tillage systems - by 0.57-1.09 mm (11.1-21.2%). A similar tendency to increase the aggregate WAD when minimizing the tillage was also established by E.V. Dubovik and others on typical chernozem [12].

The average weighted size of the agronomically valuable fraction (10,0-0,25 mm) for most of the studied treatment variants was 3,00-3,29 mm. It increased by 0,21-0,58 mm or 7,7-21,2% in comparison with initial (2,71 mm) due to a significant increase of this fraction (10,0-0,25 mm) in 0-10 cm layer. On the mouldboard system the average weighted size of the agronomically valuable fraction remained close to the initial condition - 2.70 mm.

Analysis of the structural condition of the 0-30 cm layer in general showed that with increasing the depth of the soil profile, involving (in contrast to the 0-20 cm layer) an additional lower layer of 20-30 cm, with even greater than in the 10-20 cm layer, compared with the initial state of the share of the soil clumpy structure content led to even greater than in the 0-20 cm layer reduction in the agronomically valuable structure.

At the same time in the layer 0-30 cm the highest indicators of the structural condition of the agronomically valuable fraction were on the mouldboard system of cultivation (73,2%), flat-cut (69,8%) and differentiated (66,4%) with a coefficient of structure of 1.97-2.73. The listed tillage systems provided maintenance of structural condition in comparison with initial - 68.0% and coefficient of structure - 2.13 (the mouldboard system - higher). Non-mouldboard, combined and surface tillage resulted in 9.7-15.9% decrease in the content of this structure compared to the mouldboard system and in a 0.99-1.39 decrease in the structure coefficient, mainly due to an 8.5-17.5% increase in the lump fraction content in the soil layer 10-20 cm compared to the control, 20-30 cm - by 10.3-28.1% (see Table 1).

With an increase of the soil profile depth up to 0-30 cm an increase in the total weighted average size of the aggregates up to 5.00-6.91 mm was observed even more than in the layers 0-10 and 0-20 cm, which is explained by an increase in the volume of the lumpy fraction. At the initial value of the average weighted size of the aggregates of 5.53 mm in the soil layer 0-30

Табл. 2. Величина средневзвешенного диаметра агрегатов по почвенным горизонтам в зависимости от системы основной обработки почвы по завершении 6-й ротации зернопарового севооборота

Table 2. Value of average weighted diameter of aggregates by soil horizons depending on the systems of main tillage at the end of the 6th rotation of grain and fallow crop rotation

Option number	Soil layer, cm					
	0–10		10–20		20–30	
	Average weighted diameter of the aggregates, mm					
	Total aggregates	10,00–0,25	Total aggregates	10,00–0,25	Total aggregates	10,00–0,25
Initial condition (1988)						
0	4,77	2,35	5,62	3,07	6,33	3,22
On completion of the 6th rotation (2019)						
1	2,80	2,04	5,71	3,35	6,48	3,05
2	5,38	3,53	7,07	3,05	8,29	2,05
3	4,28	2,96	6,98	2,87	7,32	2,87
4	4,26	2,97	6,49	3,29	7,29	2,78
5	3,93	2,67	5,83	3,34	7,11	3,20
6	4,22	3,12	7,18	3,09	8,06	2,31
LSD_{05}	1,44	0,95	1,36	0,50	1,10	0,29

cm by the end of the 6th rotation of the crop rotation the average weighted size decreased slightly - by 0.53 mm, or 9.6%, only on the mouldboard system of cultivation, remaining at the level with the initial state on the flat tillage - 5.63 mm. The average weighted size of the aggregates increased by 0.49-1.38 mm, or 8.9-25.0% (see Table 2).

The data of determining the weighted average size of the agronomically valuable aggregates testify to the high stability of this indicator in the soil layer 0-30 cm. For all the studied tillage systems the indicator was favorable, close to the optimal values, since the average weighted size of 2.81-3.07 mm corresponds to the granular state of the soil structure [10]. The tillage systems during the observed period of research had mainly an equal effect on the average weighted size indicator. The tendency of some increase in it in comparison with the control variant by 0.20-0.26 mm, or 7.1-9.2%, was noted only for flat-cut and differentiated systems of tillage.

Determination of grain yield in the average for the final rotation of the crop rotation (2014-2019) showed that the studied systems of main tillage provided quite a close level of grain yield - 2.85-3.07 t/ha, which indicates favorable and close conditions of structural-aggregate state, as well as conditions of moisture supply and soil density [16].

CONCLUSIONS

1. Dark gray forest soils of forest-steppe zone have favorable and stable structural-aggregate condition. During the 30-year period of arable land use the soil of 0-20 cm layer preserved the structural condition not inferior to the initial one in most of the studied variants of tillage. With an increase in the depth of the soil profile due to an increase in the proportion of lumpy fraction in the lower layer (10-30 cm) in the layer 0-30 cm the content of agronomically valuable structure in comparison with the layer 0-20 cm, as well as with the initial state on most of the studied options of tillage systems was reduced.

2. Prolonged use of arable land has led to qualitative changes in the structural state, consisting in quantitative redistribution in comparison with the initial state of the fractional composition of the structure on the soil horizons. There was a decrease in the content of the lumpy fraction (>10 mm) in the layer 0-10 cm in relative values by 23,3-69,5%. In the 10-30 cm layer, the content of the lumpy fraction increased by 25.5-85.5%. With an insignificant share in the total structure of the aggregates less than 0,25 mm, the observed changes in the content of the lumpy fraction led to an increase in the agronomically valuable fraction in the layer 0-10 cm by 15.6-22.8%, the coefficient of structure from 1.94 at baseline to 3.23-4.27 and, conversely, to a decrease in the agronomically valuable fraction of aggregates in the layer 10-30 cm by 6.7-44.8%.

3. The highest content of agronomically valuable structure in the layer 0-20 cm was noted on the mouldboard, flat-cut and differentiated systems of tillage (72.8-77.8%). For the 30-year period the content of agronomically valuable structure (10-0,25 mm) in the layer 0-20 cm increased by 6,12-13,45% for these treatments, the structure coefficient - by 21,9-60,3%. The content of this fraction (67,5-69,8%) and the structure coefficient (2,07-2,31) for the other studied tillage systems were close to the initial condition 68,6% and Cstr = 2,19. The average weighted diameter of the agronomically valuable aggregates increased from 2.71 mm at baseline to 3.00-3.29 mm (by 7.7-21.2%) due to a significant increase in the proportion of these aggregates in the layer 0-10 cm.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

(✉)**Перфильев Н.В.,** доктор сельскохозяйственных наук, главный научный сотрудник; **адрес для переписки:** Россия, 625501, Тюмень, пос. Московский, ул. Бурлаки, 2; e-mail: p.nikolay52@yandex.ru

Вьюшина О.А., научный сотрудник**;** e-mail: vyushina63@yandex.ru

AUTHOR INFORMATION

(✉)**Nikolay V. Perfilyev,** Doctor of Science in Agriculture, Head Researcher; **address:** 2, Burlaki St., Moskovskiy vil., Tyumen, 625501**,** Russia**;** e-mail: p.nikolay52@yandex.ru

Olga A. Vyushina, Researcher; e-mail: vyushina63@yandex.ru

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ОСОБЕННОСТИ АЗОТНОГО ПИТАНИЯ ОЗИМОЙ ПШЕНИЦЫ В ТЕХНОЛОГИИ ПРЯМОГО ПОСЕВА

(✉)**Бильдиева Е.А., Ерошенко Ф.В.**
Северо-Кавказский федеральный научный аграрный центр
Ставропольский край, г. Михайловск, Россия
(✉)e-mail: bildieva@rambler.ru

Представлены результаты исследования азотного питания растений озимой пшеницы, возделываемой по технологии прямого посева в зоне неустойчивого увлажнения Ставропольского края. Исследования проводили в 2020–2022 гг. в стационарном опыте по двум технологиям: прямого посева (без обработки почвы) и общепринятой технологии на различных фонах минерального питания. Изучали накопление азота в органах растений на разных этапах развития озимой пшеницы (по методике В.Т. Куркаева) и активность фермента нитратредуктазы в листьях (по методике Мульдера в модификации Б.И. Токарева). Установлено, что применение минеральных удобрений в технологии прямого посева положительно влияет на такой физиологический процесс, как минеральное питание растений озимой пшеницы, существенно увеличивая накопление в них азота. Использование почвопокровной культуры в севообороте по технологии прямого посева увеличило содержание азота в растениях озимой пшеницы на 32,7% по сравнению с вариантом, где применяли только удобрения, в общепринятой технологии – на 29,1% по отношению к варианту с тем же фоном минерального питания. Высокая активность фермента нитратредуктазы в листьях в репродуктивный период свидетельствует о наличии большого количества нитратов в растениях озимой пшеницы. Активность фермента в листьях растений, выращенных по технологии без обработки почвы на фоне применения удобрений и почвопокровной культуры, была существенно выше, чем на остальных вариантах в начале репродуктивного периода (на 72–89%), и достигала максимального значения через 14 дней (4,15 мкМ/г·ч).

Ключевые слова: озимая пшеница, технология прямого посева, почвопокровная культура, минеральное питание, содержание азота, нитратредуктаза

SPECIFIC FEATURES OF NITROGEN NUTRITION OF WINTER WHEAT IN NO-TILL TECHNOLOGY

(✉)**Bildieva E.A., Eroshenko F.V.**
North Caucasus Federal Agrarian Research Centre
Mikhailovsk, Stavropol Territory, Russia
(✉)e-mail: bildieva@rambler.ru

The results of the study of nitrogen nutrition of winter wheat plants cultivated by No-till technology in the zone of unstable moisture in the Stavropol Territory are presented. The studies were conducted in 2020-2022 in a stationary experiment using two technologies: direct seeding (no tillage) and conventional technology on different backgrounds of mineral nutrition. Nitrogen accumulation in plant organs at different stages of winter wheat development (by V.T. Kurkaev method) and nitrate reductase enzyme activity in leaves (by Mulder method modified by B.I. Tokarev) were studied. It has been established that the use of mineral fertilizers in the technology of direct seeding has a positive effect on such a physiological process as mineral nutrition of winter wheat plants significantly increasing the accumulation of nitrogen in them. The use of a cover-ground culture in the crop rotation by direct seeding technology contributed to an increase in the nitrogen content in winter wheat plants by 32.7% compared to the variant where only fertilizers were used, as well as by 29.1% compared to the variant with the same background mineral nutrition in conventional technology. High activity of nitrate reductase enzyme in leaves during the reproductive period indicates the presence of large amounts of nitrates in winter wheat plants. The enzyme activity in the leaves of plants grown according to the technology without soil treatment against the background of using fertilizers and cover-ground cultures was significantly higher than in the other variants at the beginning of the reproductive period (by 72-89%) and reached its maximum after 14 days (4.15 μM/g·h).

Keywords: winter wheat, No-till technology, cover-ground culture, mineral nutrition, nitrogen content, nitrate reductase

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Conflict of interest
The authors declare no conflict of interest.

INTRODUCTION

Degradation and reduction of agricultural land fertility is a nationwide problem [1-4]. In this regard, a national strategy is being developed to solve the problems of food security, preservation of ecosystems, adaptation to climate change, and restoration of agricultural land fertility. Currently, the transition to environmentally safe technologies is relevant, but not all agricultural producers agree to abandon the traditionally used technologies [5-9]. Therefore, it is necessary to educate farmers about the science-based approach to farming, the availability of technologies that allow to obtain high yields, preserving soil fertility and resources [10-12].

The technology of direct seeding belongs to the farming systems that allow to restore fertility [13, 14]. However, in the process of transition to this technology of crop production it is difficult to obtain high yields without fertilizers. It is possible to gradually reduce the doses of applied fertilizers by using cover-ground crops in the crop rotation, which are a constantly renewable source of biological nitrogen in the soil [15]. Cover-ground green manure crops are cold-resistant, the accumulation of their biomass occurs during the periods not used by the main crops of the crop rotation, which allows the rational use of soil and climatic conditions of the region. Cultivation of cover-ground crops allows the formation of organic fertilizers directly in the field. In the process of decomposition of organic residues there is a release of nutrients, so to restore soil fertility, it is necessary to constantly enrich the soil with fresh organic matter [16, 17].

The purpose of the research is to study the peculiarities of nitrogen nutrition of winter wheat plants when cultivated according to the technology of direct seeding.

MATERIAL AND METHODS

The studies were conducted in 2020-2022 at the experimental station of the North Caucasus Federal Research Agrarian Center, located in the third soil and climatic zone of the Stavropol Territory - a zone of unstable moisture. The soil of the experimental plot is ordinary medium heavy-loamy chernozem characterized by a low content of humus (3.87%) and nitrate nitrogen (11.9 mg/kg soil), an average content of mobile phosphorus (18.7 mg/kg) (by Machigin) and an average supply of exchangeable potassium (245 mg/kg).

The variety of soft winter wheat Victoria Odesskaya was chosen as an object of research. Winter wheat was placed in the rotation: pea - winter wheat - sunflower - corn. Plots of 300 m^2 in the experiment were placed in two layers in triple replication (record plot of 30 m^2): 1) technology of direct sowing - No-till (without tillage); 2) conventional technology (traditional technology with tillage, recommended by research institutions for the zone of unstable moisture).

Each layer is divided into three options:

1) no fertilizer;

2) fertilized ($N_{90}P_{60}K_{60}$);

3) fertilized ($N_{90}P_{60}K_{60}$) against the background of the application of cover ground crop (CGC) before sowing the forecrop of winter wheat - peas.

Winter wheat was sown at the optimum time for the soil and climatic zone - I ten-day period

of October, seeding rate - 4.5 million seeds/ha (210 kg/ha).

In the technology without tillage, sowing was performed by GIMETAL direct seeding machine with simultaneous application of mineral fertilizers. Traditional technology includes double tilling immediately after harvesting the forecrop, pre-sowing cultivation, sowing by SZ-3,6 seeding machine with the application of mineral fertilizers and rolling by ZKKSh-6. Feeding with ammonium nitrate, herbicide and fungicide treatment were made with RMG-4 and OP-2000. Harvesting was carried out by Sampo-130 combine harvester.

Winter rye was sown after corn harvesting as a cover- ground (depending on weather conditions, the sowing date varied from the second ten-day period of September to the first ten-day period of October). In spring, before sowing peas in the direct seeding technology, the rye was killed by a continuous herbicide; in the conventional technology, the soil was tilled with a disk harrow.

Nitroammophoska ($N_{16}P_{16}K_{16}$) and ammonium nitrate (N_{34}) were used as mineral fertilizers. Total dose of mineral fertilizers was ($N_{90}P_{60}K_{60}$): ($N_{60}P_{60}K_{60}$) - nitroammophoska was put 250 kg/ha before sowing and during sowing by seeder 125 kg/ha, (N_{30}) - ammonium nitrate during spring tillering was put 88 kg/ha randomly.

Indicators of growth and development of winter wheat plants were studied according to the method of state variety testing of crops. Nitrate reductase activity was determined by the Mulder method modified by B.I. Tokarev[1], nitrogen content in plants and grain by the method of V.T. Kurkaev[2] et al. Yield was measured by direct harvesting in the phase of full grain ripeness. The reliability of the obtained data was evaluated by statistical analysis using AgCStat and Microsoft Office Excel programs.

RESULTS AND DISCUSSION

One of the main physiological processes in a plant organism is nitrogen metabolism, since nitrogen is the key component of simple and complex proteins, nucleic acids, chlorophyll, enzymes, alkaloids, and many other organic substances that are important in metabolism.

Numerous studies have shown that about 70% of nitrogen accumulated by winter wheat plants during the growing season comes before the beginning of the reproductive period, then its consumption slows down, and the remaining part is absorbed during the period of grain ripening. However, analysis of the dynamics of nitrogen accumulation by winter wheat plants in our studies showed that this pattern is typical only for crops using conventional technology (see Fig. 1).

The process of nitrogen accumulation in winter wheat plants cultivated by direct seeding technology before the beginning of the reproductive period is less intensive than the conventional technology. Thus, the nitrogen content in winter wheat plants at the booting stage on the background without fertilizers according to the direct seeding technology was 27,9% lower than with the conventional technology. On the fertilized background the difference was 20.4%, and on the background with the use of a cover-ground crop - 20.0%. By the earing phase on the unfertilized background the difference in nitrogen content increased, and on the background of fertilizer application it decreased to 11.7%. At the variant where fertilizers were applied against the background of cover-ground crop application, the amount of nitrogen in the plants cultivated by the direct seeding technology was 2.4% higher than this indicator in the plants cultivated by conventional technology. At the X stage of organogenesis (e.o.) in the crops using the conventional technology, a decrease in nitrogen concentration was noted in all variants, whereas in the plants on the variants with

[1] *Tokarev B.I.* Methods for determination of nitrate reductase activity value in wheat and barley // Scientific Proceedings of the Siberian Branch of VASKHNIL. Novosibirsk, 1977. pp. 58-65.

[2] *Kurkaev V.T., Eroshkina S.M., Ponomarev A.N.* Agricultural analysis and basics of biochemistry. Moscow: Kolos, 1977. 239 p.

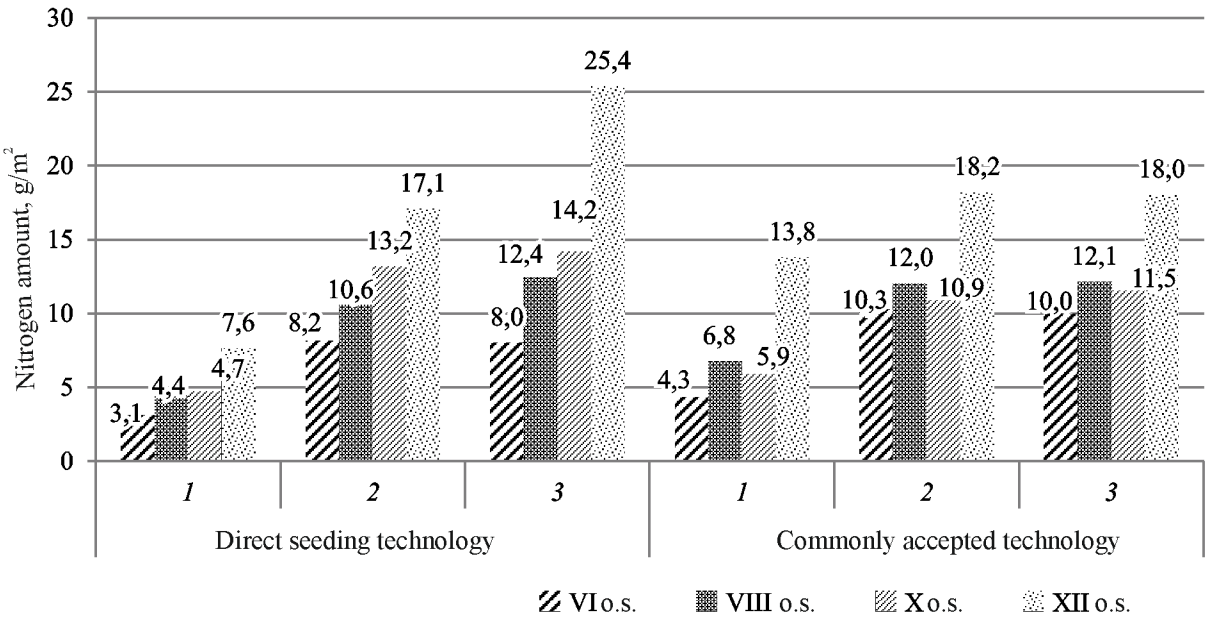

Рис. 1. Динамика накопления азота в растениях озимой пшеницы (среднее за 2020–2022 гг.) в вариантах: *1* – без удобрений; *2* – удобренный; *3* – удобренный ППК (различия значимы для $p < 0,05$, $t_{ст} = 3,5$, $t_{крит} = 2,23$)

Fig. 1. Dynamics of nitrogen accumulation in winter wheat plants (on average for 2020-2022) in the variants: *1* - no fertilizer; *2* - fertilized; *3* - CGC fertilized (differences significant for $p < 0.05$, $t_{st} = 3.5$, $t_{crit} = 2.23$)

the technology of direct seeding, in contrast, its content increased by 6.4-19.7% in relation to the VIII stage of organogenesis. By the end of vegetation, the highest nitrogen content was observed in winter wheat plants cultivated by direct seeding technology; it was 25.4 g/m^2 in the variant with fertilization against the background of a cover-ground crop, which was 29.1% higher than against the same background by the conventional technology, and 28.3% higher against the fertilized background.

The main source of nitrogen for plants, which do not fix it in symbiosis with microorganisms, are mineral forms of nitrogen in soils - nitrates, nitrites and ammonia. Higher plants assimilate inorganic nitrogen mainly in the form of nitrates, which are reduced to ammonium inside the cells and included in the synthesis of amino acids. The enzyme responsible in the plant for the reduction of nitrate to nitrite is nitrate reductase, whose activity is induced by introducing a substrate, that is, the more nitrates enter the plant, the higher the activity of the enzyme. The process of nitrate recovery is carried out in the roots and leaves of the plant. Winter wheat has a fairly high capacity for nitrate recovery by the aboveground part. Flag leaf has the highest activity in nitrogen recovery by the beginning of the reproductive period [18].

The activity of nitrate reductase in flag leaves of winter wheat was studied in all years of the research. However, if in 2020 the higher activity of this enzyme was noted in the variants where the nitrogen content by the end of the growing season was higher, in 2021 the most active nitrate reductase was in the variants without fertilizers. Therefore, in 2022 nitrate reductase activity was determined twice, at the beginning of earing (May 30) and two weeks later (June 14). It was found that at the beginning of the reproductive period the enzyme activity in leaves was quite low almost in all the variants of the experiment and varied from 0.2 to 0.51 μM/g/h, except for the variant with the use of fertilizers on the background of a cover-ground crop using the direct seeding soil technology, where it was 1.82 μM/g/h (see Fig. 2).

Analysis of nitrate reductase activity at the end of earing and the beginning of flowering of winter wheat showed a fairly high growth. In the leaves of the plants grown according to the conventional technology, nitrate reductase activity depending on the mineral nutrition background varied from 1.54 to 4.01 μM/g-h, while in the variants with the direct seeding technology it varied from 2.61 to 4.15 μM/g-h.

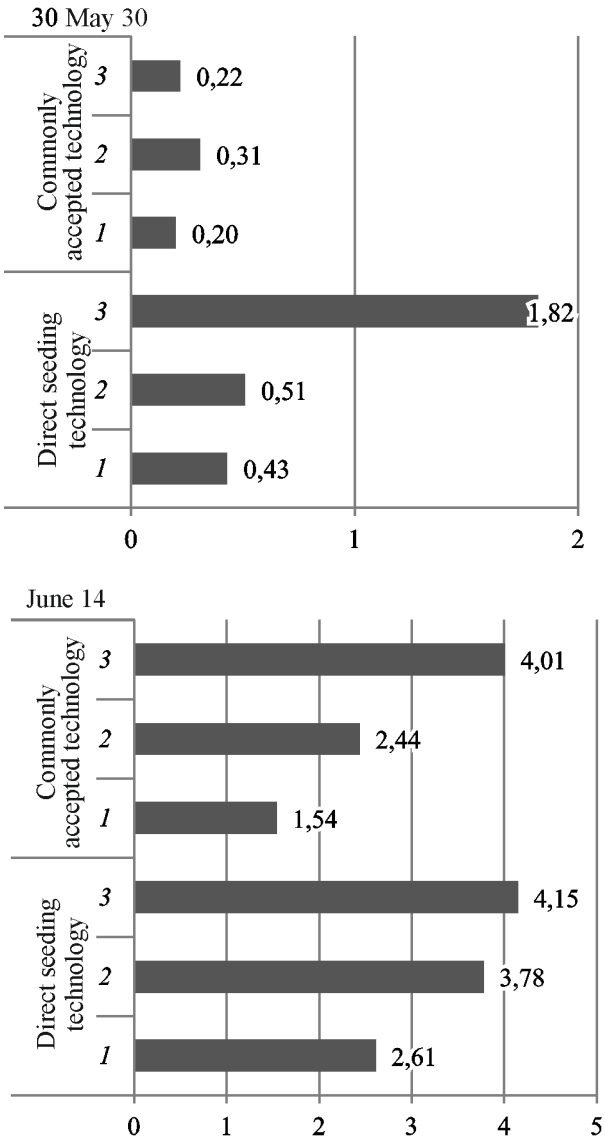

Рис. 2. Активность фермента нитратредуктаза в листьях озимой пшеницы в репродуктивный период развития растений в вариантах: *1* – без удобрений; *2* – удобренный; *3* – удобренный ППК, мкМ/г·ч

Fig. 2. Activity of the enzyme nitrate reductase in winter wheat leaves during the reproductive period of plant development in the variants: *1* - no fertilizer; *2* - fertilized; *3* - CGC fertilized, μM/g·h

The highest enzyme activity in this period of winter wheat development was noted in the variants with the use of fertilizers on the background of the cover-ground crop: 4.01 μM/g/h by conventional technology and 4.15 μM/g/h by direct seeding technology.

Not only the intensity of nitrogen consumption is important in the process of mineral nutrition, but also its content in the final product - grain (see Fig. 3). On average over the years of research, the higher content of nitrogen was noted in the grain of winter wheat cultivated by direct seeding technology with the use of mineral fertilizers on the background of cover-ground crop (19.3 g/m^2), which is 26.9% higher than on the fertilized background and 16.1% higher than in the crops according to the conventional technology with the use of fertilizers.

The result of all physiological processes in winter wheat plants aimed at their growth and development is the yield. The highest crop capacity was obtained in the variant with the use of a cover-ground crop in the direct seeding technology (5.57 t/ha), which is 2.7% (0.15 t/ha) higher than on the fertilized background (see table).

Application of a cover-ground crop in the rotation by conventional technology was ineffective, since it led to a decrease in the yield compared to the variant, where only fertilizers were used, by 2.4% (0.13 t/ha).

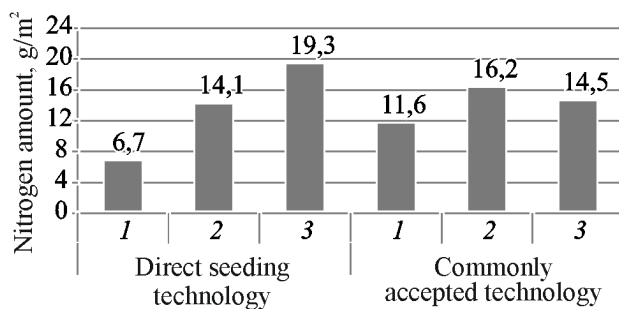

Рис. 3. Количество азота, накопленного в зерне озимой пшеницы (среднее за 2020–2022 гг.), в вариантах: *1* – без удобрений; *2* – удобренный; *3* – удобренный ППК (различия значимы для $p < 0,05$, $t_{ст} = 4,9$, $t_{крит} = 2,23$)

Fig. 3. The amount of nitrogen accumulated in winter wheat grain (on average for 2020-2022), in the variants: *1* - no fertilizer; *2* - fertilized; *3* - CGC fertilized (differences significant for $p < 0.05$, $t_{st} = 4.9$, $t_{crit} = 2.23$)

Урожайность озимой пшеницы (в среднем за 2020–2022 гг.)
Winter wheat yield (on average for 2020-2022)

Technology (A)	Background (B)	Yield, t/ha	Control add-on	
			t/ha	%
Direct seeding	No fertilizer	3,98	–	–
	Fertilized	5,42	1,43	36,0
	CGC fertilized	5,57	1,59	39,9
	Average	4,99		
Commonly accepted	No fertilizer	4,30	–	–
	Fertilized	5,36	1,06	24,6
	CGC fertilized	5,23	0,93	21,6
	Average	4,96		
$LSD_{05\ (factor\ A)} = 0,24$	$LSD_{05\ (factor\ B)} = 0,29$	$LSD_{05\ (AB)} = 0,41$		

CONCLUSION

As a result of the research the differences in the process of nitrogen accumulation by plants of winter wheat cultivated by direct seeding technology have been established:

- the intensity of nitrogen absorption in the generative period of the plant development is lower than in the case of cultivation by conventional technology;

- after earing, the intensity of nitrogen assimilation starts increasing as evidenced by high activity of nitrate reductase enzyme in the leaves;

- the intensity of nitrogen consumption does not decrease during grain formation and ripening.

Also, high efficiency of the use of a coverground crop at cultivation by direct seeding technology was revealed: the process of nitrogen accumulation in the plants increases and the grain yield increases on average by 2.7% compared to the use of fertilizers only (in some years, this increase can reach 3.5-4.0%).

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ИНФОРМАЦИЯ ОБ АВТОРАХ

✉**Бильдиева Е.А.,** кандидат сельскохозяйственных наук, ведущий научный сотрудник; **адрес для переписки:** Россия, 356241, Ставропольский край, г. Михайловск, ул. Никонова, 49; e-mail: bildieva@rambler.ru

Ерошенко Ф.В., доктор биологических наук, заведующий отделом

AUTHOR INFORMATION

✉**Evgenia A. Bildieva,** Candidate of Science in Agriculture, Lead Researcher; **address:** 49, Nikonova St., Mikhailovsk, Stavropol Territory, 356241, Russia; e-mail: bildieva@rambler.ru

Fedor V. Eroshenko, Doctor of Science in Biology, Division Head

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ЗАВИСИМОСТЬ УРОЖАЙНОСТИ НОВЫХ СОРТОВ НУТА ОТ СПОСОБА ПОСЕВА В НИЖНЕМ ПОВОЛЖЬЕ

(✉)**Маслова Г.А., Кондаков К.С., Башинская О.С.**
Российский научно-исследовательский
и проектно-технологический институт сорго и кукурузы
Саратов, Россия
(✉)e-mail: rossorgo@yandex.ru

Приведены результаты исследований в условиях Нижнего Поволжья по изучению влияния способа посева (ширина междурядий и предшественник) на урожайность сухой биомассы и семян трех сортов нута селекции Российского научно-исследовательского и проектно-технологического института сорго и кукурузы (Россорго): Бенефис, Сфера и Сокол. Выявлены различия по высоте растений и прикреплению нижнего боба: наиболее высоким из представленных оказался сорт Сокол – 69,00 и 40,13 см соответственно при междурядье 70 см и использовании в качестве предшественника ярового ячменя, далее следует сорт Бенефис – 55,30 и 29,54 см соответственно (междурядье 45 см, предшественник – яровой ячмень); затем Сфера – 55,27 и 30,80 см соответственно (междурядье 70 см, предшественник – яровая пшеница). По формированию бобов и семян на одном растении значительное преимущество показал сорт Бенефис – 55,80 бобов и 56,93 семян при междурядье 45 см, предшественнике яровая пшеница. У двух других сортов отмечены более низкие показатели: Сфера – 49,60 бобов и 46,03 семян при междурядье 45 см, предшественнике сорго зерновое; Сокол – 40,53 бобов и 38,70 семян при междурядье 60 см, предшественнике яровая пшеница. В результате анализа роста массы 1 тыс. семян, а также урожайности семян и содержания в них протеина установлена оптимальная схема посева изучаемых сортов нута: ширина междурядий 45 см, предшественник – яровая пшеница. Урожайность семян при такой схеме размещения составила: Бенефис – 4,31 т/га, Сфера – 3,89, Сокол – 3,07 т/га; содержание протеина достигало следующих значений: Бенефис – 21,15% на абс. сух. в-во, Сфера – 20,39%, Сокол – 19,32% на абс. сух. в-во.

Ключевые слова: нут, сорт, междурядье, предшественник, урожайность, сухая биомасса, протеин

YIELD DEPENDENCE OF NEW CHICKPEA VARIETIES ON THE METHOD OF SOWING IN THE LOWER VOLGA REGION

(✉)**Maslova G.A., Kondakov K.S., Bashinskaya O.S.**
Russian Research Design and Technology Institute for Sorghum and Maize "Rossorgo"
Saratov, Russia
(✉)e-mail: rossorgo@yandex.ru

The results of research in the conditions of the Lower Volga region to study the effect of the sowing method (width of row spacing and the forecrop) on the yield of dry biomass and the seeds of chickpea varieties bred by the Russian Research Design and Technology Institute for Sorghum and Maize (Rossorgo) are presented: Benefis, Sphere and Sokol. Differences in plant height and attachment of the bottom bean were found: the highest of the presented was the variety Sokol - 69.00 and 40.13 cm respectively with a row spacing of 70 cm and the use of spring barley as a forecrop, followed by the variety Benefis - 55.30 and 29.54 cm respectively (row spacing 45 cm, the forecrop - spring barley); and then Sphere - 55.27 and 30.80 cm respectively (row spacing of 70 cm, the forecrop - spring wheat). Benefis showed a significant advantage in the formation of beans and seeds per plant - 55.80 beans and 56.93 seeds at row spacing of 45 cm, the forecrop was spring wheat. The other two varieties have lower rates: Sphere - 49.60 beans and 46.03 seeds at row spacing of 45 cm, the forecrop is grain sorghum; Sokol - 40.53 beans and 38.70 seeds at row spacing of 60 cm, the forecrop is spring wheat. As a result of analysis of 1 ths seeds weight growth, as well as seed yield and protein content, the optimal seeding scheme of chickpea varieties under study was established: the width of the row spacing is 45 cm, the forecrop is spring wheat. Seed yields under this placement scheme amounted to: Benefis - 4.31 t/ha,

Sphere - 3.89 t/ha, Sokol - 3.07 t/ha; protein content reached the following values: Benefis - 21.15% a.d.m., Sphere - 20.39% a.d.m, Sokol - 19.32% a.d.m.

Keywords: chickpea, variety, row spacing, forecrop, yield, dry biomass, protein

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INTRODUCTION

The choice of agricultural producers in favor of leguminous crops is obvious - their seeds have a high content of valuable amino acid composition and degree of digestibility of protein, so they are indispensable elements in the nutrition of both humans and farm animals (in a form of balanced concentrated feed)[1] [1-4].

Such a promising legume crop as chickpea attracts special attention in modern crop production. It is of great importance for the crop rotation system in the arid conditions of the Lower Volga Region[2] [3-5], since it tolerates drought and high temperatures better than other leguminous crops [6, 7].

When expanding the areas under chickpea in the conditions of the Lower Volga region it is necessary to take into account not only biological features and adaptation to specific soil and climatic conditions, but also the technology of new varieties cultivation [3, 8, 9].

For comparative characterization, the article presents new chickpea varieties differing in the weight of 1 thousand seeds (since large-seededness is the main biological trait, which currently determines the cost of seeds [8]) - Benefis, Sphere, Sokol. These varieties are released in the Lower Volga region. In the years of the experiments (2017-2019) the amount of precipitation and temperature regime differed significantly. In the study of scientific papers, it was found that relatively hot years with dry weather conditions contribute to the reduction of the duration of the growing season in chickpea plants, lack of moisture and high air temperatures lead to a decrease in the number of beans and seeds on the plant, as well as their weight [10, 11]. It was revealed that in the years with average and high moisture the highest chickpea yield was provided by row sowing with high seeding rates. In dry periods the best results were obtained with wide-row or strip cropping methods with lower norms. Thus, the recommended seeding rates for the continuous row sowing method vary from 0.5 to 1.3 million, and for the wide-row method from 0.2 to 0.7 million germinated seeds/ha (see footnote 1) [4, 12]. Chickpea seeding rate should undoubtedly be considered in conjunction with other agrobiological factors. Based on the materials of scientific institutions and the state variety network, it was concluded that the change of seeding rates has a significant impact on the increase in the yield only in wet years. In our experiments, the optimum density was selected with seeding rate of 350 thousand germinated seeds/ha. The next element of the technology was the method of seeding [9]. The width of row spacing was: 15, 30, 45, 60, 70 cm. It was found that under the conditions of the same row spacing and increasing of the spacing between the plants

[1] *Paosypanov G.S., Dolgodvorov V.E., Zherukov B.H., Gataulina G.G., Gorbachev I.V., Arkhangelsky N.S., Bugaev P.D., Kornienko A.V.* Plant breeding. Moscow: Kolos-S. 2007. 612 p.

[2] *Shyurova N.A*. Agrobiological features and productivity of chickpea depending on cultivation methods // Modern technologies of crops cultivation: collection of scientific works. Saratov, 2002. pp. 35-40.

the height, the number of branches and beans per plant, as well as the weight of 1 thousand seeds decreases. It should also be noted the need to take into account the spatial arrangement of plants when growing chickpea (see footnote 2) [5] - in the experiment to study the yield of new chickpea varieties, observations were made in four crop rotations, in which the degree of influence of the forecrops was established: grain sorghum, corn, spring barley and spring wheat [9].

The purpose of the study was to determine the impact of the method of seeding on the dry biomass and seed yield of chickpea varieties bred by the Russian Research Design and Technology Institute for Sorghum and Maize (RosNIISK) under the conditions of the Lower Volga region.

MATERIAL AND METHODS

The research was aimed at the study of chickpea varieties of RosNIISK selection that differ in the weight of 1 thousand seeds. The experiment was laid by the type of 3-factor experiment based on the following scheme:

1. Factor A - variety: Benefis, Sphere, Sokol.

2. Factor B - width of row spacing: 15, 30, 45, 60, 70 cm.

3. Factor C - the forecrop: grain sorghum, corn, spring barley, spring wheat.

The total area of the experiment was 0.70 ha, the area of the record plot - 25 m^2, the number of variants - 60, the repetition of 4 times.

The field experiments, phenological observations, yield records, dynamics of biomass accumulation and leaf surface area by development phases were performed according to generally accepted methodological recommendations[3].

Experimental studies were conducted in the experimental field of RosNIISK in 2017-2019. The soils at this site are represented by southern low-alkaline chernozems with medium loamy texture. In the arable layer, the humus content reaches 3.3%. The chickpea farming technique is zonal, developed in RosNIISK. Soil preparation before planting included plowing, early spring harrowing (BZSS-1,0) in two tracks across the plowing direction, two pre-sowing cultivations (KPS-4 + MTZ-82) - the first at a depth of 8-10 cm, the second at the depth of seeding (6-7 cm). Seeding was carried out by SZ-3,6 (inter-row 15, 30, 45 and 60 cm) and SON-4,2 (inter-row 70 cm). Seeding rate was 350 thousand seeds/ha. Concurrently with seeding the soil was rolled with star-wheeled rollers, and on the 3rd day after seeding - pre-emergent harrowing. Yield accounting was carried out by the trial sheaves method. Weather conditions in the years of research corresponded to the average long-term values: the hydrothermal coefficient in 2017 was 1.20, 2018 - 0.68, 2019 - 0.67.

Our research on the study of new chickpea varieties, the effect of seeding methods and the choice of forecrops on their yields included phenological observations, yield structure analysis, calculation of dry biomass and seed yields, and biochemical studies (in particular, determination of protein content in seeds)[4].

RESULTS AND DISCUSSION

During the experiment, the growing season of chickpea plants varied. In 2017, the varieties had the following values: Benefis - 87 days, Sphere and Sokol - 88 days each; in 2018 (due to weather restrictions, seeding was carried out 13 days later): Benefis - 82 days, Sphere - 83 days, Sokol - 81 days (compared with 2017 reduced by 2-7 days); in 2019: Benefis - 76 days, Sphere - 77 days, Sokol - 79 days (seeding was held at the dates established in 2017, but the growing season decreased by 9-12 days).

A detailed analysis of the yield structure revealed noticeable features of the influence of the seeding method and the choice of the forecrop.

There were significant differences in the height of chickpea plants. In the variety

[3] *Dospekhov B.A.* Methodology of Field Experience. Moscow: Kolos, 2011. 290 p.

[4] GOST 10846-91. Method for determination of protein. Grain and products of its processing. Moscow: Committee for Standardization and Metrology of the USSR, 1991. 11 p.

Benefis the values were in the range of 44,37 - 55,30 cm, where the lower values were recorded when using grain sorghum as a forecrop, the highest - when using spring barley. The highest plants were recorded when seeding with row spacing of 45 and 60 cm, the lowest with row spacing of 15 and 70 cm (gradation was observed within each forecrop). A similarity with the previous variety is traced in the characteristic of the variety Sokol: the lowest values were recorded when seeding on grain sorghum (47.47 cm), the highest - on spring barley (69.00 cm). As a result of taking into account the method of seeding, it was found that the gradation from the lowest to the highest plant is observed consistently on the scheme of seeding from 15 to 70 cm (this trend was traced for each forecrop). For the variety Sphere the limit of 42.58 cm (for grain sorghum) to 55.27 cm (for spring wheat) was observed. For this variety the lowest values for the forecrop were recorded when seeding with a row spacing of 30 cm, high - with a row spacing of 70 cm. In general, the Sokol variety had the highest chickpea plants in the experiment on the variant sown with a row spacing of 70 cm for spring barley.

The highest attachment of one bean was recorded in the case of seeding with a row spacing of 70 cm for the forecrop spring barley in the variety Sokol - 40.13 cm. The other two varieties had the following indicators: Benefis - not more than 30.90 cm (row spacing 15 cm, the forecrop - spring barley); Sphere - not more than 30.80 cm (row spacing 70 cm, the forecrop - spring wheat). The height of the lowest attachment reached: the variety Benefis - 24,53 cm (inter-row 70 cm, the forecrop - spring wheat); Sphere - 24,47 cm (inter-row 15 cm, the forecrop - grain sorghum); Sokol - 32,13 cm (inter-row 15 cm, the forecrop - spring barley).

The highest number of beans per plant was formed in the variety Benefis, sown with 45 cm row spacing on the forecrops grain sorghum and spring wheat - 57.40 and 55.80 pcs. respectively. The number of seeds received from one plant in these variants was 53.93 and 56.93 pcs. respectively. In the Sphere variety the highest values for the number of beans per plant were recorded in the variants, sown with a row spacing of 45 cm: 49,60-51,58 pcs. Ear grain content of this variety was quite low and was 0.93-0.87 (46.03-44.72 seeds, respectively). However, there was a variant when seeding with a row spacing of 70 cm on the forecrop grain sorghum, which grain size was up to 1.46 (33.60 beans and 49.13 seeds per plant). The variety Sokol was distinguished by the number of beans in the variants with row spacing of 45 and 60 cm, using as a forecrop spring wheat (41,94 and 40,53 beans, respectively) and 0.86 and 0.95 (35,87 and 38,70 seeds per plant). There were variants with lower number of beans but higher number of grains at 15, 30, and 60 cm inter-row cropping method (1,20; 1,09; 1,02, respectively).

Varieties were divided into groups according to the weight of 1 thousand seeds based on the declared characteristics. The variety Benefis received larger seeds than the varieties Sphere and Sokol: respectively, 284.70-354.60 g versus 288.60-325.40 and 243.50-267.90 g on average for 3 years. Increasing row spacing to 45 cm led to an increase in the weight of 1 thousand seeds, with a standard wide-row method of seeding this figure decreased. Analysis of the effect of the forecrop on the studied chickpea varieties showed an increase in the weight of 1 thousand seeds in such a row crop as corn.

In general, by the experiment the high yield of dry biomass was noted for chickpea of Sphere variety with 45 cm row-spacing on the forecrop of spring barley - 6.45 t/ha (see Table 1). The dry biomass yield of varieties differed significantly and depended both on the forecrop and on the width of the row spacing. Thus, variants higher than the average values were noted in the arrangement of the forecrops of solid planting (spring barley and spring wheat) and with row spacing of 30, 45 and 60 cm.

According to Duncan's criterion, significant differences were revealed only by factor A: between the varieties Benefis and Sphere;

Sokol and Sphere. According to the data of the analysis of variance, there are no appreciable differences between the varieties Benefis and Sokol.

Compared to the average values for the variety in the experiments with a row spacing of 15 cm the seed yield of all chickpea varieties under study was reduced due to the deterioration of elements of the yield structure and reduction of dry biomass: Benefis - by 0.86-1.65 t/ha (the first figure was calculated for the forecrop of spring barley, the second - for corn); Sphere - by 0.34-1.14 t/ha (similar to the previous variety); Sokol - by 0.20-1.02 t/ha (see. Table 2). A similar decrease in seed yield was noted when sowing with a row spacing of 30 cm, and in the variety Benefis even with a row spacing of 70 cm. In the other studied variants, there was an increase in yield compared with the average values of the variety. The highest yield was recorded at a row spacing of 45 cm and the precursor spring wheat in all the studied varieties: Sokol - 3.07 t/ha; Sphere - 3.89 t/ha; Benefis - 4.31 t/ha on average for 3 years.

According to Duncan's criterion, significant differences were noted in factor A: between the varieties Benefis and Sokol, as well as Sphere and Sokol. According to the data of analysis of variance, there were no appreciable differences between the varieties Benefis and Sphere. By factor B: by row spacing 15 and 70; 30 and 70; 45 and 70 cm.

The average indices of protein content in the seeds of the described chickpea varieties for the 3 years of the research are in the range from 16,16 (the variety Sokol, forecrop - grain

Табл. 1. Урожайность сухой биомассы сортов нута в зависимости от величины междурядий и выбора предшественника (2017–2019 гг.), т/га

Table 1. Dry biomass yield of chickpea varieties depending on the row spacing and the forecrop choice (2017-2019), t/ha

Forecrop (factor C)	Row spacing, cm (factor B)				
	15	30	45	60	70
Benefis (*factor A*)					
Grain sorghum	2,92	4,68	4,84	4,87	3,09
Corn	2,63	5,13	5,28	4,43	3,36
Spring barley	4,70	4,85	5,75	4,79	3,73
Spring wheat	3,74	5,35	5,46	4,96	3,66
Sfera (*factor A*)					
Grain sorghum	3,34	4,99	5,62	3,99	4,52
Corn	3,12	5,54	5,72	4,58	4,25
Spring barley	5,14	5,26	6,45	4,88	5,47
Spring wheat	4,33	5,89	5,99	5,18	4,70
Sokol (*factor A*)					
Grain sorghum	2,84	3,93	4,55	3,56	3,61
Corn	2,46	4,47	4,61	4,32	3,98
Spring barley	4,62	4,32	5,22	4,55	4,66
Spring wheat	3,78	4,82	4,75	4,04	3,68
	F_{fact}	$LSD_{0,05}$			
Factor A	5,055*	0,512			
Factor B	1,891	–			
Inter. A*B	0,198	–			
Factor C	1,377	–			
Inter. A*C	0,079	–			
Inter. B*C	3,314*	1,322			
Inter. A*B*C	0,185	–			

Note. Multiple comparisons of partial averages according to Duncan's criterion: factor A – 4.41a; 4.95b; 4.14a.

Табл. 2. Урожайность семян сортов нута в зависимости от величины междурядий и выбора предшественника (2017–2019 гг.), т/га

Table 2. Seed yield of chickpea varieties depending on the row spacing and the forecrop choice (2017–2019), t/ha

Forecrop (factor C)	Row spacing, cm (factor B)				
	15	30	45	60	70
Benefis (*factor A*)					
Grain sorghum	1,20	1,84	4,19	4,03	2,30
Corn	1,12	2,14	3,95	4,01	2,24
Spring barley	1,91	2,16	3,69	3,52	2,17
Spring wheat	1,61	2,45	4,31	3,91	2,72
Sfera (*factor A*)					
Grain sorghum	1,50	1,72	3,71	2,18	2,43
Corn	1,46	1,98	3,60	2,89	2,47
Spring barley	2,26	2,00	3,44	3,23	2,85
Spring wheat	1,96	2,31	3,89	3,09	2,98
Sokol (*factor A*)					
Grain sorghum	1,09	1,13	2,83	1,87	1,91
Corn	0,99	1,29	2,77	2,38	2,27
Spring barley	1,81	1,38	2,38	2,55	2,52
Spring wheat	1,43	1,61	3,07	2,46	2,38
	F_{fact}	$LSD_{0,05}$			
Factor A	10,522*	0,346			
Factor B	3,828*	0,447			
Inter. A*B	0,290	–			
Factor C	0,570	–			
Inter. A*C	0,114	–			
Inter. B*C	7,970*	0,893			
Inter. A*B*C	0,577	–			

Note. Multiple comparisons of partial averages according to Duncan's criterion: factor A – 2.77b; 2.60b; 2.01a; factor B 2.28a; 2.16a; 2.31a; 2.61ab; 2.93b.

sorghum, inter-row width 45 cm) to 22,70% per absolute dry matter (the variety Sphere, forecrop - spring wheat, inter-row width 15 cm). When calculating the average value of the variety, taking into account the soil and climatic conditions of the Lower Volga region, it was found that the highest protein content was in Sphere (20,27% in absolute dry matter), then followed by Benefis (19,07% in absolute dry matter) and Sokol (18,72% in absolute dry matter).

CONCLUSION

The experiment revealed differences in the height of chickpea plants - the highest of the presented varieties was the variety Sokol, followed by Benefis and Sphere. There was a significant difference in height when taking into account the method of seeding: the highest plants were observed with row spacing of 45 and 60 cm, the lowest - 15 and 30 cm. Gradation by the forecrop is as follows: grain sorghum, corn, spring barley, spring wheat. Differences were also recorded in the height of attachment of the lower bean: a decrease in this indicator occurred depending on the method of seeding with row spacing of 70, 60, 45, 30 and 15 cm. The choice of the forecrop also influenced the height of the attachment, with special mention of the crops of solid planting. Among the varieties under consideration, the highest value of the studied indicator was noted in the variety Sokol, which was also the highest. The data obtained confirm that an increase in the

width of the row spacing up to 45 cm leads to an increase in the weight of 1 thousand seeds, increasing the yield of the seeds and increasing the protein content in them. It was found that for the studied varieties in the conditions of the Lower Volga region the optimal seeding width is 45 cm and the use of spring wheat as a forecrop. The yield of the studied varieties and their protein content in their seeds in the conditions of the Lower Volga region with such a scheme of planting were as follows: Benefis - 4,31 t/ha, 21,15% in abs. dry matter; Sphere - 3,89 t/ha, 20,39% in abs. dry matter; Sokol - 3,07 t/ha, 19,32% in abs. dry matter.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

✉ **Маслова Г.А.,** младший научный сотрудник; **адрес для переписки:** Россия, 410050, г. Саратов, 1-й Институтский проезд, 4; e-mail: rossorgo@yandex.ru

Кондаков К.С., кандидат экономических наук

Башинская О.С., кандидат сельскохозяйственных наук, ведущий научный сотрудник

AUTHOR INFORMATION

✉ **Galina A. Maslova,** Junior Researcher; **address:** 4, 1st Institutskiy proezd, Saratov, 410050, Russia; e-mail: rossorgo@yandex.ru

Konstantin S. Kondakov, Candidate of Science in Economics

Oksana S. Bashinskaya, Candidate of Science in Agriculture, Lead Researcher

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ЭКОЛОГИЧЕСКАЯ ПЛАСТИЧНОСТЬ И СТАБИЛЬНОСТЬ ВИКИ ЯРОВОЙ (ПОСЕВНОЙ) В УСЛОВИЯХ ЗАПАДНО-СИБИРСКОГО РЕГИОНА

Гончарова А.В., (✉)Капко Т.Н.
Сибирский научно-исследовательский институт растениеводства и селекции – филиал Института цитологии и генетики Сибирского отделения Российской академии наук
Новосибирская область, р.п. Краснообск, Россия
(✉)e-mail: tatjanakapko@mail.ru

Представлены результаты оценки урожайности и адаптивных свойств восьми сортов и линий вики яровой (посевной) (*Vicia sativa* L.). В исследование были включены Камалинская 611 (стандарт), Новосибирская, Байкальская × Льговская 34, Байкальская × Г-252, Э-1280, 4604/1-2, ГК-964, Камалинская 611 × Новосибирская. Дана оценка продуктивности растений вики в питомнике предварительного сортоиспытания и выявлены наиболее урожайные, пластичные, стабильные и адаптированные образцы. Исследование проведено в 2017–2019 гг. в условиях лесостепи Приобья (Новосибирская область). Наиболее благоприятным для формирования урожайности зеленой массы был 2017 г., для зерновой продуктивности – 2019 г. Варьирование урожайности зеленой массы составило от 25,20 (Новосибирская в 2018 г.) до 40,70 т/га (Камалинская 611 × Новосибирская в 2017 г.), семян – от 1,50 (Камалинская 611 в 2017 г.) до 3,16 т/га (Камалинская 611 × Новосибирская в 2018 г.). Наименьшей вариабельностью и наибольшим гомеостазом в формировании урожайности зерна выделились образцы Байкальская × Г-252 и Камалинская 611 × Новосибирская, по урожайности зеленой массы – линии Э-1280 и 4604/1-2. По селекционной ценности выделены Байкальская × Г-252 (по урожайности зерна); Байкальская × Льговская 34 (по биомассе) и Камалинская 611 × Новосибирская (по урожайности зерна и биомассе). К числу наиболее отзывчивых на улучшение условий и стабильных можно отнести по зерновой продуктивности Байкальская × Льговская 34 и Камалинская 611, по урожайности биомассы – Камалинская 611 × Новосибирская.

Ключевые слова: вика яровая (посевная), урожайность, пластичность, стабильность, гомеостатичность, адаптивность

ECOLOGICAL PLASTICITY AND STABILITY OF SPRING VETCH (TARE) UNDER CONDITIONS OF THE WEST SIBERIAN REGION

Goncharova A.V., (✉)Kapko T.N.
Siberian Research Institute of Plant Production and Breeding – Branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences
Krasnoobsk, Novosibirsk region, Russia
(✉)e-mail: tatjanakapko@mail.ru

The results of evaluating the yield and adaptive properties of the eight varieties and lines of spring vetch (tare *Vicia sativa* L.) are presented. Kamalinskaya 611 (standard), Novosibirskaya, Baikalskaya × Lgovskaya 34, Baikalskaya × G-252, E-1280, 4604/1-2, GC-964, and Kamalinskaya 611 × Novosibirskaya varieties were included in the study. The productivity of vetch plants in the nursery of preliminary varietal trials is evaluated and the most productive, plastic, stable and adapted samples are identified. The study was conducted in 2017-2019 in the forest-steppe conditions of the Priob'ye region (Novosibirsk region). The most favorable for the formation of green matter yields was 2017, for grain productiv-

ity - 2019. The variation in green matter yields ranged from 25.20 (Novosibirskaya in 2018) to 40.70 t/ha (Kamalinskaya 611 × Novosibirskaya in 2017), and seeds from 1.50 (Kamalinskaya 611 in 2017) to 3.16 t/ha (Kamalinskaya 611 × Novosibirskaya in 2018). Baikalskaya × G-252 and Kamalinskaya 611 × Novosibirskaya samples stood out with the lowest variability and the highest homeostasis in the formation of grain yield, while the lines E-1280 and 4604/1-2 stood out in terms of green matter yield. Baikalskaya × G-252 (by grain yield); Baikalskaya × Lgovskaya 34 (by biomass) and Kamalinskaya 611 × Novosibirskaya (by grain yield and biomass) were selected for breeding value. Among the most responsive to improving conditions and stable are Baikalskaya × Lgovskaya 34 and Kamalinskaya 611 in terms of grain productivity and biomass yield - Kamalinskaya 611 × Novosibirskaya.

Keywords: spring vetch (tare *Vicia sativa* L.), yield, plasticity, stability, homeostasis, adaptability

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INTRODUCTION

The use of balanced fodder is very important in the organization of scientifically justified feeding of farm animals [1]. In Russia the main sources of fodder are natural lands and field fodder production[1]. Favorable conditions for all components are created in mixed crops, where legumes play an important role in improving the quality and digestibility of forages [2, 3]. Among the variety of leguminous crops spring vetch (*Vicia sativa* L.) can be distinguished, which is characterized by higher yields and protein content. In addition, it is less demanding to conditions than field beans and edible peas [4, 5]. It is a versatile crop with high fodder value and palatability, rich in protein, macro- and microelements, which can be used to create a green conveyor belt, prepare highly nutritious roughage and succulent fodder for winter and for introduction into the compound feed[2] [5].

Highly productive varieties adapted to local conditions can give high yields if the optimal cultivation technology is followed. The productivity of green biomass in monoculture can be 20-25 t/ha, in the form of vetch-grass mixtures - up to 40-46 t/ha and more. The yield of hay reaches 3.5-4.0 t/ha in pure form, and up to 6.5-8.5 t/ha mixed with oats. In addition, it is possible to get up to 2.0-2.5 t/ha of protein-balanced forage grain[3-5].

Comprehensive evaluation of the breeding material is the most important part of the work on variety development in a changing climate. It is necessary for the variety to have a stable yield of green mass, hay, seeds in a wide range

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[4] *Glukhov V.M.* Annual fodder crops. Novosibirsk: West Siberian Book Publishing House, 1967. 96 p.

[5] *Goncharov P.L., Goncharova A.V., Vasyakin N.I., Glinchikov I.M., Kipreev Y.N., Khan C.* Spring vetch. Novosibirsk: Novosibirsk Publishing House, 1989. 36 p.

of environmental conditions of cultivation [6]. The specificity of the conditions of each region implies the creation of the varieties that are maximally adapted to the zone of cultivation. Creating varieties that combine high productivity with resistance to a set of environmental factors is an important direction of breeding, especially in the regions with a variable climate. In this regard, the assessment of parameters of their yield and adaptive potential plays a special role [7].

The aim of the study is to evaluate the productivity of spring vetch (cultivated) plants in the nursery of preliminary varietal trials and to identify the most productive, plastic, stable and adapted samples in the forest-steppe conditions of the Priob'ye region.

MATERIAL AND METHODS

The study was conducted in the Novosibirsk region, at the experimental field of the Siberian Institute of Plant Growing and Agricultural Production (SibNIIRS), a branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences. The study focused on eight varieties and lines of vetch that underwent preliminary testing from 2017 to 2019. The Kamalinskaya 611 variety served as the control.

The experimental plot is located in the forest-steppe zone of Western Siberia. The soil cover of the field is represented by medium loamy slightly humusized chernozem with a weakly acidic reaction (pH 6.7) in the ploughing layer and an alkaline reaction (pH 7.9) in the carbonate horizons. The humus content is 4.2%, gross phosphorus is 0.30%, total nitrogen is 0.34%, mobile phosphorus and potassium (according to Chirikov) are 29 and 13 mg/100 g of soil, respectively. The soil treatment was standard for the forest-steppe zone.

The vetch was sown in a mixture with oat of the Rovensnik variety as a supporting crop at the optimal time, taking into account agrometeorological conditions for autumn fallow. The sowing was carried out by a row seeder SSFK-7, with a sowing rate of 120 kg/ha of vetch and 40 kg/ha of oats. The experiments were conducted on the plots with an accounting area of 25 m^2 with four repetitions according to the methodology of the state variety testing[6]. The productivity of green mass was determined from areas of 10 m^2, and the biomass was harvested manually with a sickle at the stage of mass flowering of the vetch. The grain was harvested at the stage of full maturity of the vetch with a combine harvester. Only vetch yield from the sowing was taken into account when evaluating the productivity of green mass and grain, with oats being separated and not taken into account in calculations.

The adaptive properties of the studied vetch samples were calculated for yield of grain and green mass traits. The obtained results were statistically processed using the Microsoft Office Excel package, with a critical significance level of 5%. The coefficient of variation was calculated by the method of B.A. Dospelov[7]. The index of environmental conditions was determined by the method of L.A. Zhivotkov et al.[8] The ecological stability and pliability indicators were calculated by the method of S.A. Eberhart and W.A. Russell[9] (1966), as described by V.A. Zykin, et al.[10] The selection value and homeostasis were determined using the method of V.V. Khangildin[11]. The stress

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resistance and genetic flexibility of varieties were calculated by the method of A.A. Rossiel and J. Hamblin[12] (1981), as described by A.A. Goncharenko [13].

RESULTS AND DISCUSSION

Plant growth and development are determined by the conditions of growth. The highest yield of seeds and green mass of vetch is observed in the regions of the northern forest-steppe, subtaiga, and foothills with moderate precipitation and temperature (see footnote 3). The forest-steppe zone of the Novosibirsk region is characterized by uneven distribution of heat and precipitation throughout the years and in the season. Even with relatively good moisture and heat supply, insufficient amounts of precipitation during the vegetation period in certain years can lead to drought stress, especially in June [14]. One of the goals of vetch breeding is to create a system of varieties with high adaptive potential for promising cultivation regions (see footnote 2).

During the years of the experiment (2017-2019), the weather conditions were generally favorable for the growth and development of the main and supporting crops. However, in 2018, precipitation was insufficient (HTC = 0.98), in 2017 - optimal (HTC = 1.35), and in 2019 - excessive (HTC = 1.43). Nevertheless, at different stages of ontogenesis, plant requirements for heat and moisture are not uniform, so rainfall at a later stage of development with sufficient warmth can compensate for the lack of precipitation at the beginning of vegetation and vice versa. During extreme environmental conditions, the ability to assess genotypes for endurance to unfavorable natural phenomena is ensured, which is especially important in selection for adaptability [15].

Spring vetch is very demanding of moisture. The critical period is the time of flowering. Lack of moisture during this period slows down biomass growth and reduces seed productivity. Excess precipitation also has a negative effect on seed yield and their seeding qualities. Яровая вика очень требовательна к влаге. Критический период – это время цветения.

In addition, vetch forms a significant amount of biomass, which can cause the supporting crop to fall over and make mechanized harvesting difficult (see footnote 14) [4].

Yield formation is a complex process that reflects the realization of the genotype of the variety under the influence of environmental conditions. It is known that the influence of the genotype, environment, and their interaction is significant in the formation of agronomically significant traits in vetch, and the main source of variation in the yield is the conditions of the surrounding environment [8].

As a result of a 3-year study of eight varieties and hybrids of vetch, it was found that all samples realize their productivity potential differently under the influence of environmental conditions. Yield variation of green mass ranged from 25.20 (Novosibirskaya in 2018) to 40.70 t/ha (Kamalinskaya 611 × Novosibirskaya in 2017), and of seeds from 1.50 (Kamalinskaya 611 in 2017) to 3.16 t/ha (Kamalinskaya 611 × Novosibirskaya in 2018). On average, the seed yield for the years of the study was 2.48 t/ha. Four samples (Baikalskaya × G-252, 4604/1-2, GK-964, and Kamalinskaya 611 × Novosibirskaya) showed a significant increase in yield compared to the standard, while the other three showed a tendency to increase. The average yield of green mass reached 29.65 t/ha, with only two samples (lines 4604/1-2 and Kamalinskaya 611 × Novosibirskaya) showing a significant increase in yield compared to the standard variety over the three years of the

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study. The hybrid Kamalinskaya 611 × Novosibirskaya was the most productive for both characteristics during the study years. Its grain and green mass yields significantly exceeded the standard variety and the average values in each year of the study, but in 2017 and 2019, the Baikalskaya × G-252 line was not inferior to it in seed yield (see Table 1).

The most favorable weather conditions for the formation of high yields of green mass were in 2017: for all varieties and lines (except for E-1280, which had the highest yield in 2018), the yield was the highest in that year. Despite the weak moisture in May, vetch formed good vegetative mass thanks to sufficient amounts of warmth and rainfall from June to July. However, this year was the most unsuccessful for the formation of high grain yields - for all the samples under study, the yield was the lowest. The environmental condition index (I_j), which reflects how favorable the conditions of a particular vegetative period are for a crop in a given year, was -0.5 for seed yield and +3.1 for green mass. The most favorable conditions for high seed yields were in 2019 - for all varieties and lines, the yield was the highest in that year (except for the Baikal × Lgovskaya 34 and Kamalinskaya 611 × Novosibirskaya lines, which had the highest yield in 2018). On the contrary, the yield of green mass was the lowest for all samples in this year: the June drought did not allow plants to form high vegetative mass, but they were able to produce a high grain yield. The environmental condition index (I_j = -2.5) confirms the negative impact of drought during the period of intensive growth of vegetative organs on the yield of green mass. Sufficient amounts of warmth and moisture during the formation of beans contributed to a high seed yield (I_j = +0.3).

Significant differences in weather conditions during the years of the study allowed for an evaluation of the adaptive potential of the studied samples (see Table 2).

One of the criteria for determining the relative variability of yield is the coefficient of variation (*CV*), which reflects the genotype's response to environmental conditions (see foot-

Табл. 1. Урожайность вики яровой (полевой) в питомнике предварительного сортоиспытания, т/га
Table 1. Productivity of spring vetch in the nursery of preliminary variety testing, t/ha

Variety, line	Grain				Herbage			
	2017	2018	2019	X_i	2017	2018	2019	X_i
Kamalinskaya 611 (standard)	1,50	2,40	2,66	2,19	30,30	27,20	25,30	27,60
Novosibirskaya	1,80	2,52	2,64	2,32	35,80	26,60	25,20	29,20
Baikalskaya × Lgovskaya 34	1,60	2,76	2,64	2,33	29,20	28,90	27,70	28,60
Baikalskaya × G-252	2,55	2,89	2,92	2,79	32,00	28,20	26,70	28,97
E-1280	1,72	2,58	2,65	2,32	26,90	29,20	26,30	27,47
4604/1-2	2,01	2,66	2,81	2,49	33,50	29,90	28,10	30,50
GK-964	2,18	2,73	2,73	2,55	33,30	29,50	27,90	30,23
Kamalinskaya 611 × Novosibirskaya	2,55	3,16	2,90	2,87	40,70	32,90	30,20	34,60
X_j	1,99	2,71	2,74	2,48	32,71	29,05	27,18	29,65
LSD_{05}	0,49	0,28	0,14	0,29	5,11	2,31	1,99	2,73
HTC	1,35	1,43	0,98		1,35	1,43	0,98	
I_j	–0,5	0,2	0,3		3,1	–0,6	–2,5	

Note: X_j – average yield of varieties for the year; X_i – average yield of varieties by years; I_j – environmental conditions index.

note 7). This indicator is related to the measure of homeostasis (*Hom*), which expresses the organism's system of adaptive responses that stabilize a certain yield potential within a wide range of environmental conditions (see footnote 11). During the experiment, it was found that grain yield is a highly variable trait (*CV* > 20%), with only two samples (Baikalskaya × G-252 and Kamalinskaya 611 × Novosibirskaya) showing average variation (16% and 19% respectively). Meanwhile, variation in green mass yield was insignificant for all samples (*CV* < 10%). The samples Baikalskaya × G-252 (*Hom* = 0.17, *CV* = 16%), Kamalinskaya 611 × Novosibirskaya (*Hom* = 0.15, *CV* = 19%), and GK-964 (*Hom* = 0.12, *CV* = 22%) showed the greatest stability in grain yield formation. These lines were the least variable and had the highest level of homeostasis among those studied. In terms of realizing the potential productivity of green mass, the lines Baikalskaya × Lgovskaya 34 (*Hom* = 9.18, *CV* = 3%), E-1280 (*Hom* = 6.10, *CV* = 5%), and 4604/1-2 (*Hom* = 5.61, *CV* = 5%) were the most stable in response to changes in environmental conditions.

In practical breeding, the selection value (*Sc*) of a variety is used to evaluate its stability, based on comparing its productivity in limited and optimal environments with the averaged yield for all environments (see footnote 11). The higher the value, the more stable the variety [9]. Among the studied samples, the hybrids Baikalskaya × G-252 (*Sc* = 2.43 for grain productivity), Baikalskaya × Lgovskaya 34 (*Sc* = 27.13 for green mass yield), and Kamalinskaya 611 × Novosibirskaya (*Sc* = 2.32 for grain yield and 25.67 for green mass yield) showed the highest selection values. Homoeostasis and selection value are linked together. Genotypes with high values of these parameters show

Табл. 2. Параметры адаптивности, экологической пластичности и стабильности вики яровой в агроценозе (2017–2019 гг.)

Table 2. Parameters of adaptability, ecological plasticity and stability of spring vetch in agrocenosis (2017–2019)

Variety, line	*CV*	ST	CC	b_i	δ^2_d	*Sc*	*Hom*
Grain							
Kamalinskaya 611 (standard)	36	–1,16	2,08	1,40	0,02	1,23	0,06
Novosibirskaya	29	–0,84	2,22	1,06	0,00	1,58	0,08
Baikalskaya × Lgovskaya 34	34	–1,16	2,18	1,48	0,01	1,35	0,07
Baikalskaya × G-252	16	–0,37	2,74	0,48	0,00	2,43	0,17
E-1280	31	–0,93	2,19	1,21	0,00	1,50	0,07
4604/1-2	26	–0,80	2,41	0,99	0,01	1,78	0,10
GK-964	22	–0,55	2,46	0,74	0,00	2,03	0,12
Kamalinskaya 611 × Novosibirskaya	19	–0,61	2,86	0,64	0,04	2,32	0,15
Herbage							
Kamalinskaya 611 (standard)	6	–5,00	27,80	0,90	0,03	23,05	4,79
Novosibirskaya	8	–10,60	30,50	2,00	3,09	20,55	3,55
Baikalskaya × Lgovskaya 34	3	–1,50	28,45	0,24	0,31	27,13	9,18
Baikalskaya × G-252	6	–5,30	29,35	0,97	0,06	24,17	5,08
E-1280	5	–2,90	27,75	0,01	4,69	24,74	6,10
4604/1-2	5	–5,40	30,80	0,98	0,00	25,58	5,61
GK-964	6	–5,40	30,60	0,98	0,03	25,33	5,49
Kamalinskaya 611 × Novosibirskaya	7	–10,50	35,45	1,93	0,47	25,67	5,13

Note: *CV* - coefficient of variation, %; ST - level of stress tolerance; CC - compensatory capacity; b_i - regression coefficient (ecological plasticity); δ^2_d - variance (phenotypic stability); *Sc* - breeding value; *Hom* - homeostability.

weak response to worsening conditions and good response to improvement [9].

The plasticity parameters (b_i) and stability (δ^2_d) are primarily used to judge the stability of varieties in different growing conditions [10]. Varieties with a regression coefficient (b_i) close to or equal to one show weak response to environmental change and are considered plastic. When the regression coefficient is above one, the sample belongs to the intensive type. The higher the value of b_i, the more responsive the variety to improved growing conditions, but its yield decreases sharply in unfavorable conditions. When bi is less than one, the variety belongs to the neutral (extensive) type, and the lower the value, the weaker the variety's response to environmental changes [10, 11]. In terms of seed yield, the line 4604/1-2 was found to be the most plastic (b_i = 0.99), meaning that its yield changes in response to changing growing conditions (see Table 2). The Baikalskaya × Lgovskaya 34 hybrid was found to be the most responsive to improved conditions (b_i = 1.48), while the Baikalskaya × G-252 hybrid proved to be the most suitable for cultivation under extensive conditions (b_i = 0.48). In terms of green mass yield, the most plastic samples were 4604/1-2, GK-964, and Baikalskaya × G-252 (b_i = 0.98, 0.98, and 0.97, respectively). The Novosibirskaya variety and Kamalinskaya 611 × Novosibirskaya hybrid were found to be more responsive to improved conditions than other samples during the study period (b_i = 2.00 and 1.93, respectively), allowing them to be classified as intensive varieties, while the E-1280 line (b_i = 0.01) proved to be a sample that does not decrease yield in worsening growing conditions.

Stability (δ^2_d) is an adaptive response of a genotype that characterizes its degree of stability. The value of variance should tend towards zero, and the lower it is, the more stable the variety is. However, this does not indicate its intensity, but rather a better adaptation to deteriorating conditions. The lowest variance in green biomass yield was found in lines 4604/1-2 (δ^2_d = 0.00), GK-964 (δ^2_d =0.03), and Kamalinskaya 611 variety (δ^2_d =0.03). The Kamalinskaya 611 x Novosibirsk line showed a high variability in variance (δ^2_d =0.47), indicating lower stability under deteriorating conditions. All samples were very stable in grain productivity (δ^2_d =0.00-0.04).

Based on the above, genotypes with bi >> 1 and δ^2_d approaching 0 are considered the most valuable under conditions of production intensification. They are more responsive to improving conditions while being more stable under deteriorating conditions. Kamalinskaya 611 variety and Baikalskaya x Lgovskaya 34 line showed the highest grain productivity, and the Kamalinskaya 611 x Novosibirsk line was most productive in green biomass yield. It should be noted that increasing a variety's plasticity may lead to a decrease in its stability [10]. This was observed in the most intensive variety, Novosibirsk, where its stability under deteriorating conditions was one of the weakest.

For the regions with unstable hydrothermal conditions from year to year and during the growing season (such as the Novosibirsk region), it is essential to evaluate the studied samples' ecological stability. Over the years of research, the most stress-resistant lines for grain formation were Baikalskaya x G-252 (SR=-0.37), GK-964 (SR=-0.55), and Kamalinskaya 611 x Novosibirsk (SR=-0.61), and for green biomass formation, Baikalskaya x Lgovskaya 34 (SR=-1.50), E-1280 (SR=-2.90), and Kamalinskaya 611 standard variety (SR=-5.00). These samples had a smaller decrease in yield under unfavorable conditions, indicating wider adaptive possibilities than the rest.

Genetic flexibility, or compensatory ability, of a variety reflects its average yield in contrasting conditions. The higher the indicator, the higher the degree of correspondence between the genotype and environmental conditions (see footnote 13) [6]. The most genetically flexible, and therefore more suitable for the forest-steppe conditions of the Ob River region, was the line Kamalinskaya 611 × Novosibirskaya (CA = 2.86 and 35.45 for grain and green mass productivity, respectively), which among the studied samples has the highest correspondence between the genotype's requirements and the expression of agroclimatic factors.

The main criterion for the value of vetch as a

forage crop is high productivity of green mass. When creating new varieties for intensive cultivation technologies, it is necessary to combine high potential productivity and stability of yield formation under the influence of environmental conditions, including resistance to a variety of stress factors.

CONCLUSIONS

1. Climate conditions have a significant influence on the realization of the productivity potential. During the experiment, 2019 was favorable for grain yield formation (X_j = 2.74 t/ha; I_j = 0.3), while 2017 was favorable for biomass formation (X_j = 32.71 t/ha; I_j = 3.1). Throughout the study, the Kamalinskaya 611 × Novosibirskaya line was the most productive in both characteristics (but in 2019, the Baikalskaya × G-252 line was slightly more productive for grain).

2. In terms of grain yield and adaptive parameters, the Baikalskaya × G-252, GK-964, and Kamalinskaya 611 × Novosibirskaya samples stood out. They combine high productivity, stability, stress resistance, genetic flexibility, plasticity, and breeding value. However, in conditions of production intensification, the Baikalskaya × Lgovskaya 34 line and the Kamalinskaya 611 variety are the most valuable, as their level of plasticity is maximal, and their variance tends towards zero. The stable Baikalskaya × G-252 line is neutral to environmental factor changes and can be recommended for extensive conditions.

3. The Kamalinskaya 611 × Novosibirskaya line stood out for green mass yield and adaptive parameters, combining high yield (X_i = 34.60 t/ha), genetic flexibility (CA = 35.45), and breeding value (*Sc* = 25.67) with responsiveness to improving conditions (b_i = 1.93), while it can adapt to worsening conditions (δ^2_d = 0.47). The Kamalinskaya 611 × Novosibirskaya line, combining high plasticity and stability, is among the most valuable genotypes for intensive production. The stable Baikalskaya × G-252 line with low plasticity can be recommended for extensive conditions.

4. For the conditions of the Novosibirsk region and the set of characteristics, the Kamalinskaya 611 × Novosibirskaya line is recommended, combining high grain and green mass productivity with responsiveness to improving growth conditions while being able to adapt to worsening conditions. The Baikalskaya × G-252 line, which combines average grain and biomass productivity with high stability and low responsiveness to improving conditions, is recommended for cultivation with minimal costs.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

Гончарова А.В., доктор сельскохозяйственных наук, главный научный сотрудник; e-mail: goncharovaav@bionet.nsc.ru

✉ **Капко Т.Н.,** научный сотрудник; **адрес для переписки:** Россия, 630501, Новосибирская область, р.п. Краснообск, а/я 375; e-mail: tatjanakapko@mail.ru

AUTHOR INFORMATION

Antonina V. Goncharova, Doctor of Science in Agriculture, Head Researcher; e-mail: goncharovaav@bionet.nsc.ru

✉ **Tatyana N. Kapko**, Researcher; **address:** PO Box 375, Krasnoobsk, Novosibirsk Region, 630501, Russia; e-mail: tatjanakapko@mail.ru

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ИНДУКЦИЯ КАЛЛУСОГЕНЕЗА СОЦВЕТИЙ ЧЕСНОКА (*ALLIUM SATIVUM* L.) *IN VITRO*

✉**Азопкова М.А.**
Всероссийский научно-исследовательский институт овощеводства – филиал Федерального научного центра овощеводства
Московская область, дер. Верея, Россия
✉e-mail: tixanish@mail.ru

С целью расширения генетического разнообразия чеснока озимого (*Allium sativum* L.) изучена возможность использования *in vitro* технологии получения сомаклональных вариантов каллусной культуры. Представлены результаты исследования эффективности каллусогенеза соцветий чеснока озимого сорта Гладиатор в зависимости от возраста экспланта (7, 14 и 21 сут с момента выхода соцветий из листовых пазух) и сочетания регуляторов роста (2,4-дихлорфеноксиуксусной кислоты в концентрациях 0,25; 0,5; 1,0; 2,0 мг/л и кинетина в концентрации 0,25; 0,5; 1,0 мг/л). Установлено, что ступенчатая стерилизация материала, основанная на последовательной обработке стерилизующими растворами, позволяет получать 92–98% стерильных эксплантов. У соцветий чеснока раннего возраста первые признаки начала каллусогенеза отмечены на 35-е сутки культивирования, а у соцветий в возрасте 21 сут – на 50-е. Зафиксировано образование каллуса из основания соцветий при добавлении в питательную среду 0,5; 1,0 и 2,0 мг/л 2,4-дихлорфеноксиуксусной кислоты и кинетина в изучаемых концентрациях. Наиболее интенсивное каллусообразование выявлено у 7-суточных эксплантов на питательной среде Мурасига – Скуга, обогащенной 2,0 мг/л 2,4-дихлорфеноксиуксусной кислоты, а также 0,5 и 1,0 мг/л кинетина. При этом доля эксплантов с каллусом составила 94 и 96% соответственно. Каллусообразование из основания соцветий в возрасте 14 и 21 сут при использовании этих вариантов питательной среды наблюдалось у 44 и 22% эксплантов соответственно.

Ключевые слова: чеснок озимый (*Allium sativum* L.), *in vitro*, эксплант, каллус, регулятор роста

INDUCTION OF GARLIC (*ALLIUM SATIVUM* L.) INFLORESCENCE CALLUSOGENESIS *IN VITRO*

✉**Azopkova M.A.**
All-Russian Research Institute of Vegetable Growing – Branch of the Federal Scientific Center of Vegetable Growing
Vereya, Moscow region, Russia
✉e-mail: tixanish@mail.ru

In order to expand the genetic diversity of winter garlic (Allium sativum L.), the possibility of using in vitro technology to obtain somaclonal variants of a callus culture was studied. The results of the study of the efficiency of inflorescences callusogenesis of winter garlic cultivar ladiator depending on the explant age (7, 14 and 21 days from the moment when inflorescences emerge from the leaf axils) and the combination of the growth regulators (2,4-dichlorophenoxyacetic acid in concentrations 0,25; 0,5; 1,0; 2,0 mg/l and kinetin in concentrations 0,25; 0,5; 1,0 mg/l) are presented. It was found that stepwise sterilization of the material, based on sequential treatment with sterilizing solutions, allows obtaining 92-98% sterile explants. The first signs of the beginning of callusogenesis were observed in early garlic inflorescences on the 35th day of cultivation, and in the inflorescences aged 21 days - on the 50th day. Callus formation from the base of inflorescences was recorded when 0.5; 1.0 and 2.0 mg/L of 2,4-dichlorophenoxyacetic acid and kinetin were added to the nutrient medium at the concentrations studied. The most intensive callus formation was detected in 7-day-old explants on the Murashige - Skoog nutrient medium enriched with 2.0 mg/L 2,4-dichlorophenoxyacetic acid and 0.5 and 1.0 mg/L kinetin. The proportion of explants with callus was 94% and 96%, respectively. Callus formation from the base of inflorescences at 14 and 21 days of age using these nutrient media options was observed in 44 and 22% of explants, respectively.

Keywords: winter garlic (Allium sativum L.), in vitro, explant, callus, growth regulator

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INTRODUCTION

Garlic (*Allium sativum* L.), belonging to the family Amaryllidaceae and subfamily Allioideae, is a valuable vegetable and medicinal crop widely used in human nutrition and pharmaceutical industry. It contains vitamins and biologically active substances such as flavonoids, steroid saponins, and others[1]. Garlic is characterized by well-developed bactericidal and antioxidant properties[2] and the ability to accumulate high concentrations of essential elements such as selenium[3] [1] and germanium[4].

For garlic propagation, cloves are mainly used. In this case, hidden diseases are transmitted to offspring, leading to its rapid degeneration. Due to the lack of seed reproduction in garlic, it is not possible to expand the genetic diversity of this crop using traditional breeding methods.

However, modern biotechnological methods allow obtaining *in vitro* culture genotypes that are valuable breeding material. The following directions are considered promising: somaclonal variation, cell selection, and *in vitro* mutagenesis.

Methods for obtaining garlic morphogenic callus from the basal part of the clove [2], root tips[5-7] [3, 4], apical meristems [2, 3, 5], stem and leaf discs[8] [6, 7] are known.

The main purpose of the research is to select the optimal combination of factors (age of explant, concentration of growth regulators) that provide the formation of callus tissues of winter garlic variety Gladiator in *in vitro* culture.

MATERIAL AND METHODS

The experiment was conducted in the Department of Biotechnology and Innovation Projects of the All-Russian Scientific Research Institute of Vegetable Growing - a branch of the Federal Scientific Center of Vegetable Growing from 2019 to 2021.

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[8] *Haque M.S., Wada T., Hattori K., Plas L.H.W., Klerl G.J.* Garlic roots for micropropagation through *in vitro* bulblet formation // Proc. the XXV International Horticultural Congress. Brussels, 2000. Part 10: Application of biotechnology and molecular biology and breeding, *in vitro* culture. pp. 45–52.

The material for the study was the inflorescences of the winter garlic variety Gladiator, isolated on the 7th, 14th, and 21st days after they emerged from the leaf axils.

The Gladiator variety has been included in the State Register of the Russian Federation since 2011, and is characterized as a medium-ripening variety (with a vegetative period of 90-98 days), shooting out, with high winter hardiness, storage ability, and yield [8].

A stepped sterilization method was used for introduction *in vitro*. The inflorescences were washed for 30-60 minutes in a 10% soap solution, then for 15-20 minutes in a 0.1% solution of potassium permanganate, and then for 30 seconds in a 70% ethanol solution. They were then sterilized in a 1.0% solution of sodium hypochlorite for 20 minutes, followed by three rinses with sterile water[9] [9].

The explants were cultured on a Murashige-Skoog (MS)[10] nutrient medium enriched with 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin, the concentrations of which are presented in Table 1. Other characteristics of the study included a constant temperature of 20°C, light intensity of 5000 lux, and a 16/8-hour photoperiod.

Statistical processing of the experimental data was carried out using the Microsoft Office Excel software package. The plants were monitored every 7 days after the growth regulators were introduced into the culture.

Табл. 1. Концентрация регуляторов роста, мг/л
Table 1. Growth regulator concentration, mg/l

2,4-D	Kinetin			
	0	0,25	0,5	1,0
0,25	+	+	+	+
0,5	+	+	+	+
1,0	+	+	+	+
2,0	+	+	+	+

RESULTS AND DISCUSSION

During the course of the research, it was established that callus formation from the base of the inflorescence did not occur in all the variants studied. In addition, depending on the age of the explant, certain features of the process were observed.

In inflorescences of early age, the first signs of callusogenesis were observed on the 35th day of cultivation, while in inflorescences aged 21 days, they were observed on the 50th day. When cultivating garlic inflorescences isolated on the 7th day after the arrow emerged on a nutrient medium (enriched with 2.4-D and kinetin at concentrations of 2.0, 0.5, and 1.0 mg/l, respectively), the proportion of callusogenic explants was 94% and 96%, respectively (see Table 2).

Табл. 2. Доля каллусогенных эксплантов, % ± 2Sp (n = 100)
Table 2. Proportion of garlic callus explants, % ± 2Sp (n = 100)

2,4-Д, mg/l	Kinetin, mg/l	Age of explants, days		
		7	14	21
0,25	0	0	0	0
	0,25	0	0	0
	0,5	0	13,0 ± 6,7	0
	1,0	0	13,0 ± 6,7	0
0,5	0	0	13,0 ± 6,7	0
	0,25	10,0 ± 6,0	12,0 ± 4,6	7,0 ± 5,1
	0,5	12,0 ± 4,6	13,0 ± 6,7	12,0 ± 4,6
	1,0	14,0 ± 6,8	14,0 ± 6,8	12,0 ± 4,6
1,0	0	12,0 ± 4,6	15,0 ± 7,0	13,0 ± 6,7
	0,25	16,0 ± 7,3	26,0 ± 8,8	12,0 ± 4,6
	0,5	30,0 ± 9,1	27,0 ± 8,9	13,0 ± 6,7
	1,0	32,0 ± 9,3	29,0 ± 9,1	14,0 ± 6,8
2,0	0	56,0 ± 9,8	33,0 ± 9,0	21,0 ± 8,0
	0,25	58,0 ± 9,8	33,0 ± 9,0	22,0 ± 8,2
	0,5	94,0 ± 4,7	44,0 ± 9,9	22,0 ± 8,2
	1,0	96,0 ± 3,9	40,0 ± 9,8	22,0 ± 8,2

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No callus formation was observed when culturing explants on a nutrient medium containing 0.25 mg/l of 2.4-D.

When cultivating garlic inflorescences aged 7 days on a nutrient medium containing 0.5 mg/l of 2.4-D and kinetin at the studied concentrations, the proportion of callusogenic explants varied at the level of 10-14%.

The proportion of callusogenic explants cultivated on a nutrient medium containing 1.0 mg/l of 2.4-D was 12-32%, depending on the concentration of kinetin.

The largest number of callusogenic explants (44% and 40%) was recorded when cultivating winter garlic inflorescences aged 14 days on a nutrient medium containing 2.0 mg/l of 2.4-D and kinetin at concentrations of 0.5 and 1.0 mg/l, respectively.

During the cultivation of 14-day garlic inflorescences on a nutrient medium containing 0.25-1.0 mg/l of kinetin and 1.0 mg/l of 2.4-D, callus formation was observed in 26.0-27.0% of explants; 0.25-1.0 mg/l of kinetin and 0.5 mg/l of 2.4-D - in 12.0-14.0%; 0.5 and 1.0 mg/l of kinetin and 0.25 mg/l of 2.4-D - in 13.0%.

When cultivating garlic inflorescences aged 21 days on a nutrient medium enriched with growth regulators at the studied concentrations, suppression of explants was observed in all variants presented in Table 2. Callus formation was not observed on a nutrient medium containing kinetin at the specified concentrations and 0.25 mg/l of 2.4-D.

CONCLUSION

As a result of the studies aimed at identifying the optimal concentration of growth and age regulators for inducing callusogenesis in winter garlic, it has been established that inflorescences at 7 days after the emergence of the arrow from the leaf axils possess the highest callusogenic ability (94-96%). When cultured on MS nutrient medium enriched with 2.0 mg/L 2,4-D, as well as 0.5 and 1.0 mg/L kinetin, the onset of callus formation occurs 10-15 days earlier than in explants at 14 and 21 days of age.

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ИНФОРМАЦИЯ ОБ АВТОРЕ

✉ **Азопкова М.А.,** кандидат сельскохозяйственных наук, научный сотрудник; **адрес для переписки:** Россия, 140153, Московская область, дер. Верея, строение 500; e-mail: tixanish@mail.ru

AUTHOR INFORMATION

✉ **Marina A. Azopkova,** Candidate of Science in Agriculture, Researcher; **address:** 500, Vereya vil., Moscow Region, 140153, Russia; e-mail: tixanish@mail.ru

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ЭКОЛОГИЧЕСКОЕ ИСПЫТАНИЕ ЯЧМЕНЯ В СЕВЕРНОЙ ЛЕСОСТЕПИ ЧЕЛЯБИНСКОЙ ОБЛАСТИ

Пырсиков Д.А., ✉Пуалаккайнан Л.А., Глаз Н.В., Уфимцева Л.В.
ООО «Чебаркульская птица»
Челябинская область, пос. Тимирязевский, Россия
✉e-mail: lora708@yandex.ru

Представлена сравнительная оценка сортов ячменя различного эколого-географического происхождения (Челябинский 99, Яик, Омский 95 и Памяти Чепелева). Испытание сортов проводили в полевом опыте на сельхозпредприятии Челябинской области с целью подбора сортимента, обеспечивающего максимальную стабильную урожайность. Всего за годы исследований (2017–2019, 2021–2022) изучено 20 сортов ячменя челябинской, омской, тюменской, свердловской и самарской селекции. Рост и развитие ячменя проходили в контрастные по тепло- и влагообеспеченности вегетационные периоды. Сорт Яик не только показал высокий потенциал продуктивности в годы изучения, но и по размаху урожайности вместе с сортом Памяти Чепелева отмечен наиболее стабильным. Самыми устойчивыми к стрессу были сорта Омский 95 и Челябинский 99. По показателю генетической гибкости лидирует сорт Яик. По степени реакции на условия внешней среды изучаемые сорта разделились на две группы. В первой группе оказался сорт Памяти Чепелева, то есть в условиях данных исследований он слабо отзывался на внешние факторы. Во вторую группу вошли сорта Омский 95, Яик и Челябинский 99, они наиболее отзывчивы на внешние факторы. Наиболее выделился сорт Омский 95, который характеризуется наибольшей экологической пластичностью. Высокая степень стабильности реакции отмечена у сорта Яик, низкая – у остальных сортов. По результатам 5-летних исследований в условиях северной лесостепи Челябинской в целом по результатам расчетов максимальную пластичность и стабильность показал сорт Яик, который рекомендован для сельхозтоваропроизводителей.

Ключевые слова: ячмень, сорт, урожайность, экологическая пластичность, экологическая стабильность

ECOLOGICAL TESTING OF BARLEY IN THE NORTHERN FOREST-STEPPE OF THE CHELYABINSK REGION

Pyrsikov D.A., ✉Pualakkainan L.A., Glaz N.V., Ufimtseva L.V.
LLC «Chebarkulskaya Ptitsa»
Timiryazevsky settlement, Chelyabinsk region, Russia
✉e-mail: lora708@yandex.ru

A comparative evaluation of barley varieties of different ecological and geographical origin (Chelyabinsky 99, Yaik, Omsky 95 and Pamyati Chepeleva) is presented. The varieties were tested in a field experiment at an agricultural enterprise in the Chelyabinsk region in order to select the assortment which provides the maximum stable yield. A total of 20 barley varieties of Chelyabinsk, Omsk, Tyumen, Sverdlovsk and Samara breeding were studied during the years of research (2017-2019, 2021-2022). Barley growth and development took place in contrasting heat and moisture periods of vegetation. The Yaik variety not only showed high productivity potential in the years of study, but was also marked by the most stable crop yield scale together with the Pamyati Chepeleva variety. The most stress-resistant varieties were Omsky 95 and Chelyabinsky 99. In terms of genetic flexibility, the Yaik variety is the leader. The varieties under study were divided into two groups according to the degree of response to the environmental conditions. The first group included the variety Pamyati Chepeleva, that is, under the conditions of these studies, it was weakly responsive to external factors. The second group included the varieties Omsky 95, Yaik and Chelyabinsky 99, they are the most responsive to external factors. The most prominent variety was Omsky 95 which is characterized by the greatest ecological plasticity. A high degree of stability of the reaction was noted in the variety Yaik, low - in other varieties. According to the results of 5-year research in the

conditions of the northern forest-steppe of the Chelyabinsk region as a whole, the maximum plasticity and stability was shown by the variety Yaik, which is recommended for agricultural producers.

Keywords: barley, variety, productivity, ecological plasticity, ecological stability

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The authors declare no conflict of interest.

INTRODUCTION

The barley crop is very versatile in terms of its distribution area (it is cultivated from the subtropical zone to the Far North and in high mountain conditions) and in terms of its application (raw material for food, processing industry, forage production). Nowadays the fodder and food value of the crop is second only to wheat, corn and rice. In 2022 the total sown area of cereals in the Russian Federation was 28.5 mln ha. Barley occupied more than 25% of the total sown area - 7.2 mln ha. The largest barley crop area is in the Volga Federal District, followed by the Central, Siberian, Southern and Ural districts[1].

In the Chelyabinsk region spring barley crops occupy from 19 to 25% of the total sown area and depend on the demand for grain. An increase in the purchasing value of barley grain in the autumn-winter period leads to an increase in the sown area, and a decrease - to a reduction[2].

The decisive condition for obtaining high and stable yields in modern agricultural production is the use of the varieties most adapted to local conditions, capable of using the natural resources of the place of growth with the greatest efficiency [1-4].

The purpose of the study is a comparative analysis of barley varieties of different ecological and geographical origin in order to select the variety that provides the maximum stable yield in the bioclimatic conditions of the northern forest-steppe of the Chelyabinsk region.

MATERIAL AND METHODS

Testing of the varieties was carried out on the fields of the department of primary seed production of the complex crop production OOO Chebarkulskaya Ptitsa - one of the largest agricultural enterprises of the Chebarkul district of the Chelyabinsk region.

Chebarkul district is part of the northern forest-steppe zone of the region and is characterized by a continental climate with strongly pronounced seasons. The main features of the climate are cold and long winter with frequent snowstorms, dry and hot summer. Currently, there is a trend towards higher air temperatures and lower precipitation, climate change is observed [5].

Ecological barley variety trials were conducted under the conditions of a field experiment in accordance with the technique of state variety trials of agricultural crops at plots with the total area of 26.25 m^2, the accounting area - 25 m^2 in threefold repetition. Placement of plots was randomized. Seeding rate - 5.0 mln. of germinated grains/ha.

Soil in the experiments was low-humus me-

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dium leached loamy chernozem. The content of mobile forms of nitrogen and phosphorus in the soil was average, potassium - high, humus - high. The reaction of the soil solution was close to neutral. Protection of barley plots corresponded to the adopted scheme on the production crops.

Ecological plasticity and stability parameters of barley varieties were calculated by the method of S.A. Eberhard, W.A. Russell[3] and by the method of A.A. Gryaznov[4]. Research results were processed using single-factor and multivariate analysis of variance.

A total of 20 barley varieties of Chelyabinsk, Omsk, Tyumen, Sverdlovsk and Samara breeding were studied in 2017-2019 and 2021, 2022. The article presents a comparative assessment of the four most promising varieties - Chelyabinsky 99 and Yaik (FSBSI Chelyabinsk NIISKh), Omsky 95 (FSBSI Omsk ANC), Pamyati Chepeleva (Urals Research Institute of Agriculture - Branch of the Ural Federal Agrarian Research Center of the Ural Branch of the RAS).

Chelyabinsky 99. Medium-late, vegetation period 71-92 days. The plant is medium-grown. Resistance to lodging and drought is average. Moderately susceptible to barley net blotch and root rot, susceptible to loose smut. The average yield in the region was 25.6 c/ha. The maximum yield was 53,2 c/ha and was obtained in the Republic of Bashkortostan in 2001. Grain is of medium size, weight of 1000 grains is 38-47 g. Protein content in grains is 10,6-14,3%. It is included in the list of brewing varieties of the Russian Federation [6].

Omsky 95. Medium maturing, vegetation period is 73-86 days. The bush is semi-erect. The plant is medium-grown. Resistance to lodging and drought is at the level of standard varieties. It is affected in the moderate degree by kernel smut and barley net blotch, strongly by loose smut and root rot. During the years of study, the yield in the region was 25.6 c/ha. The maximum yield of 55 c/ha was obtained in the Tyumen region in 2005. Grain is of medium size, weight of 1000 grains is 40-48 g. Protein content in grains is 11,6-15,1%. Valuable by quality [7].

Yaik. Mid-late, the vegetation period is 77-91 days. The plant is medium-grown. In resistance to lodging and drought tolerance it is estimated at the level of standard varieties. Susceptible to head smut. Strongly affected by loose smut and stem rust. It was weakly affected by brown rust in the field. During the years of study, the average yield in the Ural region was 26.3 c/ha, the maximum - 59.2 c/ha, obtained in 2017 in the Chelyabinsk region. Grain is large, weight of 1000 grains is 40-50 g. The crop is of grain-fodder type. Protein content in the grain is up to 10.1% [8].

Pamyati Chepeleva. The variety is medium-ripening, the vegetation period is 79-99 days. The plant is short - of medium length. In resistance to lodging it is inferior to standard varieties, and exceeds them in drought tolerance. It is moderately resistant to stripe disease and root rot. It was affected under field conditions by loose smut, barley net blotch and net blotch to a moderate degree. The highest yield - 81.5 c/ha - showed in the Nizhny Novgorod region in 2015. The grain is large, the weight of 1000 grains is 40-51 g. The variety is valuable in quality. Protein content in the grain is 8.6-11.0% [9].

RESULTS AND DISCUSSION

Great importance in increasing the value and quality of the crop is the ability of the variety to show itself in local conditions. Evaluation of variety response to the changes in growing conditions will allow to solve the issues with a reasonable selection of varieties for each farm. To analyze the indicators of productive and adaptive potential of the varieties by varying their yields, scientists have developed a number of methods to assess the stability of varietal yields.

Multiyear field trials allow to estimate objectively enough the degree of plants reaction

[3]*Eberhart S.A.* Stability parameters for comparing varieties // Crop Sci. 1966. Vol.6. N 1. P. 36–40.
[4]*Gryaznov A.A.* Karabalyk barley. Kustanai: Kustanai printing yard, 1996. 446 p.

to climatic changes in the environment [5, 7, 10]. In our studies barley growth and development took place in the years with different heat and moisture supply during the growing season, which determined the value of varieties yield (see Table 1).

Yield is an indicator of adaptability and environmental sustainability of crop varieties. The lower the index of the yield range (*d*), the more stable the productivity of the variety under specific conditions (see Table 2).

The Yaik variety not only showed high productivity potential in the years of the study, but also was the most stable by the yield range together with Pamyati Chepeleva variety.

The difference between the minimum and maximum yields (Y_{min} - Y_{max}) shows how resistant this variety is to stress under changing meteorological conditions. This indicator has a negative value. The smaller its absolute value, the greater the adaptive capacity of the variety, that is, the higher its stress tolerance. The most stress-resistant (Y_{min} - Y_{max}) varieties were Omsky 95 and Chelyabinsky 99.

Variety stress tolerance is also characterized by the index of genetic flexibility (Y_{min} - Y_{max})/2, reflecting the degree of correspondence between the genotype of the variety and various environmental factors. According to this indicator the Yaik variety is the leader, the index of genetic flexibility is 5.6.

According to the degree of response to environmental conditions by the method of S.A. Eberhard, W.A. Russell (see footnote 3) the studied varieties were divided into two groups. The first group included the variety Pamyati Chepeleva ($b_i < 1$), that is, under the conditions of this study, it was weakly responsive to external factors. The second group consisted of the varieties Omsky 95, Yaik and Chelyabinsky 99, which have $b_i > 1$, they are most responsive to external factors. The variety Omsky 95 (b_i = 1,0474), which is characterized by the greatest ecological plasticity (see Table 3), stood out the most.

The best index of the degree of reaction stability (σ_2d - 3.61) was noted in the variety Yaik, lower indicators were characterized by other varieties. Thus, the variety Yaik in the years of research was characterized by higher stability of productivity.

Табл. 1. Урожайность ячменя, ц/га
Table 1. Barley yield, c/ha

Variety	Year of study				
	2017	2018	2019	2021	2022
Chelyabinsky 99 (standard)	61,17	42,60	34,03	57.20	64,13
Omsky 95	48,15	41,60	32,40	50,20	68,49
Yaik	57,66	41,70	39,89	55,10	70,63
Pamyati Chepeleva	58,69	43,82	38,95	49,10	68,03
LSD_{05}	1,27	1,78	1,53	0,95	3,22

Табл. 2. Адаптивный потенциал сортов ячменя
Table 2. Adaptive potential of barley varieties

Variety	Grain yield, t/ha			Yield spread, *d*, %	Stress Resistance, $Y_{min} - Y_{max}$	Genetic flexibility, $(Y_{max} + Y_{min})/2$
	max	min	average			
Chelyabinsky 99 (standard)	6,4	3,4	5,2	46,9	- 3,0	4,9
Omsky 95	6,8	3,2	4,8	52,9	– 3,6	5,0
Yaik	7,1	4,0	5,3	43,7	– 3,1	5,6
Pamyati Chepeleva	6,8	3,9	5,2	42,6	– 2,9	5,4

Табл. 3. Экологическая пластичность и стабильность урожайности сортов ярового ячменя
Table 3. Ecological plasticity and yield stability of spring barley varieties

Variety	Yield, t/ha			Ecological plasticity index		
	Lim	X_i	± to the standard, %	By the method of S.A. Eberhard, W.A. Russell		By the method of A.A. Gryaznov
				Plasticity coefficient (b_i)	Stability ($\sigma^2 d$)	EPI
Chelyabinsky 99 (standard)	3,4–6,4	5,2	100,0	1,0056	19,27	1,050
Omsky 95	3,2–6,8	4,8	– 7,7	1,0474	15,16	0,973
Yaik	4,0–7,1	5,3	+ 1,9	1,0171	3,61	1,085
Pamyati Chepeleva	3,9–6,8	5,2	± 0,0	0,9298	96,48	1,049

Табл. 4. Ранжирование сортов ячменя по показателям адаптивности, определенным разными методами
Table 4. Ranking of barley varieties according to adaptability indicators determined by different methods

Variety	By the method of V.A. Zykin	By the method of A.A. Rossielle, J. Hemblin		By the method of S.A. Eberhard, W.A. Russell		By the method of A.A. Gryaznov	The sum of ranks
	Yield range d	Stress resistance $Y_{min} - Y_{max}$	Genetic flexibility $(Y_{max} + Y_{min})/2$	Plasticity coefficient (b_i)	Stability ($\sigma^2 d$)	EPI	
Chelyabinsky 99 (standard)	3	2	4	3	3	2	17
Omsky 95	4	4	3	4	2	4	21
Yaik	2	3	1	2	1	1	10
Pamyati Chepeleva	1	1	2	1	4	3	12

The Yaik variety (IEP = 1.085) was also the best when calculating the index of ecological plasticity in the studied varieties (according to A.A. Gryaznov).

The use of different methods of determining the stability and plasticity of varieties allows getting the most complete information. For the final evaluation the principle of ranking by parameters was used, and the evaluation was carried out according to the sum of ranks obtained by each method. The significance of the rank decreases from 1 to 4 (see Table 4). In our experiment, according to the sum of ranks, the best variety was Yaik, followed by Pamyati Chepeleva, Chelyabinsky 99 and Omsky 95.

CONCLUSION

The study of varieties in specific climatic conditions and the definition of their plasticity and adaptability traits allow to make the right choice among the variety of crops proposed for use in favor of the cultivar most adapted for cultivation on this farm. According to the results of the 5-year research in the northern forest-steppe of the Chelyabinsk region as a whole, the maximum plasticity and stability was shown by the Yaik variety, which is recommended for agricultural producers.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

Пырсиков Д.А., генеральный директор

(✉)**Пуалаккайнан Л.А.,** кандидат сельскохозяйственных наук, начальник отдела; **адрес для переписки:** Россия, 456404, Челябинская область, Чебаркульский район, пос. Тимирязевский, ул. Мичурина, 3; e-mail: lora708@yandex.ru

Глаз Н.В., кандидат сельскохозяйственных наук, начальник отдела

Уфимцева Л.В., кандидат биологических наук, доцент, специалист

AUTHOR INFORMATION

Dmitriy A. Pyrsikov, Director General

(✉)**Lidiya A. Pualakkainan**, Candidate of Science in Agriculture, Department Head; **address:** 3, Michurina St., Timiryazevsky settl., Chebarkulsky District, Chelyabinsk Region, 456404, Russia; e-mail: lora708@yandex.ru

Nikolai V. Glaz, Candidate of Science in Agriculture, Department Head

Larisa V. Ufimtseva, Candidate of Science in Biology, Associate Professor, Specialist

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УСТОЙЧИВОСТЬ ЯРОВОГО ЯЧМЕНЯ К ВОЗБУДИТЕЛЯМ ЛИСТОВЫХ БОЛЕЗНЕЙ

(✉)**Дорошенко Е.С., Шишкин Н.В.**
Аграрный научный центр «Донской»
Ростовская область, г. Зерноград, Россия
(✉)e-mail: katyalevchenko1@mail.ru

Потери урожая ярового ячменя из-за эпифитотий различных листовых заболеваний могут составлять 30–50%. Самыми распространенными патогенами в Ростовской области считаются мучнистая роса и гельминтоспориозные пятнистости. Наиболее эффективный и доступный способ изучить устойчивость новых сортов – использование искусственных инфекционных фонов, где в провокационных и экстремальных условиях происходит отбор родительских форм и селекционных линий, наиболее устойчивых к патогенам. Исследования проведены в течение трех лет (2020–2022) на инфекционном стационарном участке. Целью исследования стало выявление среди изучаемых сортов и линий ярового ячменя, стабильно устойчивых и резистентных к распространенным в Ростовской области листостебельным патогенам. Объектом исследования послужили сорта ярового ячменя различного эколого-географического происхождения. Число изучаемых образцов варьировало: в 2020 г. – 215, 2021 г. – 256, 2022 г. – 196. В период исследования погодные условия были различными по температурному режиму и количеству осадков. В результате полевой оценки на искусственном инфекционном фоне выделены сорта и линии ярового ячменя с высокой устойчивостью к изученным патогенам. Высокоустойчивыми к мучнистой росе оказались KWS-11-228 и Pioner (Франция), Саншайн, Margret, Viking, Laurika и Tituringia (Германия), Perun (Чехия), Tipple (Англия), Калита, Леон и Тонус (Россия), Оболонь и Чаривный (Украина). Устойчивость к гельминтоспориозным пятнистостям проявили сорта Русь, Таловский 9, Тонус и Эльф (Россия), Эней (Украина). Также выделены образцы, имеющие устойчивость к обоим патогенам: Леон, Тонус, Эльф, Федос, Формат и Азимут (Россия), Margret, Prestige и Viking (Германия), Perun (Чехия).

Ключевые слова: яровой ячмень, устойчивость, толерантность, патоген, мучнистая роса, гельминтоспориозные пятнистости

SPRING BARLEY RESISTANCE TO LEAF DISEASE PATHOGENS

(✉)**Doroshenko E.S., Shishkin N.V.**
Agricultural Research Center "Donskoy"
Zernograd, Rostov region, Russia
(✉) e-mail: katyalevchenko1@mail.ru

Yield losses of spring barley due to epiphytotics of various leaf diseases can be as high as 30-50%. The most common pathogens in the Rostov region are powdery mildew and helminthosporium blotches. The most effective and accessible way to study the resistance of new varieties is to use artificial infectious backgrounds where parental forms and breeding lines most resistant to pathogens are selected under provocative and extreme conditions. The studies were conducted over a three-year period (2020-2022) at an infectious disease stationary site. The purpose of the study was to identify the varieties which were consistently stable and resistant to the common frondiferous pathogens among the studied varieties and lines of spring barley in the Rostov region. Varieties of spring barley of

different ecological and geographical origin were the object of research. The number of the studied samples varied: in 2020 - 215 varieties, 256 in 2021, and 196 in 2022. The weather conditions varied in temperature and precipitation during the study period. As a result of field evaluation on artificial infection background, spring barley varieties and the lines with high resistance to the studied pathogens were identified. Highly resistant to powdery mildew were the varieties KWS-11-228 and Pioner (France), Sunshine, Margret, Viking, Laurika and Tituringia (Germany), Perun (Czech Republic), Tipple (England), Kalita, Leon and Tonus (Russia), Obolon and Charivny (Ukraine). The varieties Rus, Talovsky 9, Tonus and Elf (Russia), and Eney (Ukraine) showed resistance to Helminthosporium blotches. Also, samples with resistance to both pathogens were identified: Leon, Tonus, Elf, Fedos, Format and Azimut (Russia), Margret, Prestige and Viking (Germany), Perun (Czech Republic).

Keywords: spring barley, resistance, tolerance, pathogen, powdery mildew, helminthosporium blotches

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INTRODUCTION

The Southern Federal District (SFD) is one of the main grain-producing regions of the Russian Federation. The largest areas of forage crops are sown here under barley. The SFD, in which Rostov region is located, accounts for 12-15% of the all-Russian barley production [1, 2].

Every year there is a loss of yields of cultivated varieties of barley from the impact of weather conditions, as well as the development of various fungal diseases [3]. Sharp fluctuations in climatic conditions noted in the region in recent years: significant temperature variations, abundant precipitation, alternating with drought and low atmospheric humidity, lead to a surge of occurrence of the most common and harmful diseases in natural conditions [4]. Each pathogen is dangerous in its own way: vegetative organs can be affected, leading to plant death (root rot) or reduced productivity (rust diseases, powdery mildew, various spot diseases), and reproductive organs can be affected (ergot, various types of smut) [5, 6].

According to long-term observations and studies, the prevalence of such leaf diseases as powdery mildew (causative agent - fungus *Blumeria graminis* (DC.) Speer) and helminthosporium spot diseases: net blotch (causative agent - *Pyrenophora teres* Drechsler) and dark brown (causative agent - *Bipolaris sorokiniana* Shoemaker) in spring barley crops was observed. Both pathogens are among the most harmful barley diseases. Directly and indirectly affecting plants, they cause a decrease in productivity and grain quality. Under favorable hydrothermal conditions, they may cover the entire assimilation surface of the leaves. They are found in all the regions of crop cultivation [7-9].

The basis of modern strategy in crop breeding is targeting, which is the need to create a system of varieties, climatically and environmentally differentiated, adapted to the specific

conditions of each region [10]. To create such varieties, the analysis of source material should be complete and versatile, it should be carried out under the conditions of the region, including phytosanitary [11]. It is conditioned by inseparable development of host plant and pathogen. Only in this case, it is possible to assess the interaction of all factors of obtaining quality and high yield.

The purpose of the study was to identify those varieties among the studied ones and the lines of spring barley that are stable and resistant to common frondiferous pathogens in the Rostov region.

MATERIAL AND METHODS

The studies were conducted over three years (2020-2022) at the infectious stationary site of the Laboratory of Plant Immunity and Protection of the Agricultural Research Center "Donskoy".

Varieties of spring barley of different ecological and geographical origin (Ukraine, Belarus, Germany, Holland, Czech Republic, Denmark and different regions of Russia), as well as varieties and lines of the Donskoy Agricultural Research Center breeding were the objects of the study. The number of tested samples varied from 215 in 2020, 256 in 2021 and 196 in 2022. Sowing was carried out at optimum dates by hand planters. The plots were infested with local races of the most common pathogens.

To create a provocative background of leaf diseases, the infectious area was sown in autumn with the winter variety Master, an accumulator of infection. Susceptibility of this variety is manifested as early as the end of November. It goes into winter with single signs of infestation. During the autumn-winter period, pathogens accumulate and develop on it. By the beginning of spring vegetation, plants are affected up to 10%. In spring, the wind and raindrops transfer mycelium and fungus spores to the shoots of the plants of spring varieties under study. By the stage of milk-wax maturity, their lesion can reach 80-90%.

Spring barley samples were sown by hand planters in two-row plots with a length of 1 l.m. in a 15 m long tier. The plot area was 0.7 m^2 with a single repetition. For greater infection load, a susceptible test variety of spring barley Zolotnik was placed in the nursery every 20 plots of the studied varieties.

Phytopathological evaluation was carried out dynamically (2-3 times during the growing season), starting from the phase of plant emergence to milk-wax ripeness. Gradation of varieties into resistance groups was carried out according to the maximum percentage of damage for all the years of study. Classification of resistance type in the samples was carried out according to the methods of VIR and VIZR (see Table 1)[1, 2].

Rostov region is characterized by a sharply continental climate. South-eastern winds with strong gusts prevail, causing drought and dry winds [12]. There is a steady increase in air temperature during the growing season of spring cereal crops (April - July), while the amount of precipitation during this period decreases. The tendency of climate aridity is increasing [13].

The mean daily air temperature was 16.5 °C in May 2020, which was close to the long-term average (15.4 °C). Precipitation was uneven, but the moisture reserve was sufficient for pathogen development. June was characterized by high temperature and air drought, which contributed to the termination of powdery mildew development, but did not affect the development of barley net blotch.

Spring and summer 2021 were characterized by elevated temperatures (+1.2°C above the long-term average) and abundant precipitation of 243.9 mm (+112.9 mm above the long-term average). Temperatures in May were elevated to 18.1°C (+1.6°C above the long-term average). Precipitation was 65.0 mm (+13.7 mm above the longterm average). June was also

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Табл. 1. Шкала оценки устойчивости ярового ячменя к листовым болезням
Table 1. Scale for assessing resistance of spring barley to leaf diseases

Point	Powdery mildew	Helminthosporium spot diseases
0	Highly resistant specimens: no lesions	Highly resistant specimens: no lesions
1	Nearly resistant: up to 10% of the leaf surface is affected, light swellings or single pads of fungus on lower tier leaves	Nearly resistant: sporadic spots on lower leaves
2	Slightly susceptible: 11-25% of the leaf surface is affected. Moderate number of pads on the lower tier leaves	Slightly susceptible: more than 50% of the lower leaves surface is affected and there are single spots on the second-tier leaves
3	Medium susceptible: 25-50% of the leaf surface is affected. Abundant fungus development on the lower leaves, on the upper leaves the pads are local, scattered	Medium susceptible: lower leaves die off, more than 50% of the second-tier leaf surface is affected, and there are single spots on the upper leaves
4	Highly susceptible: more than 50% of the leaf surface is affected. All leaves are severely affected, the pads are well defined, merging with abundant hyphae. Spikelet lesions	Highly susceptible: lower leaves die off, leaf surface of all the tiers is affected by more than 50%

rainy, with 103.9 mm of precipitation (+32.6 mm against the annual average). Such favorable conditions promoted prolonged development of powdery mildew and spots.

The driest conditions were in 2022. In April, 57.9 mm of rain was recorded (+15.2 mm to a long-run annual average). Favorable temperature regime - 12.7 °C (+2 °C to a long-run annual average) and air humidity - 73% (65% - a long-run annual average value) allowed pathogens to spread widely. May conditions supported their further development on plants: 19.1 mm (-32.2 mm to a long-run annual average) and 14.8 °C (-1.7 °C to a long-run annual average) up to the third ten-day period. Further harsh conditions in June resulted in desiccation of leaves and death of pathogens on the leaves: 19.1 mm (-32.2 mm to a long-run annual average) and 7.4 mm (-3.9 mm to a long-run annual average), respectively.

In general, the prevailing weather conditions made it possible to evaluate the material in terms of resistance to pathogens. Infection development was noted in varying degrees in all years of the study.

RESULTS AND DISCUSSION

In 2020, the degree of powdery mildew infestation on spring barley plants did not rise above the average (susceptible test variety was affected by 3 points). The same level was found in 37 specimens (Myar, Zernogradsky 1793, Zernogradsky 1783, Chelyabinsky 99 and Suzdalets (Russia), etc.). Most of the 101 examined specimens had a weak leaf surface lesion (Odin (Denmark), Eney (Ukraine), Khadzhibey, Rubikon, Zernogradsky 1808 and Zernogradsky 1794 (Russia), etc.). Seventy-six samples with very weak powdery mildew lesions were identified (84469/70 (Czech Republic), Dzivosny (Ukraine), Eifel (France), 3 YSBYM-03 (Denmark), Fedos × Priazovsky 9 and Zernogradsky 1638 (Russia), etc.). The unaffected pathogen was Margret (Germany) (see Fig. 1).

In 2021, a large distribution and a long period of development of powdery mildew on the plants were observed under conditions favorable for the manifestation of this pathogen in the spring barley nursery. Six specimens were severely affected (above the test variety level): Thaler and Mriya (Ukraine), Pasadena (Germany), Dolly (Canada), Mear and Medicum 11 (Russia). Average lesion (up to 50% of the leaf surface) at the susceptible variety level was present in 20 specimens: Zernogradsky 1794, Gris × Donetsk 15 and Fedos × Zernogradsky 1562 (Russia), Pasadena (Germany), etc. Most of the samples (141) studied were weakly af-

fected by the pathogen: Gris × Zernogradsky 1600, Marusya, Krasnoyaruzhsky 6, Azimut, Odessky 100, and Zernogradsky 1772 (Russia), etc. 58 samples were affected only slightly: Laurika (Germany), Zernogradsky 1726, Leon, Commander and Nutans 642 × Exploer (Russia), etc. The following 31 samples remained unaffected by the pathogen during the growing season: Kalkühl and Prestige (Germany), Tipple (England), Perun (Czech Republic), etc.

Under the conditions of 2022, development of powdery mildew on spring barley began early enough - in the middle of May. There was enough moisture for wide spreading of the pathogen on the nursery and rapid growth on the plant tiers. Drying of leaves on the plants in June caused the pathogen to die off on them. Under such conditions, all 196 tested varieties were divided into nearly resistant and weakly susceptible. Nearly resistant varieties were represented by 115 samples: Margret (Germany), Leon, Format, Magnet, Kumir, Zernogradsky 1872, Zernogradsky 1844, and Zernogradsky 1865 (Russia), etc. Weakly susceptible varieties were identified in 81 species: Lipen (Belarus), Astoria (France), Scarlett (Germany), Zernogradsky 1726, Bagrets, Nutans 129 and Medikum 11 (Russia), etc.

Drought conditions in 2020 restrained the development of helminthosporium spots in spring barley nursery and led to rapid desiccation of the leaf lamina. Under such conditions six samples were moderately affected by the pathogen (3 points): KWS-11-243 and Astoria (France), Tipple (England), Vacula, Ratnik × Donetsky 15 and Krasnoyaruzhsky 6 (Russia). 29 specimens were weakly affected by spots, mainly from the collection material: Skhidny and Mriya (Ukraine), local K-19109, Kamyshinsky 23, Omsky hograin and Zernogradsky 244 (Russia), etc. Extremely weak infestation was noted in 75 specimens: Prairia, Bios 1, Zernogradsky 385, Zernogradsky 1792 and Miar (Russia), etc. Under current condi-

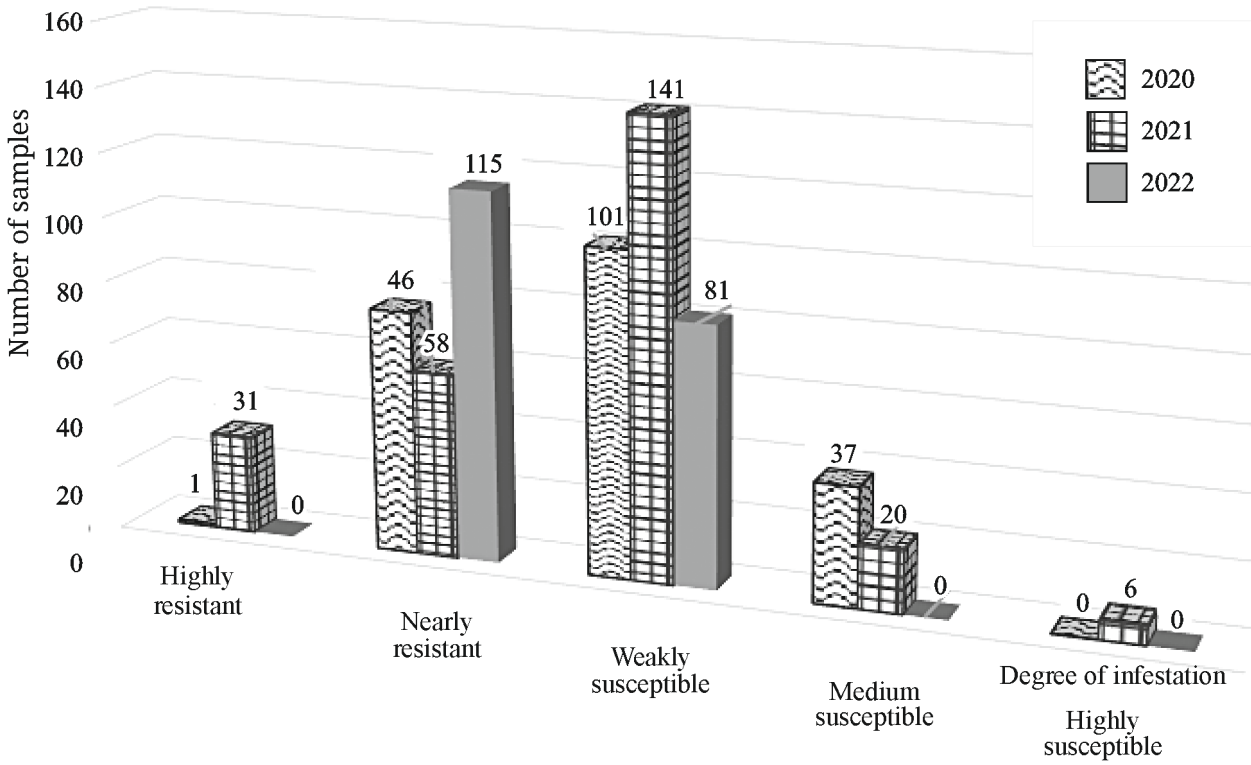

Рис. 1. Распределение изучаемых образцов ярового ячменя по поражению мучнистой росой в питомнике в 2020–2022 гг.

Fig. 1. Distribution of the studied samples according to powdery mildew infestation in the spring barley nursery (2020–2022)

tions, 105 specimens were not affected by the pathogen: Viking and Cw 102236 (Germany), Zernogradsky 1636, Zernogradsky 1825 and Vladimir (Russia), etc. (see Fig. 2).

In 2021, conditions were favorable for the development of helminthosporium spots. Their sufficiently uniform distribution was noted. Average lesion (3 points) at the level of susceptible test variety was noted in the variety Timofei (Russia). Most of the studied samples were mostly low susceptible (2 points) and very low susceptible (1 point) (123 and 129, respectively). Seven samples were found unaffected: Eifel (France), Partner (Ukraine), Zernogradsky 1874, Oskolets, Focus, Grace, and Bagrets (Russia).

In 2022, the development of leaf spot diseases began in mid-May. The June drought contributed to desiccation of the leaf lamina and halted further development of the pathogen. Practically resistant to the pathogen were 175 studied varieties: Laurika (Germany), Explorer and Pioner (France), Zernogradsky 1801, Gris, Fedos, Azimut, Format and Novik (Russia), etc. Twenty-one varieties were found to be weakly susceptible: Nord 071111, Partner, Skhidny and Commander (Ukraine), Marusya, Step and Bogatyr (Russia), etc.

According to the results of the studies, a number of varieties with complex resistance to the studied pathogens were identified (see Table 2).

CONCLUSION

As a result of field evaluation on artificial infectious background, varieties and lines of spring barley with high resistance to the studied pathogens were singled out. Highly resistant to powdery mildew were KWS-11-228 and Pioner (France), Sunshine, Margret, Viking, Laurika and Tituringia (Germany), Perun (Czech Republic), Tipple (England), Kalita, Leon and Tonus (Russia), Obolon and Charivny (Ukraine). The varieties Rus, Talovsky 9, Tonus and Elf

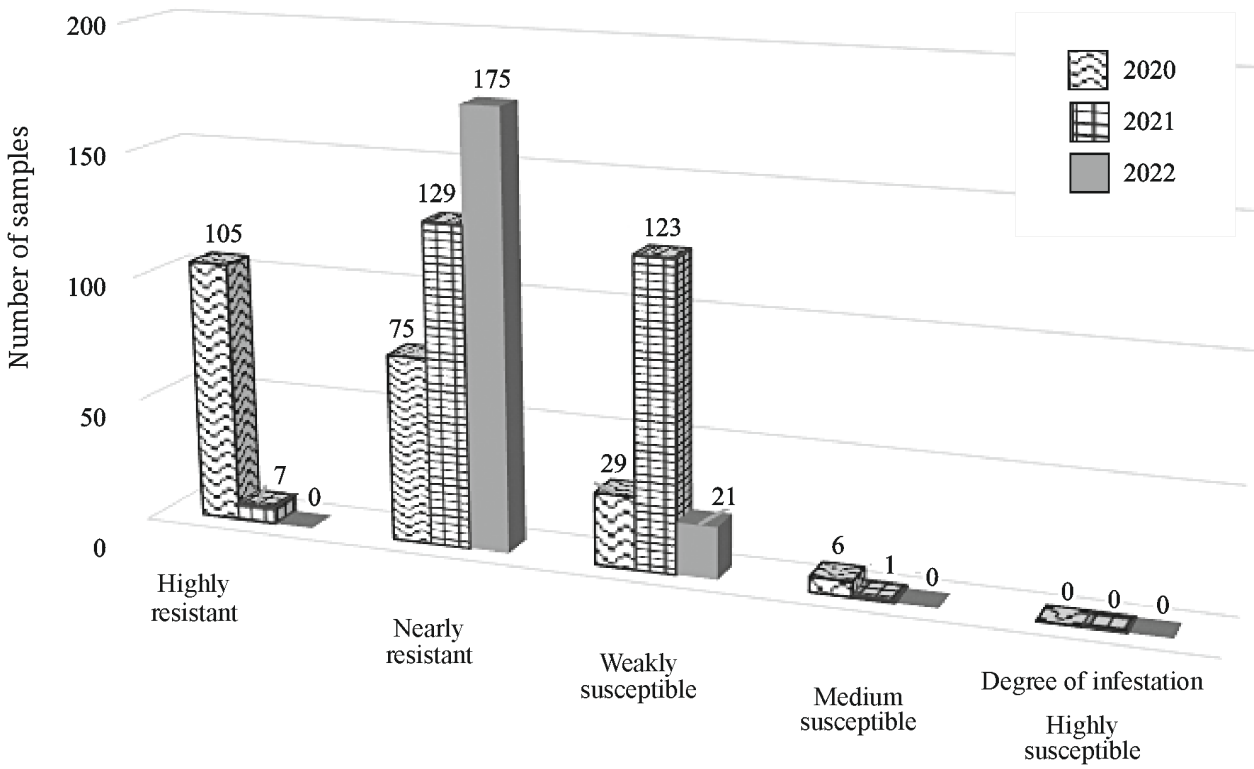

Рис. 2. Распределение изучаемых образцов ярового ячменя по поражению гельминтоспориозными пятнистостями в питомнике в 2020–2022 гг.

Fig. 2. Distribution of the studied samples according to helminthosporium blotches in the spring barley nursery (2020–2022)

Табл. 2. Иммунологическая характеристика выделившихся по комплексной устойчивости сортов ярового ячменя, балл

Table 2. Immunological characteristics of the spring barley varieties identified according to complex resistance, point

Variety/ sample	Powdery mildew			Helminthosporium spot diseases		
	2020	2021	2022	2020	2021	2022
Susceptible	3	2	2	2	2	2
Margret (Germany)	0	0	1	2	1	1
Perun (Czech Republic)	1	0	1	1	2	1
Prestige (Germany)	1	0	1	2	2	1
Viking (Germany)	1	0	1	0	2	1
Leon (Russia)	1	1	1	0	1	1
Tonus (Russia)	1	1	1	2	1	1
Elf (Russia)	1	1	1	0	1	1
Fedos (Russia)	1	1	1	0	1	1
Format (Russia)	1	1	1	0	1	1
Azimut (Russia)	3	1	1	0	2	1

(Russia), and Eney (Ukraine) showed resistance to helminthsporium spots. Also, the following varieties with resistance to both pathogens were identified: Leon, Tonus, Elf, Fedos, Format and Azimut (Russia), Margret, Prestige and Viking (Germany), and Perun (Czech Republic).

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ИНФОРМАЦИЯ ОБ АВТОРАХ

✉**Дорошенко Е.С.,** младший научный сотрудник; **адрес для переписки:** Россия, 347740, Ростовская область, г. Зеленоград, Научный городок, 3; e-mail: katyalevchenko1@mail.ru

Шишкин Н.В., кандидат сельскохозяйственных наук, ведущий научный сотрудник; e-mail: nik.shiskin.1961@mail.ru

AUTHOR INFORMATION

✉**Ekaterina S. Doroshenko**, Junior Researcher; **address:** 3, Nauchny gorodok, Zelenograd, Rostov Region, 347740, Russia; e-mail: katyalevchenko1@mail.ru

Nikolay V. Shishkin, Candidate of Science in Agriculture, Lead Researcher; e-mail: nik.shiskin.1961@mail.ru

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ИЗУЧЕНИЕ ВЛИЯНИЯ МАСЛЯНОЙ КИСЛОТЫ И ПРОПАНДИОЛА НА КИШЕЧНИК У МЫШЕЙ ICR

Афонюшкин В.Н.[1,2,3], (✉)Козлова О.С.[3], Черепушкина В.С.[1], Миронова Т.Е.[1,3], Козлова Ю.Н.[3], Ян Ф. [2], Коптев В.Ю.[1], Донченко Н.А.[1,3], Леденева О.Ю.[3]

[1]*Сибирский федеральный научный центр агробиотехнологий Российской академии наук*
Новосибирская область, р.п. Краснообск, Россия

[2]*Институт химической биологии и фундаментальной медицины Сибирского отделения Российской академии наук*
Новосибирск, Россия

[3]*Новосибирский государственный аграрный университет*
Новосибирск, Россия

(✉)e-mail: loi-2005@yandex.ru

Изучено влияние корректоров микробиоты кишечника на моторику желудочно-кишечного тракта мышей линии ICR. Использованы две кормовые добавки – композиции на основе глицерина и 1,3 пропандиола и Салколи Mono BP Dry. По принципу аналогов сформировано три группы по 10–12 гол. в каждой: две опытных и одна контрольная. Экспериментальные кормовые смеси изготавливали путем пропитывания 200 г корма подсолнечным маслом, 1,3 пропандиолом, глицерином (1-я опытная группа); кормовую смесь 2-й опытной дополнительно смешивали с кормовой добавкой Салколи Mono BP Dry. Для оценки скорости выведения фекалий мышам всех групп выпаивали флуоресцентные метки по 100 мкл (тушь флуоресцентная зеленая и красная), предварительно смешав их с водой 1 : 1. Оценивали сроки появления первых признаков флуоресценции в фекалиях у животных контрольной и опытных групп. Отмечена интенсивная флуоресценция зеленой метки при флуориметрии образцов. Наибольший прирост флуоресценции наблюдали к 4-му часу эксперимента в контрольной группе, во 2-й опытной прирост продолжался до 5-го часа. В 1-й опытной группе выведение основного количества зеленого красителя не началось даже к 5-му часу опыта. Методом с использованием флуоресцентных меток удалось выявить замедление перемешивания кормовых масс у мышей, получавших в составе корма глицерин и 1,3 пропандиол, что сопровождалось статистически значимым приростом концентрации *Escherichia coli*, – в 3 раза ($p < 0,05$). Использование препарата Салколи Mono BP Dry статистически значимо не влияло на концентрацию кишечной палочки и моторику желудочно-кишечного тракта.

Ключевые слова: *Escherichia coli,* моторика кишечника, кормовые добавки, масляная кислота, 1,3 пропандиол

STUDY OF THE EFFECTS OF BUTYRIC ACID AND PROPANEDIOL ON THE INTESTINE IN ICR MICE

Afonyushkin V.N.[1,2,3], (✉)Kozlova O.S.[3], Cherepushkina V.S.[1], Mironova T.E.[1,3], Kozlova Yu.N.[3], Yang F.[2], Koptev V.Yu.[1], Donchenko N.A.[1,3], Ledeneva O.Yu[3].

[1]*Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences*
Krasnoobsk, Novosibirsk region, Russia,

[2]*Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences*
Novosibirsk, Russia

[3]*Novosibirsk State Agrarian University*
Novosibirsk, Russia

(✉)e-mail: loi-2005@yandex.ru

The effect of gut microbiota correctors on the motility of the gastrointestinal tract of ICR mice was studied. Two feed additives were used - compositions based on glycerol and 1.3 propanediol and

Salkoli Mono BP Dry. Three groups of 10-12 animals each were formed according to the principle of analogues: two experimental and one control. Experimental feed mixtures were made by saturating 200 g of feed with sunflower oil, 1.3 propanediol, glycerol (experimental group 1); the feed mixture of experimental group 2 was additionally mixed with the feed additive Salkoli Mono BP Dry. To estimate the rate of fecal excretion, 100 μl of fluorescent tags (fluorescent ink green and red) were given to mice of all groups, previously mixed with water 1: 1. The timing of the appearance of the first signs of fluorescence in feces in animals of the control and experimental groups was estimated. Intensive fluorescence of the green label in fluorimetry samples was observed. The greatest increase in fluorescence was observed at the 4th hour of the experiment in the control group, while in the 2nd experimental group the increase lasted until the 5th hour. In the 1st experimental group, elimination of the main amount of green dye did not begin even by the 5th hour of the experiment. The method using fluorescent tags revealed a delay in mixing of feed masses in mice fed glycerol and 1,3 propanediol, which was accompanied by a statistically significant increase in *Escherichia coli* concentration - by 3 times ($p < 0.05$). The use of Salkoli Mono BP Dry had no statistically significant effect on *E. coli* concentrations and gastrointestinal motility.

Keywords: *Escherichia coli*, intestinal motility, feed additives, butyric acid, 1,3 propanediol

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INTRODUCTION

Pro- and prebiotics, as well as synbiotics - their combination, which have a favorable impact on the health of the individual through the influence on the host's own beneficial microbes[1] [1], are considered to be promising areas of correction of the intestinal microbiota. Bacteria of the genera Lactobacillus, Bifidobacterium, Pediococcus, Streptococcus, Bacillus as well as Escherichia coli and others are currently used as probiotics.

One of the representatives of the family Lactobacillaceae, Lactobacillus reuteri, is of particular interest[2] [2] because it is a widespread species of heteroenzymatic lactic acid bacteria [3, 4] and in some cases a dominant component of the normal microflora of human and animal intestines[3,4] [5].

[1]*Guarner F., Ramakrishna B.S., Szajewska H., Sanders M.E., Shanahan F., Fedorak R.* World Gastroenterology Organisation Global Guidelines // Journal of Clinical Gastroenterology. 2012. N 46 (6). C. 468–481. DOI: 10.1097/mcg.0b013e3182549092.

[2]*Zhang D., Li R., Li J.* Lactobacillus reuteri ATCC 55730 and L22 display probiotic potential in vitro and protect against Salmonella-induced pullorum disease in a chick model of infection // Research in Veterinary Science [Internet]. 2012. N 93 (1). P. 366–373. DOI: 10.1016/j.rvsc.2011.06.020.

[3]*Morita H., Toh H., Fukuda S., Horikawa H., Oshima K., Suzuki T., Murakami M., Hisamatsu S., Kato Y., Takizawa T., Fukuoka H., Yoshimura T., Itoh K., O'Sullivan D.J., McKay L.L., Ohno H., Kikuchi J., Masaoka T., Hattor M.* Comparative genome analysis of Lactobacillus reuteri and Lactobacillus fermentum reveal a genomic island for reuterin and cobalamin production // DNA Research: An International Journal for Rapid Publication of Reports on Genes and Genomes. 2008. N 15 (3). P. 151–161. DOI: 10.1093/dnares/dsn009.

[4]*Schaefer L., Auchtung T.A., Hermans K.E., Whitehead D., Borhan B., Britton R.A.* The antimicrobial compound reuterin (3-hydroxypropionaldehyde) induces oxidative stress via interaction with thiol groups // Microbiology. 2010. N 156 (6). P. 1589–1599. DOI: 10.1099/mic.0.035642-0.

L. reuteri activates CD4+ T cells and coordinates other immune cells for the regulation of the immune response, stimulates IgA synthesis, inhibits adhesion of bacteria and viruses to epithelial cells and neutralizes toxins, produces acetic acid, which lowers pH in vivo and has a pronounced antibacterial effect on many pathogens [2]. In addition, it produces bacteriocin reuterin (3-hydroxypropionaldehyde, 3-HPA)[5], a broad-spectrum antimicrobial component [6, 7], resistant to proteolytic and lipolytic enzymes, studied as a food preservative or adjuvant therapeutic agent.

L. reuteri strains have shown good potential for use as probiotics in humans and animals [8-10]. Prebiotics (oligofructose, inulin, galacto-oligosaccharides, lactulose, breast milk oligosaccharides) are used to increase the effectiveness of probiotics as well as to activate the existing normal microflora (see footnote 1). In the case of L. reuteri, the administration of exogenous sources of glycerol can be used as prebiotics (see footnote 5), since this microorganism metabolizes active reuterin in the colon in the presence of sufficient glycerol, which is a product of microbiological fermentation, lipid digestion in the gut lumen, mucus rejection and desquamated epithelial cells. As a cofactor of the enzyme that synthesizes reuterin, as well as an inhibitor of the enzymes involved in its degradation, such as 1,3-propanediol and its structural analogues that are more resistant to degradation or that bind more efficiently to the enzyme (see footnote 5).

Another promising mechanism is the use of compounds that switch glycerol metabolism to reuterin synthesis. The identification of such compounds, as well as their effective combinations will make it possible in the future to develop a new effective prebiotic or synbiotic complex for the normalization of intestinal microflora and prevention of infectious diseases of farm birds.

The purpose of the study was to investigate the effect of gut microbiota correctors on the motility of the gastrointestinal tract of ICR mice.

MATERIAL AND METHODS

The studies were carried out in the sector of molecular biology and the laboratory of diseases of young farm animals of the Institute of Experimental Veterinary Science of Siberia and the Far East SFSCA RAS. Experiments on laboratory animals were carried out in accordance with the "Rules of work with the use of experimental animals".

The experiment was performed on 22 laboratory ICR mice aged 2 months and weighing 60-65 g. Three groups of 10-12 animals each were formed according to the principle of analogues: two experimental and one control. The animals were kept in cages at 22-25ºC, humidity 70-85% with a daylight regime of 12 hours (day-night). Access to water was without restriction. Both experimental and control mice were fed with standard mice compound feed produced by the IC&G SB RAS.

Experimental feed mixtures were made by soaking 200 g of feed with sunflower oil, 1,3 propanediol, glycerin (the 1st experimental group); the feed mixture of the 2nd experimental group was additionally mixed with the feed additive Salkoli Mono BP Dry. This feed additive is based on a composition of organic acids of butyric and propionic acids with glycerol and contains monobutyrate (13%), monopropionate (52%) and silica filler (up to 100%). The maximum allowable deviation of the components does not exceed 10%. The composition of Salkoli Mono BP Dry does not include genetically engineered products. Then the prepared feed mixtures were dried at room temperature for 2 days. The control group received only standard feed without additives.

Each group of laboratory animals was given 200 g of cooked feed daily for 5 days. During the experiment, feces were collected in each of the study groups.

To estimate the fecal excretion rate (see footnote 3), 100 µl of fluorescent tags (fluorescent green and red ink) were given to mice of all groups after mixing them with water 1:1. First the red label was given and after 1 h - the green

[5]*Afonyushkin V.N., Filipenko M.L., Shirshova A.N., Maslov O.G.* Mechanisms of biological activity of Lactobacillus reuteri - reuterin system // Siberian Herald of Agricultural Science. 2013. N 4 (233). pp. 70-75.

one. In 5 h after drinking the red fluorescent ink the mice were removed from the experiment.

Fluorescently labeled fecal samples resuspended in a solution of Guandin isothiocyanate 6M and Triton X100 1% at a ratio of 1 : 5, from each of the study groups, 50 µl were placed in a 96-well microplate.

Fluorescence was measured using a CFX PCR amplifier (Bio-Rad) using FAM and HEX channels. DNA extraction from bacterial cultures and samples of intestinal contents was performed by silico-sorption method. Computer selection of primers was performed using the programs "Beakon Designer", Vector NTI with subsequent homology check with nonspecific DNA in the BLAST program. To assess the concentration of E. coli and L. reuteri, real-time PCR was performed on a CFX real-time amplifier (BioRad Laboratories) and LightCycler (Roche)[6].

Statistical processing of the obtained data was performed by the methods of variation and nonparametric statistics (mean square deviation, coefficient of variation, mean error). The obtained dependencies were described using polynomial function using Microsoft Office Excel program. Statistical significance of differences was assessed by Wilcoxon and Mann-Whitney.

RESULTS AND DISCUSSION

During the experiments, the timing of the first signs of fluorescence appearance in the feces of the control and experimental groups animals was evaluated. Intense fluorescence of the green label on the FAM channel was observed (see Fig. 1). The largest increase in fluorescence was observed by the 4th hour of the experiment in the control group, in the 2nd experimental group the increase continued up to the 5th hour. In the 1st experimental group, the main amount of green dye was not eliminated even by the 5th hour of the experiment.

In the Hex channel, the dynamics of red label excretion, which was injected earlier by 1 h, was analyzed. The process of label excretion in mice of experimental group 1 extended up to 5 h of the experiment; the decrease in fluorescence in the Hex channel was observed only by the end of the experiment. In the control group and the 2nd experimental group, most of the label was eliminated by 3 h of the experiment, and residual amounts of the label were eliminated (see Fig. 2).

It follows from Fig. 3 that there was no statistical significance between the concentrations of Lactobacillus reuteri in the intestinal microbiota in experimental group 1 and the control group; in experimental group 2 the concentration of L. reuteri was 2.69 times higher than in the control.

Based on the results shown in Fig. 1-2, it can be concluded that for the detection of fluorescent tags (ink red and green) using the CFX PCR amplifier (Bio-Rad), the most suitable channels are FAM and HEX.

The following results were obtained during the PCR study for the detection of genomic E. coli DNA: the concentration of E. coli in the intestinal microbiota in the 1st experimental

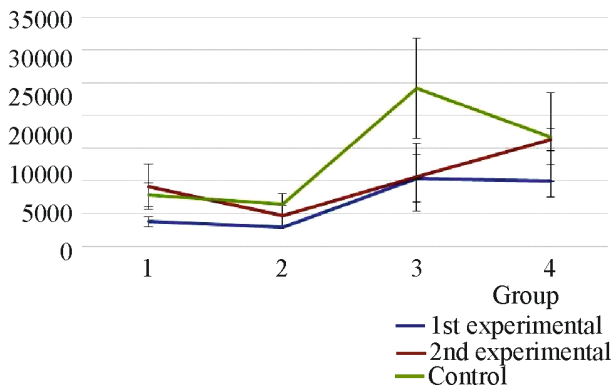

Рис. 1. Измерение флуоресценции при помощи ПЦР амплификатора CFX (Bio-Rad) с использованием канала FAM.
Продолжительность опыта: 1 – 2 ч; 2 – 3 ч; 3 – 4 ч; 4 – 5 ч

Fig. 1. Fluorescence measurement using a CFX PCR cycler (Bio-Rad) using the FAM channel.
Duration of the experiment: 1 – 2 hours, 2 – 3 hours, 3 – 4 hours, 4 – 5 hours

[6]*Cherepushkina V.S.* Development of a test to determine the representation of *L. reuteri* in the intestinal contents of humans and animals // The latest trends in agricultural science in the works of young scientists: materials of the 7th international scientific - practical conference / ed. by N.G. Vlasenko et al., Novosibirsk, 2019. pp. 250-253.

group was statistically significant compared to the control group, increased by 3 times (p = 0,02), in the 2nd experimental group - by 1.4 times (p = 0,35). High concentration of E. coli in the animals of the 1st experimental group indicates the accumulation of E. coli.

Based on the three criteria analysis of the dynamics of elimination of the intestinal contents, it can be stated that the mixture of glycerol and 1,3-propanediol can slow down the process of elimination of the intestinal contents from the body. This makes promising the use of such a feed additive to improve feed digestibility and treat digestive disorders accompanied by increased rate of evacuation of intestinal contents.

When evaluating the concentration of E. coli in the studied groups, it was found that its highest concentration was observed in the fecal samples of the 1st experimental group, higher than in the control by 3 times. In the 2nd experimental group, the concentration of E. coli was 1.4 times higher than in the control group, which may indicate some delay in the movement of the contents through the intestine.

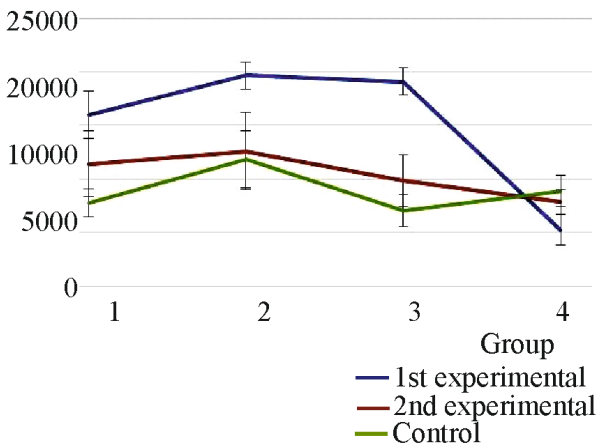

Рис. 2. Измерение флуоресценции при помощи ПЦР амплификатора CFX (Bio-Rad) с использованием канала HEX

Продолжительность опыта: 1 – 3 ч; 2 – 4 ч; 3 – 5 ч; 4 – 6 ч

Fig. 2. Fluorescence measurement with a CFX PCR cycler (Bio-Rad) using the HEX channel

Duration of the experiment: 1 –3 hours, 2 – 4 hours, 3 – 5 hours, 4 – 6 hours

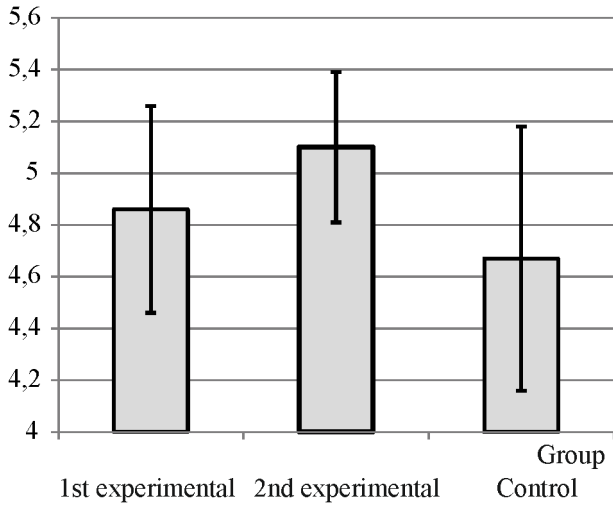

Рис. 3. Концентрации *L. reuteri* Log10 M±SD

Fig. 3. *L. reuteri* Log10 M+SD concentrations

CONCLUSION

The structure of the experiment allows to estimate the dynamics of mixing and excretion of feed and intestinal masses under the influence of different pharmacological influences. Injection of fluorescent carcass samples into feed masses revealed a slowdown in mixing of feed masses in mice receiving glycerin and 1.3 propanediol as part of the feed, which was accompanied by a statistically significant increase in E. coli concentration - by 3 times ($p < 0.05$). The use of Salkoli Mono BP Dry had no effect on the concentration of E. coli and gastrointestinal motility.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

Афонюшкин В.Н., кандидат биологических наук, заведующий сектором

(✉)**Козлова О.С.,** старший преподаватель; **адрес для переписки**: Россия, 630039, Новосибирск, ул. Добролюбова, 160; e-mail: loi-2005@yandex.ru

Черепушкина В.С., младший научный сотрудник

Миронова Т.Е., младший научный сотрудник

Козлова Ю.Н., кандидат биологических наук, младший научный сотрудник

Ян Фуди, аспирант

Коптев В.Ю., кандидат ветеринарных наук, ведущий научный сотрудник

Донченко Н.А., доктор ветеринарных наук, профессор, член-корреспондент РАН, руководитель ИЭВСиДВ

Леденева О.Ю., кандидат ветеринарных наук, доцент, заведующая кафедрой

AUTHOR INFORMATION

Vasily N. Afonyushkin, Candidate of Science in Biology, Section Leader

(✉)**Olga S. Kozlova**, Senior Lecturer; **address**:160, Dobrolyubova St., Novosibirsk, 630039, Russia; e-mail: loi-2005@yandex.ru

Victoria S. Cherepushkina, Junior Researcher

Tatyana E. Mironova, Junior Researcher

Yulia N. Kozlova, Candidate of Science in Biology, Junior Researcher

Fudi Yang, Postgraduate Student

Vyacheslav Yu. Koptev, Candidate of Science in Veterinary Medicine, Lead Researcher

Nikolay A. Donchenko, Doctor of Science in Veterinary Medicine, Professor, Corresponding Member RAS, Director IEVM&SFE

Olga Yu. Ledeneva, Candidate of Science in Veterinary Medicine, Associate Professor, Department Head

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АНАЛИЗ КАЧЕСТВА СПЕРМЫ БЫКОВ АЙРШИРСКОЙ ПОРОДЫ В СВЯЗИ С ГАПЛОТИПОМ ФЕРТИЛЬНОСТИ AH1

✉**Крутикова А.А., Позовникова М.В., Никиткина Е.В., Мусидрай А.А.**
Всероссийский научно-исследовательский институт генетики и разведения сельскохозяйственных животных – филиал Федерального научного центра животноводства – Всероссийского научно-исследовательского института животноводства имени академика Л.К. Эрнста
Санкт-Петербург, Россия
✉e-mail: anntim2575@mail.ru

Представлены результаты исследований (2019–2021 гг.) увеличения частоты встречаемости генетических мутаций, таких как гаплотипы фертильности, в популяции, которые приводят к снижению уровня воспроизводства у молочного скота. Проведена оценка качества спермы быков айрширской породы по подвижности и морфологическим показателям в связи с rs475678587 в локусе гена *UBE3B*, ассоциированного с гаплотипом фертильности AH1. Образцы спермы (n = 14) получены из Центра коллективного пользования «Генетическая коллекция спермы крупного рогатого скота отечественных и зарубежных пород» ВНИИГРЖ и принадлежали животным 2014 года рождения и старше (средний возраст быков составил 6–15 лет). Генотипирование по rs475678587 гена *UBE3B* проводили методом секвенирования по Сенгеру. Животных с гетерозиготным генотипом CT определяли как носителя гаплотипа AH1 (AH1-C), с гомозиготным генотипом CC – как свободное от гаплотипа AH1 (AH1-F). Оценку замороженно-оттаянной спермы проводили по следующим параметрам: общая и прогрессивная подвижность, процент нормальных сперматозоидов. Морфологические показатели качества спермы оценивали после дифференциального окрашивания клеточных элементов сперматозоидов коммерческим набором "Диахим-Дифф Квик" (научно-производственная фирма АБРИС+) в мазках спермы. Учитывали следующие показатели: акросомные нарушения, нарушения в области головки, хвоста и шейки сперматозоида. Данные по показателям нативной спермы (подвижность, концентрация), оцененные сразу после взятия, получены из архива технической службы племенного предприятия. Результаты исследований показали, что по физиологическим и морфологическим показателям сперма, полученная от быков-носителей гаплотипа AH1, достоверно не отличалась от аналогичных показателей у быков, свободных от мутации. По общей подвижности сперма быков-носителей гаплотипа фертильности почти на 2% отличалась более высоким показателем, чем у быков, свободных от гаплотипа. Можно отметить, что быки-носители гаплотипа фертильности в целом отличались хорошими показателями фертильности спермы.

Ключевые слова: айрширский скот, гаплотип фертильности AH1, однонуклеотидный полиморфизм гена, качество спермы, морфологические показатели качества спермы

SEMEN QUALITY ANALYSIS OF THE AIRSHIRE BULLS IN RELATION TO THE AH1 FERTILITY HAPLOTYPE

✉**Krutikova A.A., Pozovnikova M.V., Nikitkina E.V., Musidray A.A.**
Russian Research Institute of Farm Animal Genetics and Breeding – Branch of the L.K. Ernst Federal Research Center for Animal Husbandry
Saint-Petersburg, Russia
✉e-mail: anntim2575@mail.ru

The results of the studies (2019-2021) of increased incidence of genetic mutations, such as fertility haplotypes, in the population that result in lower reproductive rates in dairy cattle are presented. Semen quality of the Ayrshire breed bulls was evaluated for motility and morphological indices due to rs475678587 in the *UBE3B* gene locus associated with the AH1 fertility haplotype. Semen samples (n = 14) were obtained from the Center of Collective Use "Genetic Semen Collection of Domestic and Foreign Cattle Breeds" of the All-Russian Research Institute of Farm Animal Genetics and Breeding (ARRIFAGB) and belonged to the animals born in 2014 and older (average age of

bulls was 6-15 years). Genotyping for rs475678587 of the *UBE3B* gene was performed by Sanger sequencing. Animals with the heterozygous CT genotype were defined as carriers of the AH1 haplotype (AH1-C), and those with the homozygous CC genotype were defined as free of the AH1 haplotype (AH1-F). Frozen-thawed semen was evaluated according to the following parameters: total and progressive motility, percentage of normal spermatozoa. Morphological indices of sperm quality were assessed after differential staining of cellular elements of spermatozoa using a commercial kit "Diakhim-Diff Quick" (ABRIS+ scientific and production company) in sperm smears. The following indicators were taken into account: acrosomal abnormalities, abnormalities in the head, tail, and neck of the spermatozoon. Data on the indicators of native semen (motility, concentration), obtained immediately after collection, were received from the archive of the technical service of the breeding enterprise. The results of the studies have shown that according to physiological and morphological indices the semen obtained from the bulls carrying the haplotype AN1 did not differ significantly from those of the bulls free of the mutation. In terms of total motility, the semen of the haplotype fertility carrier bulls was almost 2% higher than that of the haplotype free bulls. It can be noted that the haplotype carriers of fertility bulls were generally characterized by good sperm fertility indices.

Keywords: Ayrshire cattle; fertility haplotype AH1; single nucleotide polymorphism of a gene; sperm quality; morphological indicators of sperm quality

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INTRODUCTION

The Ayrshire breed in Russia accounts for 2,8% of all cattle of dairy and meat production direction. In spite of small number, Ayrshire cattle take the leading place in five leading breeds of Russia as they are distinguished by high milk productivity, productive longevity, good feed conversion, resistance to infectious diseases including leucosis. Within the framework of modern intensive breeding and wide use of artificial insemination, a strict selection of stud bulls both in terms of genetic potential and level of productivity and in terms of the status of the animal with regard to the carrier of undesirable genetic defects is necessary. The use of a limited number of bulls with high breeding value on a large herd of cows will promote the spread of undesirable mutation if the breeding bull turns out to be a carrier. The focus of breeding on accelerated improvement of productive and technological qualities (milk yield, fat, udder and teat shape, etc.) has caused a decrease in reproductive function of animals associated, among other things, with active replication in the population of unfavorable alleles that have a negative impact on formation and functioning of the reproductive system of animals, which occur with the participation of a whole complex of genetic factors. More than one-third of the genes in higher mammals involved in embryonic development are critical [1]. Mutations in

such genes leading to a decrease or loss of their functions lead to embryonic mortality or the birth of non-viable progeny. Mutations in genes that determine the development of the reproductive system of animals often lead to reduced fertility [2].

In 2014, Cooper et al. [3] first reported a new genetic mutation in Ayrshire cattle that reduces reproduction in the population. The 713 kb causative region was found to be localized on the 17th KPC chromosome of cattle [4]. Then, the polymorphic region of the *UBE3B* gene identified by GWAS analysis was reliably associated with the sign of decreased reproductive function; it was identified as haplotype AH1 [3]. The portion of the *UBE3B* gene identified as significant was sequenced, which revealed several SNPs, but only the single nucleotide substitution C > T (rs475678587) was casual and associated with decreased fertility in the Ayrshire cattle. The rs475678587 mutation was found to be highly associated with the AH1 fertility haplotype. Testing of 29 bulls by rs475678587 showed that 11 bulls that were carriers of the rs475678587 mutation also carried AH1, and 18 wild-type bulls had no AH1 [4]. The C > T (rs475678587) mutation is autosomal, recessive. As a result of the C > T substitution in the exon 23 of the *UBE3B* gene, there is a disruption in the splicing process during biosynthesis of the ubiquitin protein, which plays a role in the formation of the organism during the embryonic period. Researchers have revealed that SNP C > T (rs475678587) in the homozygous state is a molecular genetic determinant of PIRM (ptosis, intellectual disability, retarded growth and mortality) syndrome in calves, which leads to death at an early age due to development of lethal pathologies [4]. AH1 fertility haplotype has been described as having a negative impact on reproduction due to increased stillbirth rate in herds, which causes significant economic losses in farms [5]. This was also confirmed by our previous studies [6].

In 2016, S. Attia et al. [7] suggested that the Ayrshire cattle fertility haplotype AH1 may be associated not only with decreased fertility of cows, but also have some effect on the semen quality of bulls, namely leading to abnormalities in sperm morphology. Sperm abnormalities can occur in the testes during spermatogenesis (primary) and in the seminal canals of the male genital tract (secondary). Fertility of bulls is less studied than that of cows. However, researchers have shown the role of genetic factors in the fertility of bulls. Thus, 15 indicators of semen quality of Holstein bulls are of hereditary character, and also genetic defects of sperm have been revealed[1].

The purpose of the research was to evaluate the semen quality of Ayrshire breed bulls by motility and morphological indices due to rs475678587 in the *UBE3B* gene locus associated with the AH1 fertility haplotype.

MATERIAL AND METHODS

The studies were conducted in 2019-2021. Semen samples (n = 14) were obtained from the CFC "Genetic semen collection of domestic and foreign cattle breeds" of the All-Russian Research Institute of Genetics and Farm Animal Breeding (VNIIGRZH) and belonged to animals born in 2014 and older (average age of bulls was 6-15 years). Seed samples were cryopreserved in paillettes and stored at -196 °C in liquid nitrogen until the study. Data on animal genotypes were obtained in our previous studies [8]. Genotyping for rs475678587 of the UBE3B gene was performed by Sanger sequencing. Animals with heterozygous CT genotype were defined as carriers of the AH1 haplotype (AH1-C), and those with homozygous CC genotype were defined as free of the AH1 haplotype (AH1-F). The primers for PCR and sequencing were designed using the BLAST NCBI[2] program. The following primers were used for the analysis: F: AGCAGCGGTCATTCTGTGAG and R: CACTGTTGACCCCATTTCCG. Sanger se-

[1] *Chenoweth P.J.* Genetic sperm defects. *Theriogenology.* 2005. Vol. 64. pp. 457–468.

[2] https://www.ncbi.nlm.nih.gov/

quencing was performed on an Applied Biosystems 3500 Genetic Analyzer using the commercial BigDye® Terminator v3.1 Sequencing Standard Kit (Applied Biosystems) according to the manufacturer's protocol. Sequences were aligned and processed using Mega-6 software [3].

Evaluation of the quality of frozen-thawed semen of bulls (n = 14) was performed after thawing at 37 °C for 1 min [9]. Assessment of physiological indices of semen was performed according to the following parameters: total and progressive motility, percentage of normal spermatozoa. Morphological indices of sperm quality were assessed after differential staining of cellular elements of spermatozoa by a commercial set "Diakhim-Diff Quick" (NPF ABRIS+) in sperm smears. The following parameters were determined: acrosomal abnormalities, abnormalities in the head, tail and neck of spermatozoa. Visualization was performed using a BA410 microscope (Motic China Group Co. Ltd., China) at 1000 magnification with immersion oil (for physiological parameters) and at 400 magnification by placing a drop of sperm on a preheated (37 °C) Makler chamber (for morphological parameters). The results were evaluated by Argus-CASA program (Argussoft, Russia). Data on native semen indices (motility, concentration) evaluated immediately after collection were obtained from the archive of the technical service of the breeding enterprise.

Statistical analysis was performed using DELL STATISTICA (data analysis software system) Dell Inc version 13 (2016, software.dell.com). ANOVA was performed at a significance level of $p < 0.05$ to determine the differences in the mean values of the variables between the analyzed groups. Comparison of qualitative indicators of bull semen was performed using the Kruskal-Wallis criterion, because the data did not pass the normality test due to the small number of groups.

RESULTS AND DISCUSSION

Studies of cryopreserved bull semen showed that the semen of AH1-C bulls had higher values for all evaluated physiological and morphological parameters than that of AH1-F bulls (see Table 1). For example, the semen of AH1-C bulls had almost 2% higher total motility than that of AH1-F bulls. Although there were no reliable differences between the compared groups, it can be noted that the bulls carrying the fertility haplotype were generally distinguished by good semen fertility indices.

The main indices of the native semen of the analyzed bulls were also analyzed, obtained when assessing semen quality immediately after collection (see Table 2). There were no significant differences between the semen of bull carriers and bulls free of AH1 mutation according to the main quality indices of the native semen.

In terms of activity index, the semen of AH1-F bulls had a higher score than that of AH1-C bulls, the difference was 0.247 points (3.0%). The native semen of AH1-F bulls had a slightly higher concentration (by 0.041 bln/ml) than that of AH1-C bulls.

AH1 fertility haplotype is the most common recessive haplotype in Ayrshire cattle. Thus, its frequency was 17.1% in Ayrshire cattle of Finland and 26.1% in Ayrshire cattle of the USA [4]. In the Russian sample of Ayrshire breed bulls we studied (n = 186), the proportion of AH1 fertility haplotype carriers was 16.66%, and analysis of the proportion of undesirable haplotype carriers depending on the birthplace of the bull breeder showed that the highest frequency (26.66%) was observed in bulls of Canadian origin [6]. Such a high frequency of this mutation has been observed since the mid-1970s. In Russia, the AH1 fertility haplotype is currently identified by PCR with allele-specific primers, as well as by full genomic screening using SNP chips and by sequencing. The number of carriers in the average breed in Russia is about 16.5% [10]. The distribution of the AH1 fertility haplotype is associated with the use of a small number of stud bulls and a high level of inbreeding against the background of a small population size [11, 12].

[3]https://www.megasoftware.net/web_help_10/index.htm#t=Citing_MEGA_In_Publications.htm

Табл. 1. Физиологические и морфологические показатели замороженно-оттаянной спермы быков в соответствии со статусом по гаплотипу AH1 (различия не были значительными)

Table 1. Physiological and morphological parameters of frozen-thawed semen of the bulls in accordance with the status of the AH1haplotype (the differences were not significant)

Indicator, %	Bull status		*p*-value
	AH1-C (*n* = 5)	AH1-F (*n* = 9)	
General mobility	64,000 ± 4,301	62,333 ± 2,920	0,686
Progressive mobility	52,600 ± 3,709	52,222 ± 3,239	0,892
Number of normal sperm cells	87,044 ± 1,732	81,597 ± 2,096	0,095
Absence of acrosomes	0,917 ± 0,399	1,512 ± 0,399	0,504
Swollen acrosomes	2,379 ± 0,780	2,916 ± 0,717	0,841
Wrinkled acrosomes	7,969 ± 0,965	11,543 ± 1,927	0,317
Tail and neck disorders	1,066 ± 0,410	1,664 ± 0,331	0,181
Head disorders	0,485 ± 0,359	0,761 ± 0,312	0,657

Note. Here and in Table 2. AH1-C – carrier of the fertility haplotype AH1; AH1-F – not carrier of the fertility haplotype AH1.

Quite often, mutations that cause a decrease in reproduction in herds can be the cause of impaired sperm morphology and, accordingly, low fertility of bull carriers. Bulls with semen with a high percentage of morphological abnormalities reduce reproduction rates in the herd. Mutations leading to decreased fertility are often associated with reproductive dysfunction of bulls rather than cows, in particular with sperm morphology disorder [13]. It has been proved that the number of normal spermatozoa in the ejaculate has a positive correlation with the frequency of calving, thus, the proportion of spermatozoa with normal morphology in the ejaculate is related to the bovine fertility indicators [8, 13]. Bulls whose semen has a higher content of spermatozoa with morphological disorders have a reduced level of fertility. However, the results of our studies showed that by physiological and morphological parameters of the quality of native and frozen-thawed semen the bulls-carriers of AH1 haplotype did not differ significantly from similar parameters in the bulls free of mutation, and by such parameters as total motility, progressive motility and percentage of normal sperm, even exceeded them. Thus, the results we obtained were opposite to the statement of S. Attia et at. [7] that AH1 haplotype of Ayrshire cattle fertility leads to pathological changes in sperm morphology. Studies of the effect of carriage of various genetic defects on both productivity and semen quality conducted in other breeds have shown that, for example, heterozygotes (carriers) of the DUMPS mutation have significantly higher genetic potential for milk productivity, and semen quality indicators of Holstein bulls do not change depending on their HCD status [14].

Табл. 2. Показатели качества нативной спермы быков в соответствии со статусом по гаплотипу AH1 (различия не были значительными)

Table 2. Indicators of the quality of native bull sperm in accordance with the status of the haplotype AH1(the differences were not significant)

Indicator	Bull status		*p*-value
	AH1-C (*n* = 5)	AH1-F (*n* = 9)	
Mobility, point	8,133 ± 0,047	8,380 ± 0,162	0,088
Concentration, bln/ml	1,169 ± 0,076	1,210 ± 0,160	0,870

CONCLUSION

The presence of the rs475678587 mutation located in the exon 23 locus of the *UBE3B* gene, defined as a marker mutation of the AH1 fertility haplotype, in the genome of stud bulls according to our data does not affect the decrease of physiological and morphological quality parameters of both native and frozen-thawed semen. Most of the biological and economic indices considered in this work [6] were better in the bulls carrying the AH1 haplotype compared to the bulls free of the mutation. Thus, the assumption made by S. Attia et al. in 2016 that the AH1 haplotype of Ayrshire cattle fertility may be associated with decreased semen quality in bulls, namely leading to abnormal sperm morphology, is not consistent with our results, which were the opposite. The studies presented in this work are of practical importance, as the results obtained can be widely used in breeding and pedigree work with Ayrshire breed of cattle.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

✉**Крутикова А.А.,** кандидат биологических наук, старший научный сотрудник; **адрес для переписки:** Россия, 196625, Санкт-Петербург, пос. Тярлево, Московское шоссе, 55а; e-mail: anntim2575@mail.ru

Позовникова М.В., кандидат биологических наук, старший научный сотрудник

Никиткина Е.В., кандидат биологических наук, ведущий научный сотрудник

Мусидрай А.А., кандидат биологических наук, младший научный сотрудник

AUTHOR INFORMATION

✉**Anna A. Krutikova,** Candidate of Science in Biology, Senior Researcher; **address:** 55a, Moskovskoe shosse, Tyarlevo, Saint-Petersburg, 196625, Russia; e-mail: anntim2575@mail.ru

Marina V. Pozovnikova, Candidate of Science in Biology, Senior Researcher

Elena V. Nikitkina, Candidate of Science in Biology, Lead Researcher

Artem A. Musidray, Candidate of Science in Biology, Junior Researcher

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ВЛИЯНИЕ ПРОБИОТИЧЕСКОГО ПРЕПАРАТА НА КАЧЕСТВО МОЛОКА КОЗ

(✉)**Функ И.А., Дорофеев Р.В.**
Федеральный Алтайский научный центр агробиотехнологий
Барнаул, Россия
(✉)e-mail: funk.irishka@mail.ru

Изучено влияние экспериментального пробиотического препарата «Плантарум» на физико-химические и микробиологические показатели молока помесных коз молочного направления продуктивности. Данный препарат разработан в лаборатории микробиологии молока и молочных продуктов Сибирского научно-исследовательского института сыроделия Федерального Алтайского научного центра агробиотехнологий. В состав препарата включены чистые культуры пробиотических штаммов лакто- и пропионовокислых бактерий из «Сибирской коллекции микроорганизмов». Научно-хозяйственный опыт проведен в 2018–2020 гг. на территории Первомайского района Алтайского края. В качестве объектов исследования выступали помесные козы молочного направления продуктивности. С целью оценки влияния экспериментального пробиотического препарата «Плантарум» на качественные показатели молока подопытных животных методом групп аналогов были сформированы четыре группы (по 20 гол. в каждой) сукозных коз: одна контрольная и три опытных. Козам опытных групп дополнительно с основным рационом скармливали пробиотический препарат «Плантарум» в дозах 0,4; 0,6 и 0,8 мл/кг массы тела в сутки. Результаты исследований показали, что содержание массовой доли жира и белка в образцах молока всех подопытных групп находилось в пределах нормы для данного вида животных, регламентируемой ГОСТ 32940–2014. Необходимо отметить, что использование разных доз пробиотика позволило увеличить массовую долю жира в молоке за одну лактацию от 0,06 до 0,13 абс.% и массовую долю белка на 0,12 абс.% относительно контроля. Физико-химические показатели (сухой обезжиренный молочный остаток, титруемая кислотность, соматические клетки) молока подопытных коз находились в пределах нормы; существенные различия по группам не установлены. При определении количества мезофильных аэробных и факультативно анаэробных микроорганизмов выявлена тенденция к снижению бактериальной контаминации молока в опытных группах по сравнению с контролем.

Ключевые слова: козье молоко, пробиотик, физико-химические показатели, микробиологические показатели

EFFECT OF PROBIOTIC PREPARATION ON GOAT MILK QUALITY

(✉)**Funk I.A., Dorofeev R.V.**
Federal Altai Scientific Center for Agrobiotechnology
Barnaul, Russia
(✉)e-mail: funk.irishka@mail.ru

The effect of the experimental probiotic drug "Plantarum" on the physico-chemical and microbiological parameters of milk of crossbred dairy goats was studied. This preparation was developed in the laboratory of microbiology of milk and dairy products of the Siberian Research Institute of Cheese Making of the Federal Altai Scientific Center for Agrobiotechnology. The preparation includes pure cultures of probiotic strains of lacto- and propionic acid bacteria from the "Siberian collection of microorganisms". Scientific and economic experiment was conducted in 2018-2020 on the territory of the Pervomaisky district of the Altai Territory. The objects of the study were crossbred dairy goats. In order to evaluate the effect of the experimental probiotic drug "Plantarum" on the milk quality of the experimental animals, four groups (20 animals in each group) of pregnant goats were formed: one control and three experimental. The goats of the experimental groups were fed the probiotic drug "Plantarum" in addition to the basic diet at the doses of 0.4, 0.6, and 0.8 ml/kg of body weight/day. The results showed that the content of the mass fraction of fat and protein in the milk samples of all experimental groups were within the norm for this type of animals regulated by GOST 32940-2014. It should be noted that the use of

different doses of probiotic increased the fat mass fraction in milk from 0.06 to 0.13% and the protein mass fraction by 0.12% relative to the control. Physicochemical parameters (dry skimmed milk residue, titratable acidity, somatic cells) of milk of the experimental goats were within normal limits; no significant differences between the groups were found. There was a tendency to reduce the bacterial contamination of milk in the experimental groups compared to the control when determining the number of mesophilic aerobic and facultatively anaerobic microorganisms.

Keywords: goat milk, probiotic, physical and chemical parameters, microbiological parameters

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Conflict of interest
The authors declare no conflict of interest.

INTRODUCTION

At present, the principle of healthy nutrition is becoming more and more popular, which leads to an increase in consumer interest in goat milk due to its dietary and therapeutic-prophylactic properties. Goat milk and products based on it can be widely used in the diet of the population of all age categories, including elderly people and children [1–3].

Dairy goat breeding accounts for a small share in animal husbandry as a whole, but the growth of consumer demand stimulates further development of this industry. The successful development of dairy goat farming requires not only the realization of the genetic potential of the animals, but also their balanced and rational feeding. A promising direction in this area is the use of safe and environmentally friendly biologically active preparations, in particular probiotics[1] [4-7]. Probiotics include representatives of normal microflora of the gastrointestinal tract of animals, which contribute to optimization of digestion, better absorption of nutrients and positive dynamics in the field of productivity[2, 3] [8].

The main product of dairy goat breeding is milk. Therefore, when assessing the dairy productivity of goats, it is important to consider both the quantity and quality of milk, since changes in milk productivity (increase, decrease) can positively or negatively affect the quality of milk produced. Milk quality is characterized by physical, chemical and microbiological indicators that determine its biological usefulness[4, 5] [9, 10]. In this regard, the analysis of the effect of probiotic preparations on milk quality of dairy goats is very relevant, and is of theoretical and practical relevance.

The purpose of the research is to study the effect of the experimental probiotic drug "Plantarum" on the milk quality of dairy goats.

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MATERIAL AND METHODS

To evaluate the effect of the drug "Plantarum" developed by the Siberian Research Institute of Cheesemaking (SibNIIS) on the milk quality of crossbred dairy goats, four groups (20 animals each) of pregnant goats were formed: one control and three experimental groups. The animals of the experimental groups were given probiotic preparation in the dosages of 0,4; 0,6 and 0,8 ml/kg body weight per day in addition to the basic diet. The probiotic was fed during the period from the 3rd to the 4th month of pregnancy for 28 days. Physico-chemical and microbiological parameters of goat milk of the experimental groups were determined according to the generally accepted methods on the basis of SibNIIS.

RESULTS AND DISCUSSION

For effective action of probiotic drugs, their composition should include strains of microorganisms attributed to the normal microflora of the gastrointestinal tract of animals and resistant to aggressive conditions of the internal environment [6]. Probiotic preparation "Plantarum" was created by the specialists of the laboratory of microbiology of milk and dairy products of SibNIIS. It includes pure cultures of lactobacilli (*Lactobacillus* spp.) and multistrain culture of propionic acid bacteria (*Propionibacterium* spp.). Probiotic cultures from the "Siberian collection of microorganisms" (SCM) of the Federal Altai Scientific Center of Agrobiotechnologies were selected according to their technologically valuable properties: amount of probiotic microflora, antagonistic activity, active acidity, etc. The content of probiotic microflora in Plantarum remained at a therapeutically significant level for 60 days (1×106 CFU/cm^3).

The fat mass fraction and protein mass fraction are important indicators of milk quality when evaluating the milk productivity of goats. The results of the effect of the probiotic drug "Plantarum" on the milk quality of goats are shown in Fig. 1 and 2.

The study found (see Fig. 1) that the mass fraction of fat in all experimental groups was within the norm regulated by GOST 32940-2014 (not less than 3.2%). The fat content during the lactation period (10 months) was 4.03 ± 0.10% in group 1, 4.00 ± 0.08 in group 2, 4.09 ± 0.24 in group 3, and 4.16 ± 0.22% in group 4. At the same time in the 3rd and 4th experimental groups this index was higher than in the control group by 0,06 and 0,13 abs.% respectively.

It should be noted that milk from goats of groups 3 and 4 surpassed milk from the control group in terms of milk fat content in all the months of lactation. Thus, the difference between the 3rd and the 1st experimental groups was from 0.01 to 0.17 abs.%, between the 4th

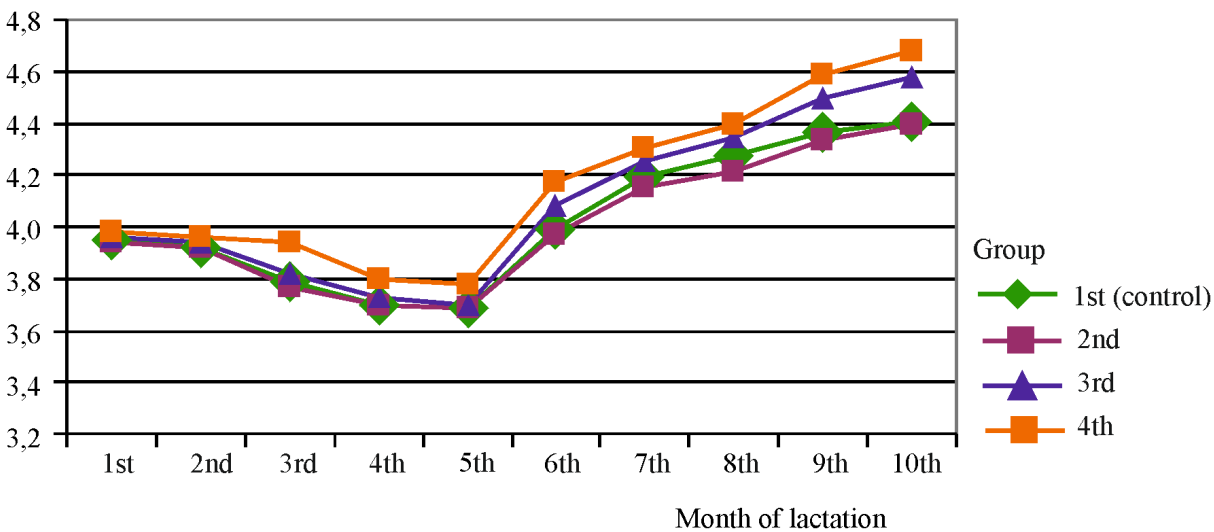

Рис. 1. Содержание жира в молоке в среднем по месяцам лактации, %

Fig. 1. Fat content in goat milk on average for lactation months, %

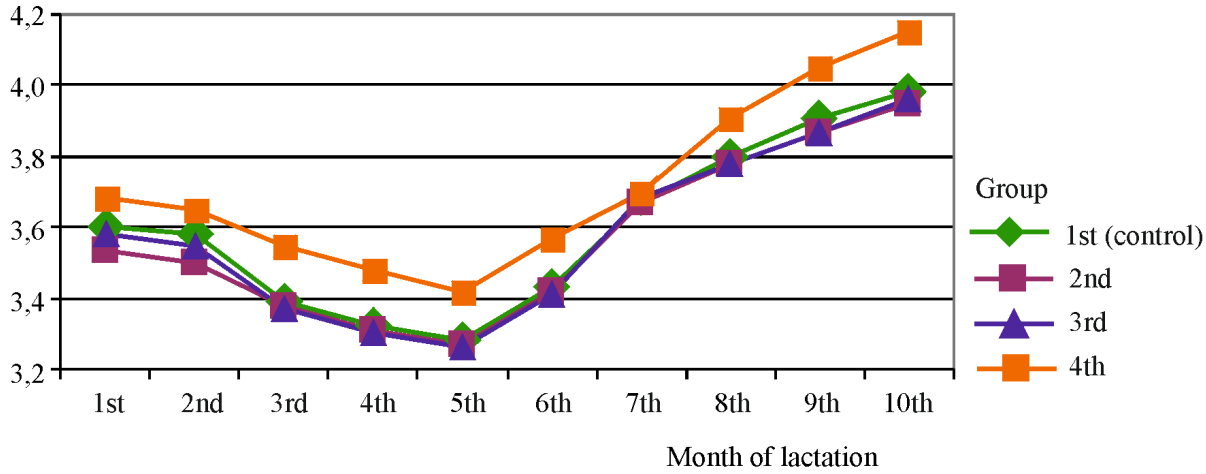

Рис. 2. Содержание белка в молоке-сырье в среднем по месяцам лактации, %

Fig. 2. The content of protein in the raw milk of goats on average for the months of lactation, %

and the 1st - from 0.03 to 0.27 abs.%. The results show that from the 1st to the 5th month of lactation there was a decrease in the fat mass fraction in milk of goats of all the experimental groups (see Fig. 1). This was caused by a natural increase in average daily milk yields. Subsequently (from the 6th month), there was a gradual increase in the amount of fat in milk, and it reached its maximum in the 10th month of lactation. Despite the fact that in all experimental groups there is an inverse relationship between the average daily milk yield and the mass fraction of fat (with an increase in the average daily milk yield decreases the mass fraction of fat), it should be emphasized that the growth of milk productivity in the experimental groups had no effect on the percentage of fat in milk.

The protein content (see Fig. 2) in all experimental groups was also within the normal range for this animal species (at least 2.8%). The mass fraction of protein during lactation ranged from 3.57 ± 0.11 to 3.72 ± 0.22%. Group 2 and 3 animals were 0.03 and 0.02 abs.% lower in protein content in milk than their control group counterparts, respectively, and Group 4 goats were 0.12 abs.% higher than the control group.

Such physico-chemical parameters of raw milk as MSNF (milk solids non-fat), titratable acidity and somatic cells were within the normal range; no significant differences between the groups were established. The microbiological index QMA&OAMO (quantity of mesophyll aerobic and optional-anaerobic microorganisms) in the four milk samples of the experimental groups of Saanen goats were also within normal limits (5×105 CFU/cm^3). However, there was a tendency to reduce the bacterial contamination of milk in the experimental groups as compared to the control. The lowest bacterial contamination was detected in the sample from the goats of group 4 (5×103 CFU/cm^3), where the maximum dose of probiotic (0.8 ml/kg body weight per day) was introduced into the animals' diet. It can be assumed that the reduction of bacterial contamination of milk is due to the antagonistic activity of probiotic microorganisms contained in Plantarum against sanitary-positive microflora (E. coli group bacteria, QMA&OAMO).

CONCLUSION

Thus, the results obtained in the course of the experiment testify to the positive effect of the experimental probiotic preparation "Plantarum" on the quality indicators of milk of dairy goats, which is reflected in the increase of fat mass fraction during the lactation period from 0,06 to 0,13 abs.% and protein mass fraction by 0,12 abs.%.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

(✉)**Функ И.А.,** кандидат сельскохозяйственных наук, младший научный сотрудник; **адрес для переписки:** Россия, 656910, г. Барнаул, Научный городок, 35; e-mail: funk.irishka@mail.ru

Дорофеев Р.В., кандидат сельскохозяйственных наук, старший научный сотрудник

AUTHOR INFORMATION

(✉)**Irina A. Funk,** Candidate of Science in Agriculture, Junior Researcher; **address:** 35, Nauchny gorodok, Barnaul, 656910, Russia; e-mail: funk.irishka@mail.ru

Roman V. Dorofeev, Candidate of Science in Agriculture, Senior Researcher

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НОВЫЕ ПЕРСПЕКТИВНЫЕ ШТАММЫ *BACILLUS SUBTILIS*, ВЫДЕЛЕННЫЕ ИЗ МЕРЗЛОТНЫХ ПОЧВ ЯКУТИИ

Тарабукина Н.П., (✉)Былгаева А.А, Степанова А.М., Парникова С.И., Неустроев М.П.
Якутский научно-исследовательский институт сельского хозяйства им. М.Г. Сафронова – Федеральный исследовательский центр «Якутский научный центр Сибирского отделения Российской академии наук»
Якутск, Россия
(✉)e-mail: agrobiotex@mail.ru

Скрининг природных микроорганизмов, обладающих сочетанием антагонистической и ферментативной активности, – одна из основных задач в разработке агробиотехнологических препаратов. С целью поиска перспективных в современной биотехнологии штаммов бактерий рода *Bacillus* проведены микробиологические исследования мерзлотных почв Центральной Якутии. Из среднесуглинистой мерзлотной почвы Хангаласского улуса выделено 14 изолятов. Из них три изолята при идентификации по физиолого-биохимическим свойствам отнесены к виду *Bacillus subtilis*, что подтверждено молекулярно-генетическими исследованиями по 16S рРНК. Установлено, что новые штаммы *B. subtilis* Вас-1р, *B. subtilis* Вас-2р, *B. subtilis* Вас-4р обладают выраженными антагонистическими свойствами по отношению к возбудителям сальмонеллезных и стрептококковых инфекций: *Sal. abortus equi* БН-12, *Str. equi* 5/1. Новые штаммы способны также к выработке ряда гидролитических ферментов (амилаза, ксиланаза, фитаза). В качестве контроля при определении количественных показателей ферментативной активности использованы штаммы бактерий *B. subtilis* ТНП-3 и *B. subtilis* ТНП-5. Данные штаммы депонированы в коллекции микроорганизмов, используемых в животноводстве и в ветеринарии и являющихся основой пробиотических препаратов. Установлено, что штаммы *B. subtilis* Вас-1р, *B. subtilis* Вас-2р, *B. subtilis* Вас-4р по амилолитической активности несколько уступают контрольным штаммам, но по ксиланазной и фитазной активности значительно их превосходят. Результаты проведенных исследований показали перспективность новых штаммов *B. subtilis* для дальнейшего изучения и возможность использования при разработке биологических препаратов для сельского хозяйства.

Ключевые слова: *Bacillus subtilis*, культурально-морфологические свойства, антагонистическая активность, ферментативная активность, амилаза, ксиланаза, фитаза

NEW PROMISING STRAINS OF *BACILLUS SUBTILIS* ISOLATED FROM FROZEN SOILS OF YAKUTIA

Tarabukina N.P., (✉)Bylgaeva A.A., Stepanova A.M, Parnikova S.I., Neustroev M.P.
M.G. Safronov Yakut Scientific Research Institute of Agriculture - Division of Federal Research Centre "The Yakut Scientific Centre of the Siberian Branch of the Russian Academy of Sciences"
Yakutsk, Russia
(✉)e-mail: agrobiotex@mail.ru

Screening of natural microorganisms possessing a combination of antagonistic and enzymatic activities is one of the main tasks in the development of agrobiotechnological preparations. Microbiological studies of permafrost soils of Central Yakutia were carried out with the aim of finding bacterial strains of the *Bacillus* genus that are promising in modern biotechnology. 14 isolates were isolated from the middle loamy permafrost soil of the Khangalassky ulus. Of these three isolates were identified as *Bacillus subtilis* species by their physiological and biochemical properties which was confirmed by molecular genetic studies on 16S рРНК. The new strains of *B. subtilis* Bac-1p, *B. subtilis* Bac-2p, and *B. subtilis* Bac-4p were found to possess marked antagonistic properties against Salmonellosis and streptococcal pathogens: *Sal. abortus equi* BN-12, *Str. equi* 5/1. New strains are also capable of producing a number of hydrolytic enzymes (amylase, xylanase, phytase). *B. subtilis* TNP-3 and *B. subtilis* TNP-5 bacterial strains were used as controls for determining quantitative indicators of enzymatic activity. These strains are deposited in the

collection of microorganisms used in animal husbandry and veterinary medicine and are the basis of probiotic preparations. *B. subtilis* Bac-1p, *B. subtilis* Bac-2p, and *B. subtilis* Bac-4p strains were found to be somewhat inferior to the control strains in amylolytic activity, but they were significantly superior in xylanase and phytase activities. The results of these studies have shown the potential of new *B. subtilis* strains for further study and the possibility of using them in the development of biological preparations for agriculture.

Keywords: Bacillus subtilis, cultural and morphological properties, antagonistic activity, fermentation activity, amilase, xylanase, phytase

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INTRODUCTION

Bacillus spore-forming bacteria are a promising group of microorganisms that produce a wide range of biologically active substances (BAS): antibiotics, enzymes, growth regulators and other compounds with antimicrobial and growth-stimulating properties [1]. The ability of *Bacillus* to synthesize substances of antibiotic nature is one of the key factors determining the nature of antagonism. BAS production determines high bactericidal and bacteriostatic activity against pathogenic Gram-positive and Gram-negative bacteria as well as fungicidal activity against phytopathogenic fungi. In recent years, scientists have been actively studying the lytic enzyme complex of bacilli as one of the factors involved in the manifestation of antagonism. Bacteria of the genus *Bacillus* attract the attention of researchers due to their wide distribution in nature, development cycle, unusual resistance of their spores to chemical, physical and biological agents. Nowadays the *Bacillus* strains are used for production of enzymes, antibiotics, highly purified biological preparations, detergents, probiotics, bio-insecticides, including food additives and functional foods.

The study of enzymes is of practical importance due to their high catalytic activity. Amylases, lipases, proteases, lactamases, cellulases, xylanases, chitinases and pectinases are among the enzymes with high catalytic activity at low temperatures. Cold-active proteases, which are widely used in the food and pharmaceutical industries and in the production of detergents, are of great interest for researchers [2]. The second most important group of enzymes are amylases, used in food, textile and paper industries. The third group of widely used enzymes is represented by lipases, which play the role of biocatalysts in the food, paper and textile industries, the production of detergents, etc. [3].

At present, the search for the most promising microorganisms - highly active enzyme producers - is relevant, since the production of preparations based on them occupies one of the leading places in modern biotechnology.

The purpose of the study is to identify promising strains of *Bacillus subtilis* microorganisms isolated from frozen soils of Yakutia.

The research objectives are to:

– identify isolates of the isolated *B. subtilis* strains by their physiological and biochemical properties;

– study their antagonistic properties;

– determine their enzymatic activity.

MATERIAL AND METHODS

Isolates of the aerobic spore-forming bacteria of the *Bacillus* genus were isolated from frozen medium-loamy soil samples from the Khangalassky District of the Republic of Sakha (Yakutia), taken using the point sampling method, according to GOST[1].

Bacillus isolates were identified according to the classification proposed by Gordon[2]. Microscopy of smears of the isolated and Gram-stained isolates was used in the study of cultural and morphological properties. Mobility of the isolated bacteria was determined by the hanging drop method. The presence of globules in cell protoplasm of the cultures was determined using meat-peptone agar (MPA) with 1% glucose. Halophilicity was determined on meat-peptone broth (MPB) containing 2 and 7% sodium chloride. Acetylmethylcarbinol, an intermediate formed during decomposition of glucose, was determined using the Voges-Proskauer reaction (Clark's medium).

Starch hydrolysis was studied on potato-peptone agar with lugol solution by the appearance/absence of the light zones of starch hydrolysis. Casein hydrolysis was studied on milk agar by the appearance of the hydrolysis zones (transparent).

To study the antagonistic activity of the spore-forming aerobic bacteria of the genus *Bacillus*, FBH-agar (TU 9385-058-39484474-2009) and FBH-broth (TU 9385-059-39484474-2009) were used. Bacterial strains *Salmonella abortus equi* BN-12, *Streptococcus equi* 5/1 deposited in the All-Russian collection of microorganisms (VGNKI of veterinary preparations, Moscow), provided by the laboratory of veterinary biotechnology of the Yakutsk Scientific Research Institute of Agriculture, were used as test cultures.

The antagonistic activity of the isolates was studied by the diffusion method on an agar plate containing one-day broth cultures of test strains: MPA for Salmonella and MPA with 1% glucose added, and 10% horse blood serum for Streptococci. Then by means of a sterile puncher the wells were made with a diameter of 7 mm, on which 0.3-0.5 ml of the culture liquid of the isolated isolates was put; for the control cup sterile NaCl solution was put into the well. The cups with seeds (in three replicates) were placed in the thermostat at 37 °C for 24 hours. The results were counted according to the presence of lysis zones of the test cultures around the well with the studied isolates of the bacteria genus *Bacillus*.

Toxicity and toxigenicity of the isolates were determined in purebred white mice. Laboratory animals were observed for 10 days in strict compliance with interstate standards[3].

Enzymatic activity of the isolates was studied in 1, 2, 3, 4, and 5-day culture liquid after centrifugation and filtration. For this purpose, the isolates were cultured on MPB at 37 °C for 5 days. Samples of culture fluid were taken every 24 hours.

Enzymatic activity was determined using spectrophotometer SFEK UV-1280, amylolytic, phytase and xylanase activities were established according to GOST [4-6]. Enzymatic activity of enzymes was determined by the change in optical density and expressed in units/ml of culture liquid.

RESULTS AND DISCUSSION

In order to identify the isolated isolates, their cultural, morphological and physiological and biochemical properties were studied. As a result, six isolates with typical properties of *Bacillus* genus bacteria characteristic of the

[1]GOST 17.4.4.02-2017. Soils. Methods of sampling and sample preparation for chemical, bacteriological, helminthological analysis. M., 2019. 12 p.

[2]Bergey's bacteria identifier: a handbook; 9th ed. Moscow: Mir, 1997. 26 p.

[3]GOST 33216-2014 Test methods on the effects of chemical products on the human body. Acute oral toxicity. M, 2019.

[4]GOST 34440-2018. Enzyme preparations for food industry. Methods for determination of amylolytic activity. M., 2019.

[5]GOST 31487-2012. Enzyme preparations. Methods for determination of the enzymatic activity of phytase. M., 2013.

[6]GOST R 55302-2012. Enzyme preparations for the food industry. Methods of determination of xylanase activity. M., 2012.

first morphological group (according to Gordon) were selected. All isolates are aerobic, spore-forming, Gram-positive, mostly motile, do not form intracellular globules (endospores), and are catalase-positive. The strains hydrolyze starch, partially decompose casein, mainly cause starch hydrolysis, are halophilic: many grow in the presence of 7% NaCl, partially ferment carbohydrates (glucose, mannitol, lactose, arabinose, xylose) with acid formation. The identified isolates somewhat differ in biochemical parameters, which, according to many researchers, is characteristic of the species of this genus [4]. Three isolates had promising properties for possible further use: *B. subtilis* Bac-1p, *B. subtilis* Bac-2p, and *B. subtilis* Bac-4p.

According to literature data, it is established that aerobic spore-forming bacteria of the genus *B. subtilis* are natural antagonists of many pathogenic and conditionally pathogenic microorganisms[7] [5]. The factor of their antagonism is based on the ability to synthesize a wide range of biologically active substances such as antibiotics, enzymes, bacteriocins that inhibit the growth and development of pathogens [6, 7]. In this connection, the antagonistic activity of the identified isolates against test cultures of *Sal. abortus equi* BN-12, *Str. equi* 5/1 was studied. The results of the experiment showed the presence of antagonistic properties of the isolated strains with respect to the causative agents of salmonellosis and streptococcal infections (see the table).

The studies showed that the highest antagonistic activity was noted in the *B. subtilis* Bac-2p strain: the lysis zone of the test cultures was 20 mm each.

In the next series of the experiments, the toxicity and toxigenicity of the isolated strains were determined. No mortality of laboratory animals was recorded. There were also no signs of intoxication in mice throughout the experiment. No statistically significant differences were found between the animals that received the studied strains and the control animals in terms of body weight and body weight gain in either males or females. Thus, the tested strains of *B. subtilis* Bac-1p, *B. subtilis* Bac-2p, *B. subtilis* Bac-4p are safe and have no potentially toxic effect when administered repeatedly intragastrically.

In the final stage of the research, the enzymatic activity of the isolated *B. subtilis* strains was determined, since it is known that the representatives of this genus are among the most active producers of hydrolytic enzymes [8].

Amylolytic activity is based on the ability of the bacterial α-amylase enzyme to catalyze the hydrolysis of extracellular polysaccharide to dextrins [9]. The amylolytic activity of the new *B. subtilis* Bac-1p, B. subtilis Bac-2p, and B. subtilis Bac-4p strains was determined in comparison with the reference strains *B. subtilis* TNP-3 and *B. subtilis* TNP-5, which are in the laboratory of microbial preparation development at the YSRIA[8].

Антагонистическая активность изолятов бактерий рода *Bacillus*, выделенных из мерзлотных почв, мм
Antagonistic activity of *Bacillus* genus bacterial isolates isolated from permafrost soils, mm

B. subtilis bacterial strain	Lysed zone	
	Sal. abortus equi	*Str. equi*
B. subtilis Bac-1p	15	15
B. subtilis Bac-2p	20	20
B. subtilis Bac-4p	18	18

[7] *Lelyak A.A., Shternshis M.V.* Antagonistic potential of Siberian strains of Bacillus spp. against pathogens of animals and plants // Bulletin of the Tomsk State University. Biology. 2014. N 1. pp. 42-55. 8TU 9384-003-00670203-06.

[8] TS 9384-003-00670203-06

Comparative analysis of five *B. subtilis* strains showed that they had different amylolytic activity (see Fig. 1). The highest α-amylase activity in the first day of cultivation was exhibited by *B. subtilis* Bac-1p (32.53 units/dm^3) and *B. subtilis* TNP-5 (55.95) strains, the middle one by *B. subtilis* TNP-3 (26.76), *B. subtilis* Bac-4p (25.303), the lowest by *B. subtilis* Bac-2p (20.1 units/dm^3). It should be noted that *B. subtilis* Bac-1p, *B. subtilis* Bac-2p, and *B. subtilis* Bac-4p strains, although they have amylolytic activity, are inferior to the reference strains in the number of amylolytic complex enzymes.

It is known that microorganisms with amylolytic activity can also produce other extracellular hydrolytic enzymes, such as xylanase, pectinase, chitinase, etc. [10]. Xylan is the main structural and second most abundant polysaccharide in plant cells [11]. The enzyme that hydrolyzes xylan is xylanase, which belongs to the hydrolytic group. The determination of xylanase activity is based on (detection) determination of the initial rate of reducing sugars formation using the Schomodi-Nelson method from xylan.

The peak of xylanase activity of the strains under study falls on the 3rd day of cultivation (from 100 units). These strains were *B. subtilis* Bac-4p (136.85 units/dm^3), *B. subtilis* Bac-2p (129.625), and *B. subtilis* TNP-5 (103.18 units/dm^3) (see Fig. 2). By the end of the observation, on day 5, a decrease in xylanase activity was recorded in all *B. subtilis* strains studied. Thus, it can be assumed that the xylanase activity of *B. subtilis* strains gained high activity on day 2-3 of cultivation, then gradually decreasing. The new *B. subtilis* Bac-1p, *B. subtilis* Bac-2p, and *B. subtilis* Bac-4p strains are superior to the recognized *B. subtilis* TNP-3 and *B. subtilis* TNP-5 strains in xylanase activity.

Phytase activity is determined by inorganic phosphate formed during hydrolysis of phytic acid by phytase enzyme. Phytic acid (phytate) is characterized by a strong negative charge in

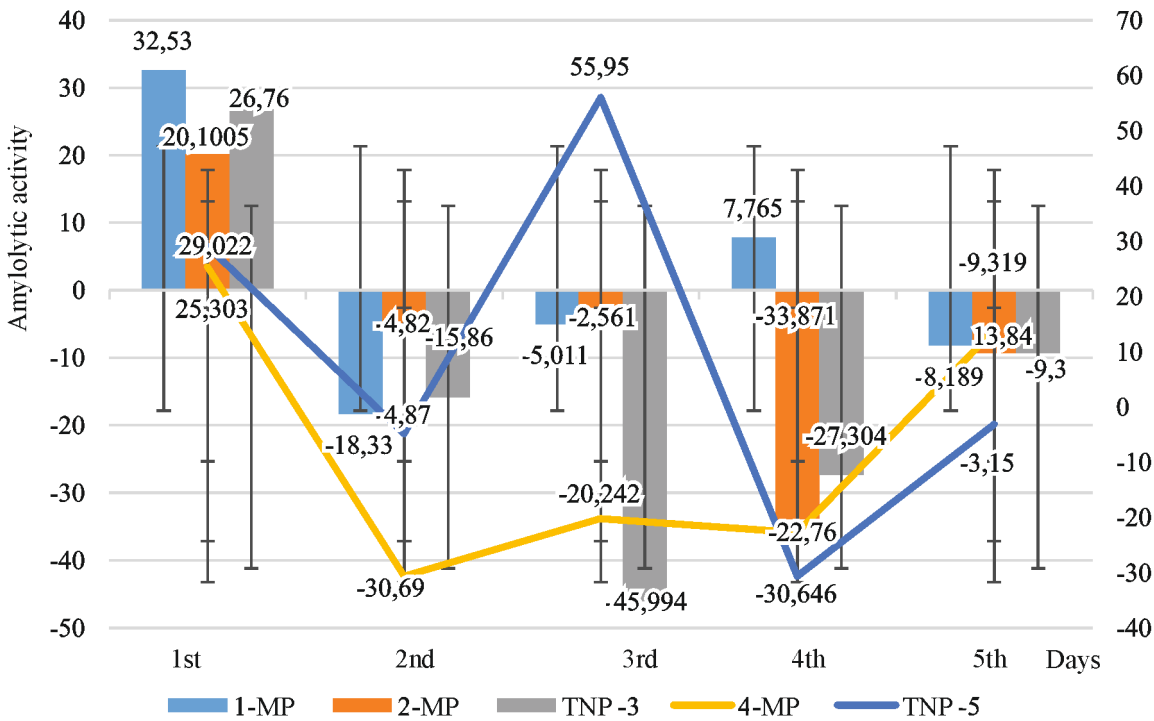

Рис. 1. Амилолитическая активность штаммов *B. subtilis* в зависимости от времени культивации, ед./дм^3

Fig. 1. Amylolytic activity of *B. subtilis* strains depending on the cultivation time, units/dm^3

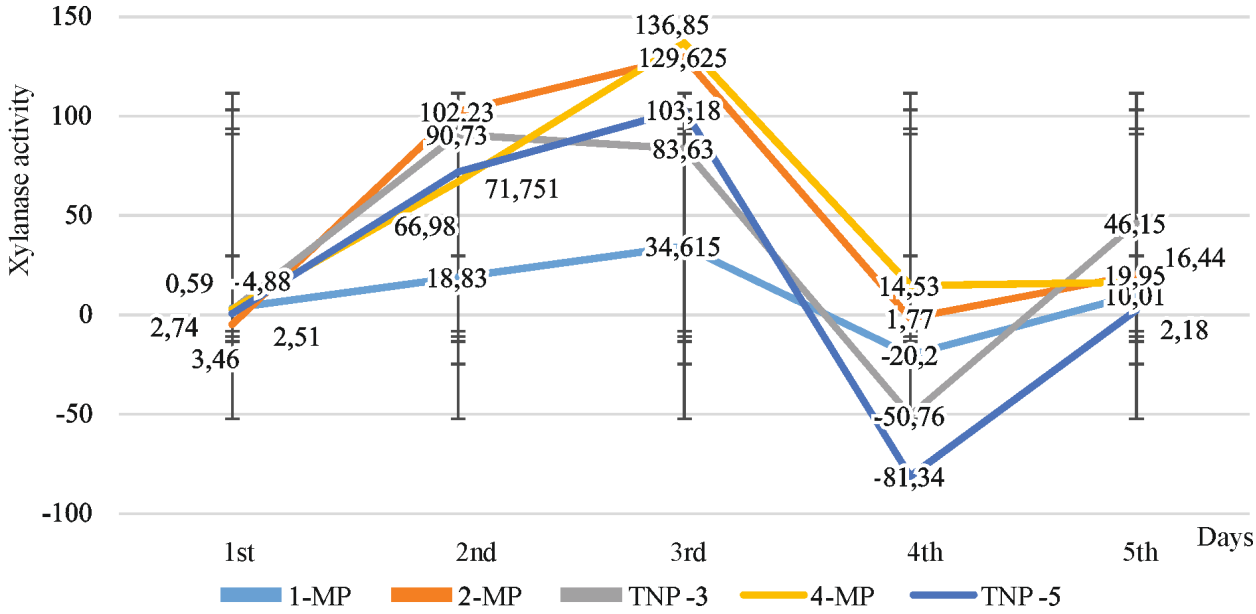

Рис. 2. Ксиланазная активность штаммов *B. subtilis* в зависимости от времени культивации, ед./дм^3
Fig. 2. Xylanase activity of *B. subtilis* strains depending on the cultivation time, units/dm^3

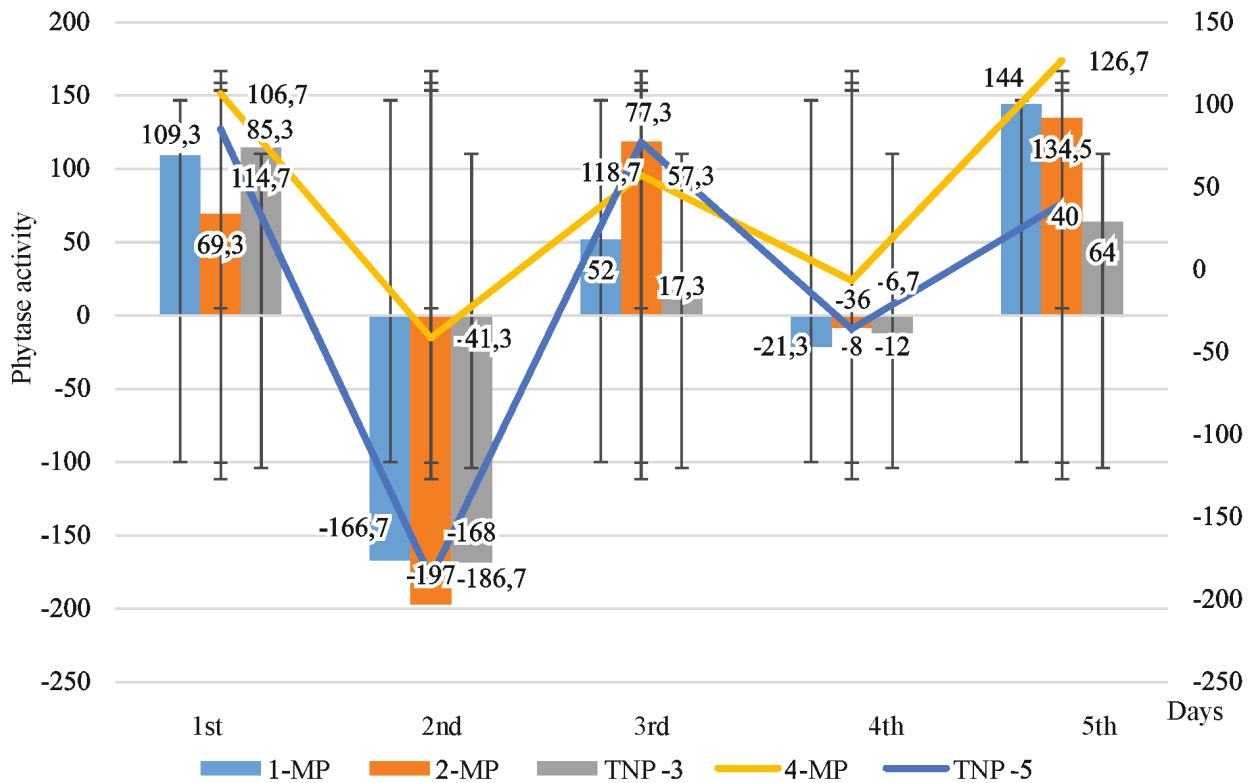

Рис. 3. Фитазная активность штаммов *B. subtilis* в зависимости от времени культивации, ед./дм^3
Fig. 3. Phytase activity of *B. subtilis* strains depending on the cultivation time, units/dm^3

its structure, which reduces the bioavailability of phosphorus, calcium, other cations, some amino acids and carbohydrates, and triggers a number of undesirable chain physiological reactions [12].

The analysis showed that *B. subtilis* strains had different phytase activity (see Fig. 3). The highest phytase activity, established on day 5, was in *B. subtilis* strain Bac-1p (144.0 units/dm^3), *B. subtilis* Bac-2p (134.5) and *B. subtilis* TNP-3 (126.7 units/dm^3). Peak phytase activity of the reference strains was found on day 1: *B. subtilis* TNP-3 (114.7 units/dm^3) and *B. subtilis* TNP-5 (85.3 units/dm^3).

CONCLUSION

Fourteen isolates of *Bacillus* bacteria were isolated from the permafrost medium-loamy soils of Yakutia. Three of them were identified by physiological and biochemical properties and confirmed by molecular genetic study as *B. subtilis* Bac-1p, *B. subtilis* Bac-2p, and *B. subtilis* Bac-4p.

New strains of *B. subtilis* Bac-1p, *B. subtilis* Bac-2p, and *B. subtilis* Bac-4p isolated from frozen soils of Yakutia have a combination of antagonistic and enzymatic activities and are promising for the development of drugs, enzymes, and feed additives.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

Тарабукина Н.П., доктор ветеринарных наук, профессор, заведующая лабораторией; e-mail: hotubact@mail.ru

(✉)**Былгаева А.А.,** кандидат ветеринарных наук, старший научный сотрудник; **адрес для переписки:** Россия, 677001, Республика Саха (Якутия), ул. Бестужева-Марлинского, 23/1; e-mail: agrobiotex@mail.ru

Степанова А.М., кандидат ветеринарных наук, старший научный сотрудник; e-mail: hotubact@mail.ru

Парникова С.И., кандидат ветеринарных наук, старший научный сотрудник; e-mail: hotubact@mail.ru

Неустроев М.П., доктор ветеринарных наук, профессор, заведующий лабораторией; e-mail: mneyc@mail.ru

AUTHOR INFORMATION

Nadezhda P. Tarabukina, Doctor of Science in Veterinary Medicine, Professor, Laboratory Head; e-mail: hotubact@mail.ru

(✉)**Andzhela A. Bylgaeva,** Candidate of Science in Veterinary Medicine, Senior Researcher; **address:** 23/1, Bestuzheva-Marlinskogo St., Republic of Sakha (Yakutia), 677001, Russia; e-mail: agrobiotex@mail.ru

Anna M. Stepanova, Candidate of Science in Veterinary Medicine, Senior Researcher; e-mail: hotubact@mail.ru

Svetlana I. Parnikova, Candidate of Science in Veterinary Medicine, Senior Researcher, e-mail: hotubact@mail.ru

Mikhail P. Neustroev, Doctor of Science in Veterinary Medicine, Professor, Laboratory Head; e-mail: mneyc@mail.ru

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ИЗМЕНЕНИЕ ЭКСТЕРЬЕРНЫХ ПРИЗНАКОВ МЕДОНОСНЫХ ПЧЕЛ В ТЕЧЕНИЕ СЕЗОНА

(✉)**Березин А.С.**
Федеральный научный центр пчеловодства
Рязанская область, г. Рыбное, Россия
(✉)e-mail: mellifera@yandex.ru

Изучены экстерьерные признаки медоносных пчел, используемые для определения породной принадлежности в селекционной работе. Отмечено, что изменение экстерьерных признаков обусловлено различными факторами (географическими, межпородными, внутрисемейными и др.). Опыт проведен на пасеке, расположенной в Рязанской области, в 2021 г. Пробы молодых пчел в течение сезона получали с использованием рамочного изолятора. Препарирование и измерение отдельных частей хитинового скелета пчел провели по принятой методике измерения экстерьерных признаков с использованием программного обеспечения, которое позволяет проводить измерение по изображению объекта. По каждой пчелиной семье в отдельности рассчитаны средняя и ее стандартная ошибка. На основе полученных средних рассчитаны непараметрические критерии для множественных сравнений (Фридмана и Крускала-Уоллиса) с использованием программного обеспечения Statistica 13.0. Анализ полученных средних показал, что экстерьерные признаки изменялись в незначительной степени в течение сезона, но это изменение было меньше, чем изменчивость между особями одной пробы. Проведенное сравнение полученных средних с помощью непараметрических критериев в большинстве случаев показало незначительные различия между пробами, отобранными от одной пчелиной семьи в течение сезона. Только в трех случаях отмечены достоверные различия. Изменение экстерьерных признаков в течение сезона незначительно и связано оно с неравномерным смешиванием спермы трутней в половых путях пчелиной матки. Причем, чем больше эта неравномерность смешивания, тем больше различия. Рекомендуется проводить гомогенизацию спермы трутней при использовании инструментального осеменения.

Ключевые слова: полиандрия, пчела медоносная, изменчивость, экстерьер, *Apis mellifera*, Altami Studio

CHANGES IN THE EXTERIOR FEATURES OF HONEY BEES DURING THE SEASON

(✉)**Berezin A.S.**
Federal Beekeeping Research Centre
Rybnoe, Ryazan region, Russia
(✉)e-mail: mellifera@yandex.ru

The exterior traits of honey bees used to determine breed affiliation in breeding work were studied. It has been noted that changes in exterior traits are caused by various factors (geographic, interbreeding, intrafamily, etc.). The experiment was conducted on an apiary located in the Ryazan region in 2021. Young bees were sampled during the season using a frame isolator. Individual parts of the chitinous skeleton of bees were dissected and measured according to the accepted method of measuring exterior features using software that allows to measure from the image of the object. The average and its standard error were calculated for each bee family separately. Non-parametric tests for multiple comparisons (Friedman and Kruskal-Wallis) were calculated using Statistica 13.0 software. Analysis of the obtained averages showed that the exterior traits change to a small extent during the season, but this change was less than the variability between the individuals of the same sample. Comparison of the averages obtained using non-parametric tests in most cases showed insignificant differences between the samples taken from the same bee family during the season. Only three cases showed significant differences. The change in exterior traits during the season is insignificant, and it is associated with the uneven mixing of drone bees' sperm in the genital tracts of the queen bee. Moreover, the greater this unevenness of mixing, the greater the differences. It is recommended to homogenize the sperm of drones when using instrumental insemination.

Keywords: polyandry, honey bee, variability, exterior, *Apis mellifera*, Altami Studio

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INTRODUCTION

Morphometric traits are formed under the influence of genotype-environment interaction. The honey bee (*Apis mellifera* (Hymenoptera: Apidae)) is distributed over most of the Earth's land surface. Its exterior traits, including those used to determine their breed (race), are subject to variability (geographic, seasonal, etc.)[1] [1].

Currently, various data on the question of seasonal variability of bee exterior are presented in the literature. Geographic variability associated with changes in altitude above sea level can be attributed to geographic variability. Thus, S. Radloff et al.[2], using a multivariate analysis of morphometric traits of worker bees from natural populations, divided them into three groups according to their altitude above sea level. A.R.S. Sousa et al. [2] showed that families of africanized bees from several areas of Brazil had marked morphometric differences and that their body size varied with altitude.

A.S. Mikhailov[3] began studying the seasonal variability of bees by analyzing several generations obtained from a single queen in one season by a number of traits. A.L. Dulkin and G.F. Treskova[4] determined that wing length, wing width, and length of the third tergite in the Ural bee were greatest in spring, and proboscis length in mid-summer. I.D. Mumladze[5] showed that in populations of gray mountain Caucasian bees, the linear dimensions of most traits decrease by the fall. Other authors believe that, on the contrary, the traits increase towards the end of the season. For example, N.I. Krivtsov[6] points out that the values of exterior traits of Middle Russian bees are significantly higher in autumn than in summer. E.D. Bozina[7] in comparing samples taken from bee families of four breeds in May and September found that, in general, the proboscis length of the fall generation is longer than that of the spring generation, but there are exceptions. Research data on length and width of wax mirror and wing size mainly confirm the results on proboscis variability. Foreign authors have found that the exterior of honeybees obtained in the dry season and in the rainy season from the same population differs [3]. A. Janczyk and A. Tofilski [4] found that differences between families in wing shape were much greater than seasonal differences, hence the influence of seasonality on identification is insignificant.

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The purpose of the study is to evaluate the variability of the exterior appearance of bees of the inbreed type of the Middle Russian Prioksky breed during the season and to establish its possible causes.

MATERIAL AND METHODS

The experiment was carried out in an apiary located in Rybnoe, Ryazan Region, in 2021. A group of 11 inbred bee colonies of the Middle Russian Prioksky breed was created for the experiment. By the end of the experiment, 5 families remained, the remaining 6 families dropped out during the season because of the change of queens.

To study the exterior, samples were taken from 3 to 5 days old bees[8]. To do this, the honeycomb with emergent brood was placed in a frame insulator, which was then placed in the nest for 24 hours. At the end of 24 hours, the honeycomb with brood was removed, the bees were shaken off onto the foil, and the honeycomb was returned to the family. The resulting day-old bees were poured into the isolator, a feeding frame was given, and then the isolator was placed in the nest for 5 to 7 days. Bees were sampled once every 2 weeks, but sometimes there was a shift if the planned date coincided with a weekend or bad weather. An additional sample was taken on July 14 because there were few bees in some of the previous samples. The final sampling of bees in the ninth series was done directly from the families, without the use of insulators (since there was no brood at this time). The date of this sampling is the typical date of bee sampling for analysis.

The work on dissection and measurement of exterior features was performed according to the modified and supplemented method of Alpatov [5]. The licensed certified software Altami Studio was used to measure the exterior features of bees. The arithmetic mean (M) and its standard error (m) were calculated in the Microsoft Office Excel software, using the package Data Analysis - Descriptive Statistics. Calculation of the nonparametric criteria: Friedman ANOVA (X_r^2) and Kruskal-Wallis criterion (H) was performed using Statistica 13.0 software. Criterion X_r^2 allows for multiple comparison of an indicator measured on one sample several times during a certain period of time. Criterion H also allows a multiple comparison of an indicator measured on several (more than two) samples[9]. These criteria are nonparametric analogues to single factor analysis of variance (with repeated measures (X_r^2) and multiple group measures (H)[10]). The parallel use of the criteria for independent and dependent samples is justified by the fact that the measurements are made on different samples and can be considered independent on the one hand, and on the other hand, bees in different samples from the same family are related at least in the maternal line[11].

RESULTS AND DISCUSSION

The arithmetic mean (M) and standard error (m) for all the measured traits were determined for each sample and presented in Table 1.

Exterior traits in each bee family vary slightly in a wave-like manner during the season (see Table 1). At the same time, these changes are not always synchronous. For example, a trait may decrease in one bee family and increase in another bee family during the same period of time.

To determine the significance of these changes, the Friedman (X_r^2) and Kruskal-Wallis (H) criteria were calculated from the results of Table 1 (see Table 2). Two opposite hypotheses were proposed, which were tested by calculating X_r^2: the null hypothesis (H0) and the alternative hypothesis (H_1). Hypothesis H_0 indicates that the results of measuring the exterior trait during the season do not differ, i.e. the exterior trait does

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Табл. 1. Изменение экстерьерных признаков пчел ($M \pm m$) в течение сезона
Table 1. Change in the exterior characteristics of bees ($M \pm m$) during the season

№ of the bee family	Date of sampling									
	01.06.2021	15.06.2021	06.07.2021	14.07.2021	20.07.2021	03.08.2021	17.08.2021	31.08.2021	14.09.2021	06.10.2021
Proboscis length (n_{sample} = 26-30)										
2	6.61 ± 0,019	6,59 ± 0,020	6,49 ± 0,018	6,59 0,019	6,63 ± 0,012	6,62 ± 0,017	6,56 ± 0,013	6,44 ± 0,020	6,69 ± 0,026	6,58 ± 0,026
15	6,58 ± 0,018	6,62 ± 0,022	6,49 ± 0,019	6,57 ± 0,018	6,54 ± 0,021	6,44 ± 0,013	6,52 ± 0,018	6,52 ± 0,019	6,52 ± 0,032	6,49 ± 0,021
18	6,61 ± 0,023	6,55 ± 0,019	6,52 ± 0,021	–	6,56 ± 0,019	6,51 ± 0,027	6,55 ± 0,019	6,58 ± 0,020	6,59 ± 0,025	6,55 ± 0,019
35	6,57 ± 0,019	6,56 ± 0,022	6,43 ± 0,023	–	6,54 ± 0,021	6,56 ± 0,019	6,52 ± 0,025	6,55 ± 0,017	–	6,55 ± 0,020
40	–	–	6,48 ± 0,027	–	6,58 ± 0,021	6,50 ± 0,018	6,53 ± 0,021	6,50 ± 0,020	6,57 ± 0,019	6,49 ± 0,026
Conditional width of the third dorsal plate (n_{sample} = 26–30)										
2	4,74 ± 0,013	4,75 ± 0,022	4,70 ± 0,014	4,74 0,014	4,82 ± 0,020	4,83 0,018	4,71 ± 0,018	4,77 ± 0,015	4,78 ± 0,021	4,77 ± 0,021
15	4,75 ± 0,026	4,77 ± 0,025	4,91 ± 0,020	4,95 ± 0,022	4,85 ± 0,029	4,75 ± 0,028	4,85 ± 0,027	4,81 ± 0,023	4,90 ± 0,020	4,70 ± 0,027
18	4,66 ± 0,019	4,68 ± 0,012	4,73 ± 0,020	–	4,70 ± 0,017	4,71 ± 0,014	4,77 ± 0,015	4,75 ± 0,013	4,77 ± 0,026	4,73 ± 0,014
35	4,78 ± 0,019	4,76 ± 0,021	4,72 ± 0,024	–	4,83 ± 0,024	4,82 ± 0,022	4,82 ± 0,018	4,81 ± 0,020	–	4,81 ± 0,019
40	–	–	4,58 ± 0,020	–	4,71 ± 0,021	4,73 ± 0,022	4,71 ± 0,021	4,69 ± 0,022	4,78 ± 0,021	4,72 ± 0,018
Length of the right front wing (n_{sample} = 26–30)										
2	9,16 ± 0,021	9,20 ± 0,020	9,06 ± 0,021	9,10 0,019	9,21 ± 0,017	9,25 ± 0,018	9,17 ± 0,020	9,16 ± 0,019	9,30 ± 0,026	9,27 ± 0,027
15	9,22 ± 0,020	9,23 ± 0,023	9,20 ± 0,026	9,26 ± 0,022	9,25 ± 0,024	9,14 ± 0,033	9,24 ± 0,027	9,20 ± 0,027	9,27 ± 0,027	9,08 ± 0,023
18	9,11 ± 0,014	9,14 ± 0,018	9,10 ± 0,024	–	9,14 ± 0,019	9,12 ± 0,019	9,15 ± 0,020	9,23 ± 0,020	9,11 ± 0,028	9,16 ± 0,021
35	9,20 ± 0,020	9,07 ± 0,025	9,05 ± 0,022	–	9,24 ± 0,025	9,20 ± 0,021	9,22 ± 0,023	9,18 ± 0,029	–	9,23 ± 0,020
40	–	–	9,16 ± 0,027	–	9,25 ± 0,020	9,22 ± 0,021	9,26 ± 0,018	9,21 ± 0,020	9,33 ± 0,018	9,34 ± 0,021
Length of the FG segment of the medial vein on the right forewing (n_{sample} = 26–30)										
2	0,24 ± 0,004	0,25 ± 0,005	0,25 ± 0,004	0,25 ± 0,005	0,26 ± 0,005	0,25 ± 0,005	0,24 ± 0,006	0,25 ± 0,004	0,26 ± 0,004	0,27 ± 0,005
15	0,23 ± 0,005	0,24 ± 0,004	0,24 ± 0,005	0,23 ± 0,003	0,23 ± 0,004	0,23 ± 0,005	0,24 ± 0,004	0,23 ± 0,004	0,25 ± 0,003	0,24 ± 0,004
18	0,28 ± 0,005	0,28 ± 0,005	0,27 ± 0,004	–	0,27 ± 0,005	0,27 ± 0,004	0,26 ± 0,003	0,27 ± 0,005	0,25 ± 0,005	0,28 ± 0,005
35	0,26 ± 0,005	0,26 ± 0,004	0,25 ± 0,003	–	0,27 ± 0,003	0,25 ± 0,004	0,24 ± 0,004	0,26 ± 0,003	–	0,26 ± 0,004
40	–	–	0,24 ± 0,005	–	0,25 ± 0,004	0,25 ± 0,004	0,24 ± 0,005	0,25 ± 0,004	0,26 ± 0,004	0,25 ± 0,004

not change significantly during the season. In the case when the calculated value of X_r^2 exceeds the critical value (for the chosen significance level and the corresponding number of degrees of freedom), then H_0 is rejected and H_1 is accepted (i.e. the differences are not random). Since X_r^2 is used for dependent samples, and given that there are empty cells in Table 1, we calculated X_r^2 for different numbers of families and different numbers of sampling.

We also tested two hypotheses with criterion H: H_0 - there are only random differences in the level of the studied trait between the samples from one bee family, and H_1 - there are non-random differences in the level of the studied trait between the samples from one bee family.

Due to the fact that these criteria in large samples (with a large number of pairs being compared) or with a large number of cases have a distribution close to X^2, the empirical values of these criteria were compared with the critical values of X^2 (see footnote 9). Using the X_r^2 criterion, significant differences were established: in one case for the Proboscis Length trait and in the other case for the FG Segment Length trait. Differences according to criterion H were significant only for the Proboscis Length trait. The fact that the differences are significant, i.e., non-random only in single cases, testifies in favor of the hypothesis of unequal sperm mixing.

Kendall's coefficient of concordance (or consistency) (W) is similar to Spearman's R, but unlike R it shows the dependence between several variables (see footnote 10). In our case it shows the consistency of the changes in exterior traits during the season. Significance of the coefficient W was checked by Pearson's criterion (X^2) calculated according to the formula $X^2_{calc} = N (C-1) W$ [6].

Then X^2calc was compared with the tabulated X^2 (see footnote 9). If $X^2_{calc} > X^2_{tabl}$, then W was found to be significant. The W coefficient was significant in two cases and had a medium degree of consistency of change, which may

Табл. 2. Сравнение результатов измерения экстерьерных признаков с помощью непараметрических критериев

Table 2. Comparison of the results of measuring exterior features using non-parametric criteria

C	*N*	*df*	Friedman criterion		Concordance coefficient		Kruskal-Wallis criterion (*df* = 9, *N* = 44)	
			X_r^2	*p*	*W*	*r*	*H*	*p*
Proboscis length								
10	2	9	10,71	0,296	0,595	0,190	21,12*	0,012
9	3	8	14,38	0,072	0,599	0,399		
8	4	7	14,36*	0,045	0,513*	0,350		
6	5	5	9,48	0,091	0,379	0,224		
Conditional width of the third dorsal plate								
10	2	9	5,71	0,769	0,317	0,366	6,81	0,657
9	3	8	8,85	0,355	0,369	0,053		
8	4	7	9,02	0,251	0,322	0,096		
6	5	5	2,75	0,739	0,109	0,113		
Length of the right front wing								
10	2	9	9,05	0,432	0,503	0,006	11,04	0,273
9	3	8	7,38	0,496	0,307	–0,039		
8	4	7	11,42	0,121	0,408	0,210		
6	5	5	10,03	0,074	0,401	0,251		
Length of the FG segment of the medial vein on the right forewing								
10	2	9	11,19	0,263	0,622	0,243	5,42	0,796
9	3	8	9,07	0,336	0,378	0,067		
8	4	7	12,67	0,081	0,452	0,269		
6	5	5	15,40**	0,009	0,616**	0,520		

Note. *C* – number of cases; *N* – number of pairs compared; *df* – number of degrees of freedom; *r* – average rank.

have been due to unrecorded factors and could therefore be considered random, because in other combinations (most cases) of number of bee colonies and sampling dates, the coefficient was not significant.

Previously, we found that intrafamily variability, determined by the coefficient of variation, is generally significantly greater than seasonal variability [7]. For example, in bee family No. 18, the proboscis length Cv varied from 1.52 to 2.28% (depending on the date of sampling), while the seasonal Cv was 0.5%. In family No. 15, this index ranged from 1.08 to 2.68 and 0.78%, respectively, and in family No. 2, from 0.96 to 2.16 and 1.07, respectively. On the trait Length of the segment FG of the medial vein on the right forewing, depending on the date of sampling, Cv in family No. 18 (depending on the date of sampling) was from 6.48 to 11.38, and seasonal - 3.65%. In families № 15 and № 2 (depending on the date of sampling) were variations of this index from 6,96 to 12,03 and from 7,72 to 13,05, and seasonal - 2,26 and 3,16% respectively. This can be explained by the fact that the queen bee mates with several drones[12], and the seasonal variation is due to irregular (or partial) mixing of drones' semen in the genital tracts of queen bees[13]. In this connection, the reliable differences established with the help of nonparametric criteria, can also be attributed to irregularity of sperm mixing (see footnote 13).

CONCLUSION

The variation of exterior traits during the season is not due to climatic conditions or nutrition, but to the irregularity of drones' sperm mixing in the seminal receptacle of the queens, because the coefficient of variation between individuals of one sample is greater than between the samples of bees taken from the same bee family. In the case of instrumental insemination, semen homogenization is necessary to ensure that the offspring composition of the queen is even throughout the whole period of its use [8]. Comparison of the progeny of the queens inseminated with homogenized semen is a possible topic of our further research. It is recommended that the samples in the families formed in the current year should be taken after the change of bees to the progeny of a new queen, and that a frame isolator should be used to obtain the bees. Sampling can be done at any time of the season, but the most convenient time is in the fall (late August to September). The progeny from the best producing bee colonies (regardless of the type of mating) should be obtained in the year they achieve high productivity (see footnote 13).

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ИНФОРМАЦИЯ ОБ АВТОРЕ

(✉)**Березин А.С.,** научный сотрудник; **адрес для переписки:** Россия, 391110, Рязанская область, г. Рыбное, ул. Почтовая, 22; e-mail: mellifera@yandex.ru

AUTHOR INFORMATION

(✉)**Andrey S. Berezin,** Researcher; **address:** 22, Pochtovaya St., Rybnoe, Ryazan Region, 391110, Russia; e-mail: mellifera@yandex.ru

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РАЗРАБОТКА ДВУХСТУПЕНЧАТОГО ЦИКЛОНА

Пшенов Е.А., (✉)Блёскин С.С.
Новосибирский государственный аграрный университет
Новосибирск, Россия
(✉)e-mail: Moonlait2510@mail.ru

Исследован процесс сепарации транспортируемого материала от несущего газового потока в циклонных аппаратах на зерноперерабатывающих предприятиях. Производство комбикормов сопровождается образованием мелкодисперстной мучной пыли, которая является ценным компонентом ферментированных кормов. Системы аспирации миникомбикормовых заводов не обеспечивают ее улавливание в полном объеме, что приводит к загрязнению помещений, а также потере части продукта. В ходе анализа конструкций и путей совершенствования центробежных аппаратов определено, что перспективна установка в выхлопном патрубке циклона дополнительных закручивающих устройств для очистки воздушного потока от мелкой пыли. Предложена конструкция циклона с дополнительной ступенью очистки воздушного потока от мелкой мучной пыли в выходном патрубке, используемая в системе пневмотранспорта миникомбикормового завода. Диаметр осевого патрубка увеличен для размещения внутри его отбойного конуса и вихревой воронки. При этом диаметр выходного патрубка должен быть меньше диаметра верхнего основания вихревой воронки для снижения выноса мелкой фракции из циклона. Разработана экспериментальная установка, включающая загрузочный бункер, пневмоприемник-отвод, материалопровод, циклон, фильтр и всасывающий вентилятор. В качестве материала использовано зерно пшеницы влажностью 16%, измельченное на молотковой дробилке с диаметром отверстий в решете 3 мм. Произведены сравнительные испытания предлагаемого циклона с циклонами типа ЦР и SNT, результаты которого доказывают эффективность предложенной конструкции. В исследуемом диапазоне (от 0,026 до 0,33 кг/с) эффективность двухступенчатого циклона по подаче транспортируемого материала была выше, чем у серийно выпускаемых циклонов разгрузителей ЦР.

Ключевые слова: миникомбикормовый завод, пневмотранспорт, циклон разгрузитель, мучная пыль

DEVELOPMENT OF A TWO-STAGE CYCLONE

Pshenov E.A., (✉)Bleskin S.S.
Novosibirsk State Agrarian University
Novosibirsk, Russia
(✉)e-mail: Moonlait2510@mail.ru

The process of separating the transported material from the carrying gas flow in cyclones at grain-processing enterprises is studied. Feed production is accompanied by the formation of fine flour dust which is a valuable component of fermented feed. Aspiration systems of mini-fodder plants do not provide its full capture, which leads to contamination of the premises, as well as the loss of some of the product. During the analysis of designs and ways to improve centrifugal apparatuses, it was determined that installation of additional swirling devices in the cyclone exhaust pipe is promising for cleaning the air flow from fine dust. The design of cyclone with an additional stage of cleaning the air flow from fine flour dust in the outlet nozzle, used in the pneumatic conveying system of mini-fodder plant, is proposed. The diameter of the axial pipe socket is enlarged to ac-

commodate the baffle cone and vortex funnel inside it. The diameter of the outlet nozzle should be smaller than the diameter of the upper base of the vortex funnel to reduce the fine fraction escape from the cyclone. An experimental unit was developed including a charging hopper, pneumatic receptor-diverter, material pipeline, cyclone, filter, and a suction fan. The material used was wheat grain with a moisture content of 16%, crushed on a hammer crusher with a hole diameter of 3 mm in the sieve. Comparative tests of the proposed cyclone with cyclones of the DC and SNT types were carried out, the results of which prove the effectiveness of the proposed design. In the investigated range (from 0.026 to 0.33 kg/s) the efficiency of the two-stage cyclone in feeding the transported material was higher than that of the commercially available discharger cyclones DC.

Keywords: mini-fodder plant, pneumatic conveying, discharger cyclone, flour dust

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INTRODUCTION

Currently, industry (enterprises of chemical, metallurgical, mining and food industries) has a great impact on the atmosphere, lithosphere and hydrosphere of the planet. Due to the release of various wastes into the environment, there is state control over pollution. That is why every enterprise is interested in ecological production not only from the ethical point of view, but also because of possible sanctions. In this regard, the organization of production requires systems of cleaning, disposal, and deposition of waste.

The main factors affecting the state of the atmosphere are emissions of the exhaust gas stream after an insufficiently effective system of its cleaning. One of the main emissions of feed mills into the atmosphere is a fine component of grain milling products. Results of researches at mixed fodder productions confirm that one of the harmful conditions of work is air dustiness[1, 2]. Thus, the dustiness of exhaust gases during processing of grain crops can reach 2 to 3 g/m^3.

Separate attention should be paid to mini feed mills designed for the preparation of complete feed with various additives in the conditions of small and medium-sized farms, with a capacity of 0.8 to 5 t/h of finished mixed fodder. As a collector of fine flour dust fabric filters in the form of bags are used which are unable to carry out effective air purification are used at such mills.

The efficiency of the fine component capture not only affects the health of workers and the explosion hazard of the premises. This component can be used as a feed additive. However, the fine component, on the one hand, is dangerous for animals, and its content in bulk feed should not exceed permissible norms. On the other hand, it is a valuable component, in terms of making full use of the potential of grain dust, in the production of fermented feed, such as grain molasses[3]. The presence of

[1] *Ananyev V.A., Baluyeva L.N., Galperin A.D., Gorodov A.K., Eremin M.Y., Zvyagintseva S.M., Murashko V.P., Sedykh I.V.* Ventilation and air conditioning systems. Theory and practice. Moscow: Euroclimate, 2003. 416 p.

[2] *Butkovsky V.A.* Peculiarities of milling plants in Russia in modern conditions // Khleboprodukty. 2005. N 5. pp. 2-4.

[3] *Pshenov E.A., Blyoskin S.S., Nechaev N.A*. Improvement of forage grain milling quality by hammer crushers // Proceedings of the scientific and practical conference of teachers, graduate students, undergraduates and students of the Novosibirsk SAU, Novosibirsk, October 21-23, 2019 Novosibirsk: IC NSAU " Zolotoy Kolos", 2019. pp. 112-114.

flour dust in the feedstock in the production of pelleted and extruded feed does not affect the quality of the finished product and does not have a negative impact on animal health.

Therefore, it is important to ensure a high degree of air purification with subsequent deposition and processing of the flour dust. Low efficiency of catching fine component and return of dust particles to the exhaust pipe is the most urgent and difficult to solve problem of development of centrifugal dust collector technology, and consequently, ensuring environmental and fire safety of an enterprise and full use of the caryopsis potential.

A.G. Titov's thesis[4] is devoted to a similar problem, in which secondary entrainment of dust in the exhaust nozzle of the cyclones at industrial enterprises, as well as during purification of waste gases from dust in electric power industry and in the experiments on reduction of entrainment are noted [1]. V.V. Kuzmin, V.A. Markov, D.I. Misyulya [2, 3] made an attempt to solve the problem of secondary dust carry-over in chemical industry by introducing certain spinners directly into the cyclone supply nozzle.

The object of this research is the process of separation of the transported material from the carrier gas flow in cyclones at grain processing plants.

The purpose of the research is to increase the efficiency of separation in the inertial dust collector by reducing the dust backflow into the cyclone exhaust nozzle.

The research objectives are to:

1. Analyze ways to improve cyclone dust collectors.

2. Develop an improved cyclone apparatus design.

3. Experimentally verify the effectiveness of the proposed technical solution.

MATERIAL AND METHODS

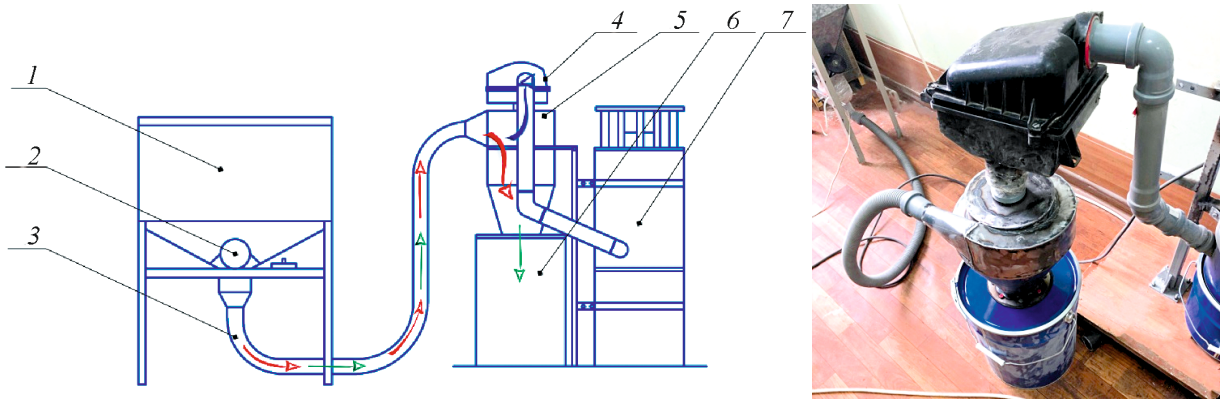
The solution of these problems is based on the application of a comparative critical analysis of the current state of development of centrifugal separation, the methodology of technological equipment design, as well as experimental research.

To check the efficiency of various cyclone designs, an experimental unit (see Fig. 1) was made at the Engineering Institute (Department of Mechanization of Animal Husbandry and Processing of Agricultural Products) of the Novosibirsk State Agrarian University.

The experimental unit includes a charging hopper with an agitator (to prevent piling) and an adjustable damper. Material is taken in and transported due to air rarefaction created by a suction fan. Pneumatic conveying of material is carried out by a pneumatic collector-diverter of U2-BPO type and a material pipeline, connected to the cyclone under study. To estimate the cyclones efficiency, an automobile air filter was installed at the outlet pipe, weighing of the filtering element allowed to determine the mass of the dust carried out.

The material used was crushed wheat grain with moisture content of 16%, obtained on a hammer crusher ShIK B-600 with installed sieves with a hole diameter of 3 mm. According to the results of sieve analysis according to GOST 13496.8-72 (Combined fodders. Methods of Determination of Milling Coarseness and Content of Ungrindable Seeds of Cultivated and Wild Plants) the average particle size distribution of the product was determined: mass of residue on a 3 mm sieve - 2,3 g, 2 mm - 31,6 g, 1 mm - 42,1 g, a tray - 23,9 g.

[4] *Titov A.G.* Intensification of the dust collection process in an electrocyclone by reducing secondary entrainment: extended abstract of candidate's thesis in Chemistry in Chemistry: 05.17.08. Tomsk, 2014. 20 p.



а *б*

Рис. 1. Экспериментальная установка:

а – схема экспериментальной установки: *1* – загрузочный бункер; *2* – ворошитель; *3* – материалопровод; *4* – корпус воздушного фильтра; *5* – циклон; *6* – емкость для сбора отделяемого продукта; *7* – всасывающий вентилятор; *б* – общий вид экспериментальной установки

Fig. 1. Experimental unit:

a - experimental unit scheme: *1* - charging hopper; *2* - agitator; *3* - material conduit; *4* - air filter casing; *5* - cyclone; *6* - container for collecting separated product; *7* - suction fan; *б* - general view of an experimental unit

Relevance of the study

Cyclone dust collectors and bag filters are the most widespread as dust and gas cleaning systems[5]. There are also combined cleaning systems, in which the efficiency of bag filters is increased by including a cyclone in the dust and gas cleaning scheme[6]. Industrial enterprises use various schemes of emission purification systems using cyclone dust collectors[7]. The areas of application, basic design features, as well as the classification of existing cyclones are considered in works [5, 6].

The main advantages of cyclone dust collectors include the following[8]:

− no moving parts;

− dry dust trapping capacity;

− ability to trap abrasive dusts, for which the active surfaces of cyclones are coated with special materials;

− ability of cyclones to operate at high pressures;

− hydraulic resistance has a stable value;

− simple design and repair capability;

− increased dust concentration does not reduce the fractional efficiency of the unit.

The main disadvantages are relatively high hydraulic resistance, which reaches 1250-1500 Pa, and low efficiency when catching particles < 5 microns in size. Thus, to improve the efficiency of dust collection of polydisperse particles, there are the following directions[9] of improvement of centrifugal apparatuses [4, 7], presented in Fig.2.

[5]*Bleskin, S.S. Pshenov E.A.* Operation of cyclone dust collectors // State and innovations of technical service of machines and equipment: Materials of the 11th regional scientific-practical conference of students, graduate students and young scientists dedicated to the 75th anniversary of the Engineering Institute, Novosibirsk, November 11-12, 2019. Novosibirsk: NSAU Publishing Center " Zolotoy Kolos ", 2019. pp. 38-41.

[6]*Bushumov S.A., Korotkova T.G.* Increasing the efficiency of bag filters by including cyclone in the technological scheme of dust and gas cleaning // Proceedings of the International Scientific-Practical Conference, Krasnodar, June 21-22: Sustainable development, environmentally safe technologies and equipment for processing of food agricultural raw materials. Krasnodar: OOO Ecoinvest, 2016. pp. 75-77.

[7]*Kochetov O.S., Soshenko M.V., Zubkova V.M.* Cyclone dust collectors of emission treatment systems // Regularities and trends of innovative development of society: collection of articles of the International Scientific and Practical Conference: in 6 parts, Magnitogorsk, December 20, 2017. Magnitogorsk: OOO Aeterna, 2017. pp. 66-68.

[8]Advantages and disadvantages of cyclones // KS-technology elevator and mill equipment URL: http://ksmash. ru/priyemushchestva-i-nedostatki-tsiklonov (accessed: 10.04.2022).

[9]*Chekalov L.V., Guzaev V.A., Smirnov M.E.* Basic ways of increasing the efficiency of gas-cleaning equipment in the cement industry // Alitinform: Cement.Concrete.Dry Mixtures. 2008. N 3-4 (4-5). pp. 28-37.

The main direction of work to improve the quality of dust separation is to change the overall design of the device. Design forms of cyclone dust collectors are various. There is a certain tendency of development of the cone-shaped part, since the cyclone shape affects the aerodynamics of the air flows, occurring in the apparatus. In a cone-shaped cyclone, the distribution of velocities along the cyclone radius, as well as the changes in the air currents inside the device, are of interest. Consequently, in order to improve the quality of purification different spinning devices and inserts are used, which in one case reduce the hydraulic resistance without changing the quality of filtration [8], and in the other case increase the hydraulic resistance. This insignificantly increases the efficiency of purification.

Unscrewing devices are installed at the supply nozzle, at the bottom of the cyclone or at the outlet nozzle. Both quality and energy efficiency of the dust collector depend on the place

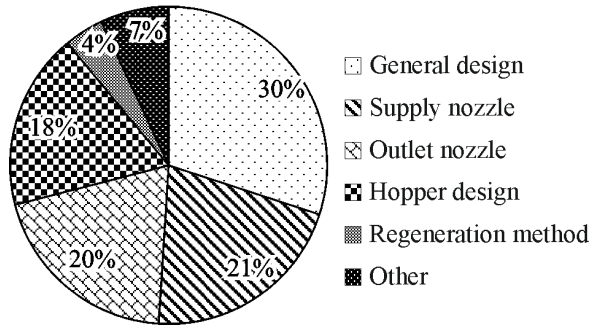
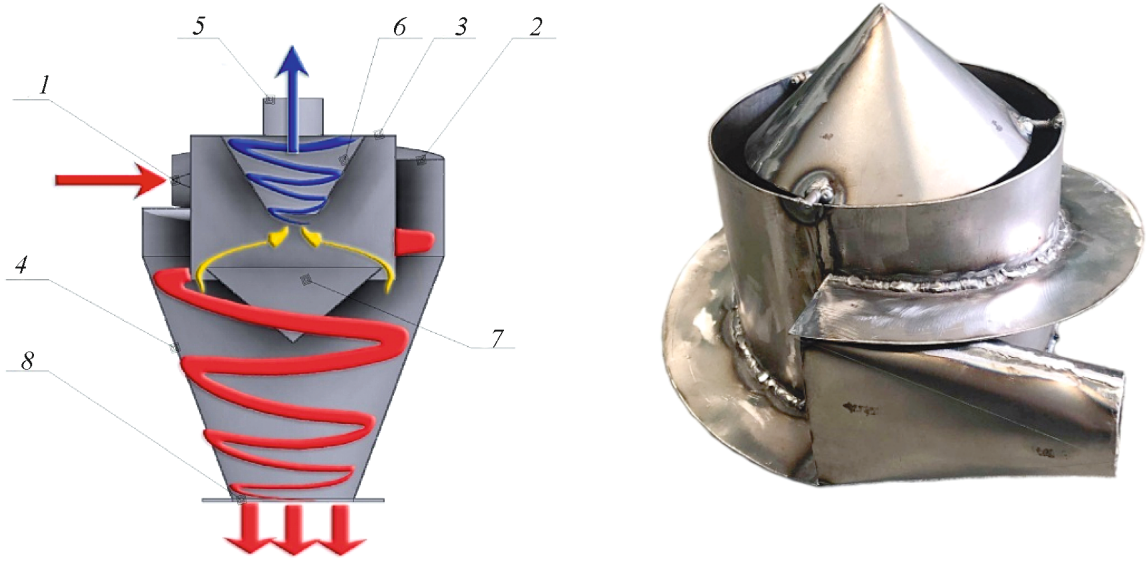


Рис. 2. Направления совершенствования центробежных аппаратов

Fig. 2. Directions for improvement of centrifugal machines

of installation. Thus, when installing additional spinning devices at the supply nozzle or at the bottom part of a cyclone, there is a noticeable reduction of hydraulic resistance, leading to escape of a fine component to the exhaust nozzle, and when installing it at the outlet nozzle - to an insignificant pressure drop [8]. Designs of devices to reduce power consumption, as well



а *б*

Рис. 3. Двухступенчатый циклон:

а – схема движения частиц в циклоне:*1* – входной патрубок; *2* – спиральная улитка; *3* – осевой патрубок; *4* – коническая часть корпуса; *5* – выходной патрубок; *6* – коническая вихревая воронка; *7* – отбойный конус; 8– разгрузочное окно; *б* – общий вид предлагаемой конструкции спиральной улитки с отбойным конусом

Fig. 3. Two-stage cyclone

a - scheme of particles motion in a cyclone:*1* – supply nozzle; *2* - spiral volute; *3* - axial socket; *4* - conical body part; *5* - outlet nozzle; *6* - conical vortex funnel; *7* - baffle cone; *8* - unloading aperture; *б* - general view of the proposed design of the spiral volute with a baffle cone

as ways to reduce power consumption of cyclones by introducing various spinning devices and secondary use of inertia of vortex flow are presented in works [9-11].

Model offer

Based on the analysis of the current state of gas purification apparatuses used in feed production, a cyclone with an additional purification stage, installed in the outlet nozzle, was developed.

The proposed cyclone works as follows (see Fig. 3, *а*). The separated flow is introduced into the cylindrical cyclone body through the supply nozzle into the spiral volute. The dust and gas flow are swirled in the spiral volute and directed to the conical part of the cyclone body. The rectangular cross section of the supply nozzle and the annular channel formed by the axial socket and the inside wall of the spiral volute create the directed downward flow along the inner wall of the cyclone casing surface. When the dust and gas flow is swirling, centrifugal force is generated, which causes coarse particles to move along the helical spiral in radial direction downwards and to go into the unloading aperture through the conical part of the housing.

Fine dust particles with the ascending gas flow rush upwards and meet the baffle cone (see fig. 3, *б*). Moving along its forming, the particles get into the annular channel between the axial socket and the baffle cone. The conical vortex creates a low-pressure area along the cyclone axis. This leads to a sharp change in the direction of gas flow in the annular channel. Dust particles, moving by inertia, touch the inner wall of the axial socket and fall out of the flow.

The conical vortex has a larger diameter at the top and a smaller diameter at the bottom than the outlet nozzle. Thus, a vortex with variable peripheral velocity is created in the funnel. Dust particles, entrained in the vortex, under the action of centrifugal force rotate at a larger diameter than the outlet nozzle and are not carried out of the cyclone. The cleaned gas flow exits through the outlet nozzle[10].

Methodology for comparative tests

The maximum gate position of the feed hopper, at which the stable pneumatic conveying of the feed material to the cyclones is performed, is preliminarily determined. Also, the minimum position of the flap, which facilitates stable feeding of the material into the pneumatic feeder-diverter, is pre-determined. After that, three intermediate positions were marked between the minimum and maximum positions, which together made five variants of material load on cyclones.

The experiment was conducted in three repetitions at five positions of the charging hopper slide. Before each repetition, the initial mass of material (about 11 kg) was weighed. To determine the material load on the cyclone, the time from the beginning of the flap opening to the full passage of crushed grain in the pneumatic conveying system was measured.

The total cyclone capture factor η is chosen as the separation efficiency criterion. Since the feed material is represented by four fractions, η is determined by the formula

$$\eta = 1 - \eta_1 \cdot \eta_2 \cdot \eta_3 \cdot \eta_4,$$

where η_1, η_2, η_3, η_4 – fractional capture coefficients, respectively: more than 3 mm, 3 to 2 mm, 2 to 1 mm and less than 1 mm.

Capture coefficient η_1, η_2, η_3, η_4 is characterized by the ratio of the number of actually captured particles $M_{y\text{-}i}$ to its amount in the initial mixture $M_{0\text{-}i}$, is by the formula

$$\eta_1\text{–}4 = M_{y\text{-}i} / M_{0\text{-}i}.$$

Before each repetition, the automobile filter was disassembled, the filter element was blown with compressed air and weighed, and after the next experiment was repeatedly weighed to determine the mass of the extracted dust.

[10]Useful model patent N 208117 U1 Russian Federation, IPC B04C 9/00, B01D 45/12. Cyclone: N 2021106836: application. 15.03.2021: publ. 03.12.2021 / E.A. Pshenov, A.A. Mezenov, M.L. Vertey, A.A. Didenko, S.S. Bleskin, A.G. Khristenko; applicant Federal State Budgetary Educational Institution of Higher Education "Novosibirsk State Agrarian University"

RESULTS AND DISCUSSION

During the comparative tests the following dependencies, presented in the graph (see Fig. 4) were received.

When processing the obtained results, it was determined that the trapping coefficients η_1, η_2 and η_3 for fractions over 3 mm, from 3 to 2 mm and from 2 to 1 mm for all three cyclones are equal to 1. Then the capture coefficient η_4 becomes determinative. Considering that at the sieve analysis the weight of a sample was 100 g, the weight of a fraction less than 1 mm in the pallet is a mass fraction of a component in a mixture and makes 23.9 %, that corresponds to M_0 = 2629 g of a flour dust in the initial mixture with weight 11 kg.

According to the research data, the cyclone unloader CR showed practically stable operation in the studied range of material feeding. An increase in efficiency was noted at a material load of 0.16 kg/s, the mass of dust carried out was 0.66 g, which corresponds to $\eta = \eta_4 = 0.99975$.

Cyclone SNT showed the best results at low concentrations of material (0.026 kg / s) - 0.74 g (η = 0.99972). However, as the material flow rate increased, the dust mass carried into the outlet nozzle also increased and reached 4.33 g (η = 0.99835) at 0.32 kg/s. This indicates that its design can be attributed to the dust collectors, operating at low dust concentrations.

The proposed two-stage cyclone, on the contrary, at low concentrations showed the worst results (about 1.7 g) of the dust discharged into the outlet nozzle (η = 0.99935), and as the load increases, it showed the best results in the remaining range as compared with the tested samples. At 0.33 kg/s, the mass of the dust discharged was 0.92 g (η = 0.99965), which was 32% less than the nearest counterpart of the cyclone unloader CR 1.36 g (η = 0.99948) under the same initial conditions.

CONCLUSIONS

1. According to the results of the analysis of ways to improve centrifugal dust collectors, the

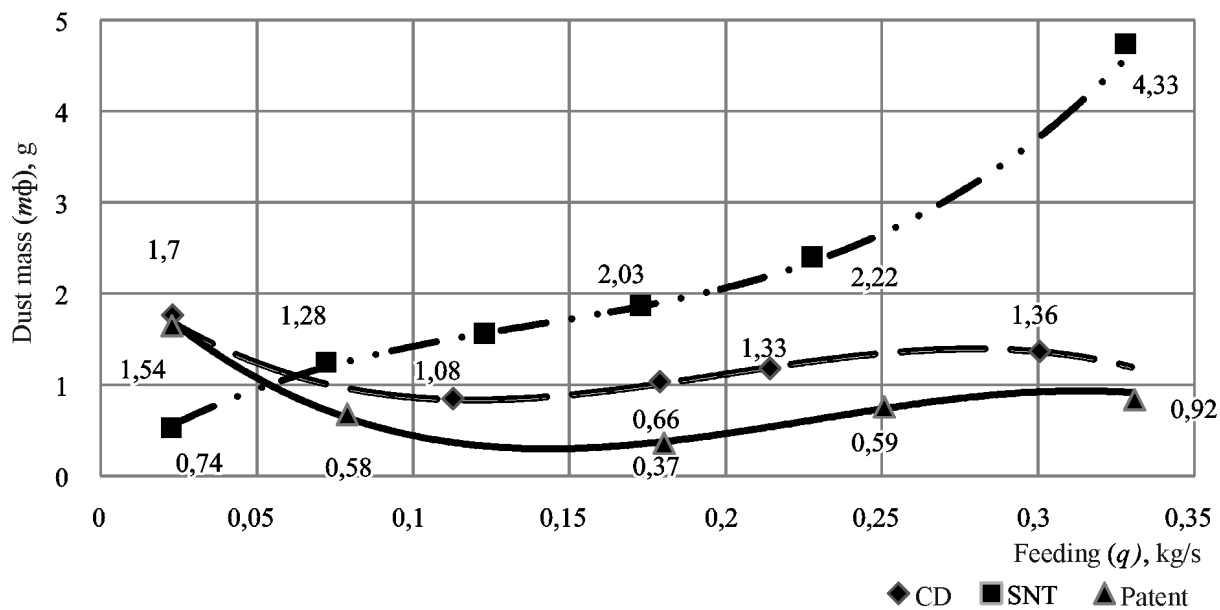


Рис. 4. Результаты сравнительных испытаний циклонов:
мф – масса пыли, оставшейся на фильтре; *q* – подача материала в циклон;
Патент – предложенное конструкторское решение циклона.

Fig. 4. Results of the comparative cyclone tests:
mф – mass of the dust remaining on the filter; *q* – feeding the material into the cyclone;
Patent – the proposed cyclone design solution.

direction associated with the installation of additional swirlers in the cyclone outlet pipe to increase the twist of the flow and increase the degree of gas purification from fine particles is highlighted.

2. Based on the analysis of modern cyclone designs, a technical solution in the form of a two-stage cyclone to improve the efficiency of a mini feed mill has been developed.

3. It has been experimentally established that the proposed design of the two-stage cyclone should be attributed to cyclones dischargers operating at high concentrations of material.

4. In the investigated range (from 0.026 to 0.33 kg/s) in terms of the transported material, the efficiency of the two-stage cyclone was higher than that of commercially available cyclones of CR unloaders (η = 0.9998-0.99965).

Thus, further research will be aimed at determining the hydraulic resistance of the cyclone, as well as determining the rational design-mode parameters of the cyclone under development.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

Пшенов Е.А., кандидат технических наук, доцент

(✉) **Блёскин С.С.,** аспирант; **адрес для переписки:** Россия, 630039, Новосибирск, ул. Добролюбова, 160; e-mail: Moonlait2510@mail.ru

AUTHOR INFORMATION

Evgenii A. Pshenov, Candidate of Science in Engineering, Associate Professor

(✉) **Sergey S. Bleskin,** Postgraduate Student; **address:** 160, Dobrolyubova St., Novosibirsk, 630039, Russia; e-mail: Moonlait2510@mail.ru

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ПОДБОР ИНТРОГРЕССИВНЫХ ЛИНИЙ ПШЕНИЦЫ И ТРИТИКАЛЕ ПО КАЧЕСТВУ ЗЕРНА И УСТОЙЧИВОСТИ К БОЛЕЗНЯМ ДЛЯ ИСПОЛЬЗОВАНИЯ В ОРГАНИЧЕСКОМ ЗЕМЛЕДЕЛИИ

(✉)**Ержебаева Р.С., Абекова А.М., Базылова Т.А., Масимгазиева А.С., Мереева Т.Д., Кожахметов К.К., Бастаубаева Ш.О., Слямова Н.Д.**
Казахский научно-исследовательский институт земледелия и растениеводства
Алматинская область, пос. Алмалыбак, Республика Казахстан
(✉)e-mail: raushan_2008@mail.ru

Изучены 17 интрогрессивных линий и сортов озимой пшеницы и 2 линии озимой тритикале (коллекция Казахского научно-исследовательского института земледелия и растениеводства) для определения возможности их применения в органическом земледелии. Исследования проведены в 2021, 2022 гг. в предгорной зоне юго-востока Республики Казахстан на базе научно-полевого стационара Казахского научно-исследовательского института земледелия и растениеводства. Анализ на содержание генетически модифицированных источников показал отсутствие в пробах регуляторных элементов 35S и NOS. Осуществленная методом ПЦР ДНК-идентификация с целью обнаружения эффективных генов бурой, стеблевой и желтой ржавчины (*Lr9*, *Lr26/Sr31/Yr9*, *Lr34/Yr18/Sr57*, *Lr35*/*Sr39*, *Sr2*, *Sr36*) позволила выделить четыре образца (1633-40, 1675-170, 1723-11, 2041-7) с ценными генами *Lr34/Yr18/Sr57/Pm38* и один образец (1127-7) с генами *Lr26/Sr31/Yr9/Pm8.* Фитопатологическая оценка устойчивости зерновых культур выполнена на естественном фоне по соответствующим шкалам учета пораженности растений бурой стеблевой и желтой ржавчиной. В результате обнаружены девять устойчивых к двум видам ржавчины (бурая и желтая) образцов с типом реакции R и нулевым процентом поражения (1127-7, 1675-170, 1676, 2005-13, 2041-13, 2046-1, KZ231, Т-409-1, Т-989-1). Стеблевая ржавчина в годы исследований не была зафиксирована. Оценка качества зерна проведена на основании требований соответствующих ГОСТов по следующим параметрам: натура зерна, стекловидность, содержание протеина и клейковины, качество клейковины. Установлено, что все рассматриваемые образцы по показателям стекловидности и содержанию клейковины соответствуют классу сильных пшениц. Выделены образцы с высокими показателями натуры зерна (≥ 800 г/л; 1674-27, 1675-149, 1675-170), содержания белка (≥ 16%; 2041-13, 2005-13, 2041-7, 1716-24, 1675-149, 1127-7, 1633-31, 1717-27, KZ231), качества и количества клейковины (1127-7, KZ231). По итогам комплексной оценки отмечены две линии (1127-7, KZ231), продемонстрировавшие хорошую устойчивость к двум видам ржавчины и высокое качество зерна. Данные линии рекомендованы для использования в органическом земледелии.

Ключевые слова: интрогрессивные линии пшеницы, генетически модифицированные источники, качество зерна, устойчивость к болезням, ДНК-идентификация

SELECTION OF INTROGRESSIVE WHEAT AND TRITICALE LINES FOR GRAIN QUALITY AND RESISTANCE TO DISEASES FOR USE IN ORGANIC FARMING

(✉)**Yerzhebaeva R.S., Abekova A.M., Bazylova T.A., Massimgaziyeva A.S., Mereyeva T.D., Kozhakhmetov K.K., Bastaubayeva Sh.O., Slyamova N.D.**
Kazakh Research Institute of Agriculture and Plant Growing
Almalybak, Almaty region, Republic of Kazakhstan
(✉)e-mail: raushan_2008@mail.ru

17 introgressive lines and varieties of winter wheat and 2 lines of winter triticale (collection of the Kazakh Research Institute of Agriculture and Plant Growing) were studied to determine the

possibility of their use in organic farming. The studies were conducted in 2021-2022 in the foothill zone of the south-east of the Republic of Kazakhstan on the basis of the research field stationary of the Kazakh Research Institute of Agriculture and Plant Growing. Analysis for genetically modified sources showed the absence of 35S and NOS regulatory elements in the samples. DNA identification by PCR to detect effective brown, stem and yellow rust genes (*Lr9, Lr26/Sr31/Yr9, Lr34/Yr18/Sr57, Lr35/Sr39, Sr2, Sr36*) made it possible to identify 4 samples (1633-40, 1675-170, 1723-11, 2041-7) with valuable *Lr34/Yr18/Sr57/Pm38* genes and 1 sample (1127-7) with *Lr26/Sr31/Yr9/Pm8* genes. Phytopathological assessment of the resistance of cereal crops was carried out on the natural background using the appropriate scales of recording the infestation of plants by brown stem rust and yellow rust. As a result, 9 samples resistant to 2 types of rust (brown and yellow) with reaction type R and zero percent were found (1127-7, 1675-170, 1676, 2005-13, 2041-13, 2046-1, KZ231, T-409-1, T-989-1). Stem rust in the years of research was not recorded. Grain quality assessment was carried out on the basis of the requirements of relevant GOSTs on the following parameters: grain nature, vitreousness, protein and gluten content, gluten quality. It was found that all the samples under consideration in terms of vitreousness and gluten content correspond to the class of strong wheat. Samples with high grain natures (≥800 g/l; 1674-27, 1675-149, 1675-170), protein content (≥16%; 2041-13, 2005-13, 2041-7, 1716-24, 1675-149, 1127-7, 1633-31, 1717-27, KZ231), quality and quantity of gluten (1127-7, KZ231) were selected. According to the results of comprehensive evaluation, 2 lines (1127-7, KZ231), which showed good resistance to 2 types of rust and high grain quality, were noted. These lines are recommended for use in organic farming.

Keywords: introgressive wheat lines, genetically modified sources (GMS), grain quality, disease resistance, DNA identification

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INTRODUCTION

Wheat is a strategically important grain crop in Kazakhstan. The area of its cultivation in the country in 2021 amounted to 12,863.8 thousand hectares[1]. Stable increase in the production of wheat grain is one of the important directions of food security of the country. It is actively used for food, fodder and technical purposes. High involvement of this crop in the production process, vast areas under crops,

[1]Agency for Strategic Planning and Reforms Bureau of National Statistics of the Republic of Kazakhstan: official website URL: https://stat.gov.kz/

and a significant share in the human diet make the problem of organic wheat production very urgent. Such properties of wheat as high nutritive value, taste, resistance to diseases, and ecological plasticity are important. Since most of the modern varieties are bred for conventional farming, supporters of organic farming have to look for or even create their own varieties that work well in organic systems[2] [1].

The stock of soft wheat genetic material controlling resistance to the effects of harmful factors is currently almost exhausted. Limitation of gene diversity creates conditions favorable for disease development, which is one of the limiting factors of the breeding work[3] [2, 3]. The richest reserve of genetic diversity is represented in the gene pool of wild species and relatives of wheat. Many of them have been successfully used to transfer useful traits (disease resistance, drought and salt tolerance, winter hardiness, high protein content) to soft and hard wheat[4]. The effectiveness of introducing high disease resistance from wild relatives has been proven by a number of researchers [4, 5]. Wheat lines and varieties with good disease resistance are valuable material for organic farming due to the ban on the use of pesticides, synthetic mineral fertilizers, growth regulators and genetically modified organisms (GMOs).

A unique constant material of winter and spring wheat with *T. militinae Zhuk*., *T. timopheevii Zhuk*., *T. kiharae Dorof. et Migusch*., *Ae. cylindrical* L., *Ae. triaristata Willd* [6], which can be tested in organic agriculture, was created in the Kazakh Research Institute of Agriculture and Crop Production (KazNIIZiR).

The purpose of the study is to investigate winter wheat and triticale introgressive lines in terms of grain quality, disease resistance, lack of genetic modification for use in organic farming.

MATERIAL AND METHODS

he material for the experiment was a collection of 17 introgressive lines and varieties of winter wheat and 2 winter triticale lines selected by KazNIIZiR specialists. Isogenic wheat lines carrying genes *Lr9, Lr26, Lr34, Lr35, Sr36, Sr39, Sr2* obtained from the Laboratory of Plant Immunity and Protection of KazNIIZiR (CIMMYT material) were used as positive control for PCR-identification.

Experiments on phytopathological assessment of the collection of introgressive wheat and triticale lines for resistance to fungal diseases were carried out in 2021, 2022 in the natural background of the research field stations of KazNIIZiR, located in the piedmont zone of the Almaty region (43°N, 77°E, 740 m above sea level).

Resistance to the stem rust pathogen was assessed according to the Stakman and Levine scale[5], leaf rust according to the Maines and Jackson scale[6], and yellow rust according to the Gassner and Streib scale[7]. Degree of lesion was determined according to Peterson scale[8]. Susceptible wheat varieties Saratovskaya 29 and Morocco were used as a standard. Counting was performed from the moment of disease

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appearance every 10 days until the phase of milky-wax ripeness of grain.

Genomic DNA was isolated from the 2nd leaf of 11-12-day-old triticale seedlings using the S.L. Delaporta method[9].

Analysis of the content of genetically modified sources (GMI) was carried out according to ST RK1346-2005[10]. Polymerase chain reaction (PCR) method was used to detect targets - 35S promoter (from cauliflower mosaic virus (CaMV 35S)), NOS terminator (from *Agrobacterium tumefaciens*). Certified standard soybean samples were used as positive (ERM-BF410ep) and negative (ERM-BF410ap) controls (reference samples).

Identification of resistance gene carriers was also performed by the PCR method. The analysis was performed in an "Eppendorf Mastercycler pro" amplifier (Germany). Molecular markers to the genes *Lr9* [11,12], *Lr26/Sr31/Yr9/Pm8* [13,14], *Lr34/Yr18/Sr57/Pm38* [15], *Lr35/Sr39* [16] [7], *Sr2* [17] [8], *Sr36* [18] were used. Reaction medium for PCR amplification consisted of the following: 2 µl (50 ng) of the tested DNA, 2 µl of the reaction buffer (10 × TagBuffer), 1 µl dNTP (4 mM), a mixture of four dNTPs, 250 µM each of two primers, 2 µl (25 mM) MgCl2, 0.5 µl (5u/µl) *Taq* polymerase (Biosan Ltd. Novosibirsk, Russia), 10.7 µl of sterile nuclease-free water (Biotechnology Grade).

Amplification products were separated in 1.5-2.0% agarose gels (Sigma Life Science, USA) and 8% acrylamide gel (Sigma Life Science, China) stained with ethidium bromide. Amplification products were visualized in a gel-documentation chamber (QUANTUMST 4, France). DNA markers Step50 plus and Step100 (Biolabmix LLC, Novosibirsk, Russia) were used as molecular weight markers.

Samples from the harvests of 2020 and 2021 were used to analyze grain quality. Grain body was evaluated according to GOST 10840-64 [19], vitreousness - according to GOST 10987-76[20].

Protein content was determined by the Kjeldahl method according to GOST 10846-91[21]. Evaluation of the quantity and quality of gluten in the flour was carried out in the laboratory according to GOST 27839-2013[22]. For gluten washing the MOK-1 system was used, for quality assessment the IDK-4M device (gluten deformation meter) was applied. Mean value (X) and standard deviation (σ) were calculated using the Microsoft Office Excel program.

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RESULTS AND DISCUSSION

GMO Identification. In order to confirm that the samples in question do not contain GMOs, a PCR-analysis for GMI content (according to ST RK1346-2005) was carried out. This method aims to detect regulatory elements that are the most commonly used in genetically engineered constructs: 35S promoter of cauliflower mosaic virus (CaMV 35S) and NOS terminator (from *Agrobacterium tumefaciens*). PCR identification showed that characteristic fragments of 195 bp for 35S and 190 bp for NOS were not found in any of the samples studied. The presence of at least one of them suggests the presence of a gene-modified insertion. Amplification of the 35S and NOS fragments occurred only with HMI-containing products in the reference samples. The results of the HMI identification by PCR in the examination of 19 samples are shown in the table. Figure 1 shows the data of the PCR-analysis of nine samples.

Identification of valuable fungal disease resistance genes. Molecular analysis using PCR method for detection of effective leaf (*Lr*-), stem (*Sr*-) and yellow (*Yr*-) rust genes (*Lr9, Lr26/Sr31/Yr9, Lr34/Yr18/ Sr57, Lr35/Sr39, Lr34, Sr2, Sr36*) in 17 introgressive winter wheat lines and two triticale lines was performed.

Two markers, J13 and SCS5, were used in the PCR analysis for the detection of *Lr9* carriers. It was found that amplification of the expected fragments with lengths of 1100 and 550 bp, respectively, was detected only in the positive control Phyton *Lr9*.

Lr26/Sr31/Yr9/Pm8 genes responsible for resistance to leaf rust, stem rust, yellow rust, and powdery mildew, respectively, are located on the short arm of chromosome 1 of rye and are closely linked to each other. These genes in the wheat-rye translocations 1BL.RS (Petkus), 1AL.1RS (Insave), 1BL.RS and 1DL.1RS (Imperial) were successfully transferred to the cultivars and lines of *Triticum aestivum* L. DNA identification using Iag95 and P6M12 markers was performed to check their presence in the studied samples. Pamirskaya rye variety was used as a positive control sample. The results of the PCR-analysis with the marker P6M12 revealed the presence of 260 and 360-bp fragments in five introgressive wheat lines (1127-7, 1633-31, 1676, 1717-27, 2005-13) and one triticale line (T-409-1) (see Fig. 2, *а*). Amplification of a 1,050-bp long fragment with the Iag95 marker was recorded only for line 1127-7. Analysis based on the two markers made it possible to identify line 1127-7 as a carrier of *Lr26/Sr31/Yr9/Pm8*. The other five lines are carriers only of the *Lr26* gene (see the table).

To detect the presence of *Lr35/Sr39* genes in the studied wheat and triticale lines, a PCR

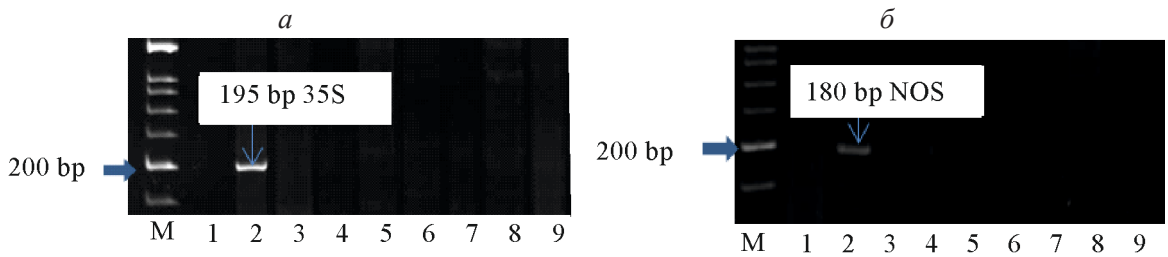

Рис. 1. Результаты идентификации регуляторных элементов 35S и NOS у образцов интрогрессивных линий озимой пшеницы и тритикале:
а – с маркером на 35S; *б* – с маркером на NOS
M – ДНК-маркер Step50 plus (1500 п. н.); 1 – стандартный образец ERMBF410ap (отрицательный контроль); 2 – стандартный образец ERM-BF410ep (положительный контроль); 3 – холостая проба (H_2O); 4 – 1716-24; 5 – 1717-27; 6 – 1723-11; 7 – 2005-13; 8 – 2041-7; 9 – 2041-13

Fig. 1. Results of identification of regulatory elements 35S and NOS in accessions of introgressive lines of winter wheat and triticale:
а – with a marker at 35S; *б* – with a marker on NOS
M – DNA marker Step50 plus (1500 bp); 1 – Standard sample ERMBF410ap (negative control); 2 – Standard sample ERM-BF410ep (positive control); 3 – Blank sample (H_2O); 4 – 1716-24; 5 – 1717-27; 6 – 1723-11; 7 – 2005-13; 8 – 2041-7; 9 – 2041-13

Результаты оценки интрогрессивных линий озимой пшеницы и тритикале по устойчивости к ржавчине и качеству зерна
Results of evaluation of introgressive lines of winter wheat and triticale in terms of rust resistance and grain quality

Catalog number	Origin	GMO of the target	The extent of lesion, type of rust resistance reaction		Fungal disease resistance genes					Natural weight g/l	Vitreousness, %	Protein, %	Gluten, %	FDM, units
		35S/ NOS	leaf	yellow	*Lr9*	*Lr26/ Sr31/Yr9/ Pm8*	*Lr34/ Yr18/ Sr57/ Pm38*	*Lr35/ Sr39*	*Sr2, Sr36*					
1127-7	Przhevalskaya × AD 221-10 Japan	–	0R	0R	–	+	–	–	–	707	71,0	16,3	34,8	80,0
1633-31	(Awnless 1 × *Ae. triaristata Willd*) × Awnless 1	–	5–10R	0R	–	+	–	–	–	774	73,0	16,3	49,4	115,0
1633-40	(Awnless 1 × *Ae. triaristata Willd*) × Awnless 1	–	1–5R	0R	–	–	+	–	–	789	62,0	15,4	47,0	120,0
1674-27	(Zhetysu × *T. kiharae*) × Almaty	–	15–20MR	0R	–	–	–	–	–	825	74,5	13,6	42,4	92,5
1675-149	(Erythrospermum 350 × *T. kiharae*) × Erythrospermum 350	–	20–25MR	0R	–	–	–	–	–	815	80,6	16,4	47,7	106,0
1675-170	(Erythrospermum 350 × *T. kiharae*) × Erythrospermum 350	–	0R	0R	–	–	+	–	–	807	78,0	15,9	44,0	91,6
1676	Vitreous 24 × *T. timopheevii*	–	0R	0R	–	+	–	–	–	762	68,6	15,9	46,1	106,6
1716-23	(Awnless 1 × *Ae. cylindrical*) × Karlygash	–	40–50MS	20–25MR	–	–	–	–	–	770	72,0	13,7	44,0	105,0
1716-24	(Awnless 1 × *Ae. cylindrical*) × Karlygash	–	40–50MS	0R	–	–	–	–	–	790	66,0	17,0	47,0	95,0
1717-27	(Awnless 1 × *Ae. cylindrical*) × Стекловидная 24	–	5–10R	0R	–	+	–	–	–	742	75,0	16,1	42,6	95,0
1723-11	(Awnless 1 × *Ae. cylindrical*) × *T. kiharae*	–	15–20MR	0R	–	–	+	–	–	772	69,5	14,5	43,4	107,5
2005-13	(Erythrospermum 121 × *Ae. triaristata Willd*) × Erythrospermum 121	–	0R	0R	–	+	–	–	–	763	83,0	17,3	49,6	105,0
2041-7	(PEG 347 × *T. kiharae*) × Zhadyra	–	10–15MR	0R	–	–	+	–	–	763	83,0	17,3	49,6	105,0
2041-13	(PEG 347 × *T. kiharae*) × Zhadyra	–	0R	0R	–	–	–	–	–	761	67,0	19,0	59,0	105,0
2046-1	(Vitreous 24 × *T. timopheevii*) × Karlygash	–	0R	0R	–	–	–	–	–	798	63,6	15,1	44,9	110,0
KZ231	(Awnless 1 × *Ae. triaristata Willd*) × Karlygash	–	0R	0R	–	–	–	–	–	785	64,0	16,0	38,6	82,5
Век	6583 × *T. timopheevii*	–	25–30MR	5–10R	–	–	–	–	–	754	63,0	14,2	43,0	120,0
T-409-1	(NAD 508 × AD 206) × Taza	–	0R	0R	–	+	–	–	–	690	55,0	14,0	30,0	95,0
T-989-1	(AD 322 × 119 AD) × Progress × Taza	–	0R	0R	–	–	–	–	–	740	–	14,0	27,7	105,0

analysis using two markers, *Sr39*, BE500705 (marker for the absence of the gene) was performed. Isogenic wheat line *RL5711 Kerber* (with *Sr39* gene) was used as a positive control. Analysis using the dominant SCAR marker *Sr39* showed that the amplification of the expected fragment of 900 bp occurred only in the DNA of the isogenic line *RL5711 Kerber*; it was not detected in all other tested lines (see Fig. 2, *б*). The EST marker BE500705 is a dominant marker that identifies a single 166 bp long band[23] also associated with the original wheat segment (susceptible allele). Therefore, this marker is used to confirm the absence of the *Lr35/Sr39* genes. PCR analysis revealed that a 290 bp fragment was present in all the diagnosed samples, but not 166 bp in length (see Fig. 2, *в*). Some authors [9] use exactly 290 bp fragments to identify the absence of the allele sought. Thus, based on the analysis with two

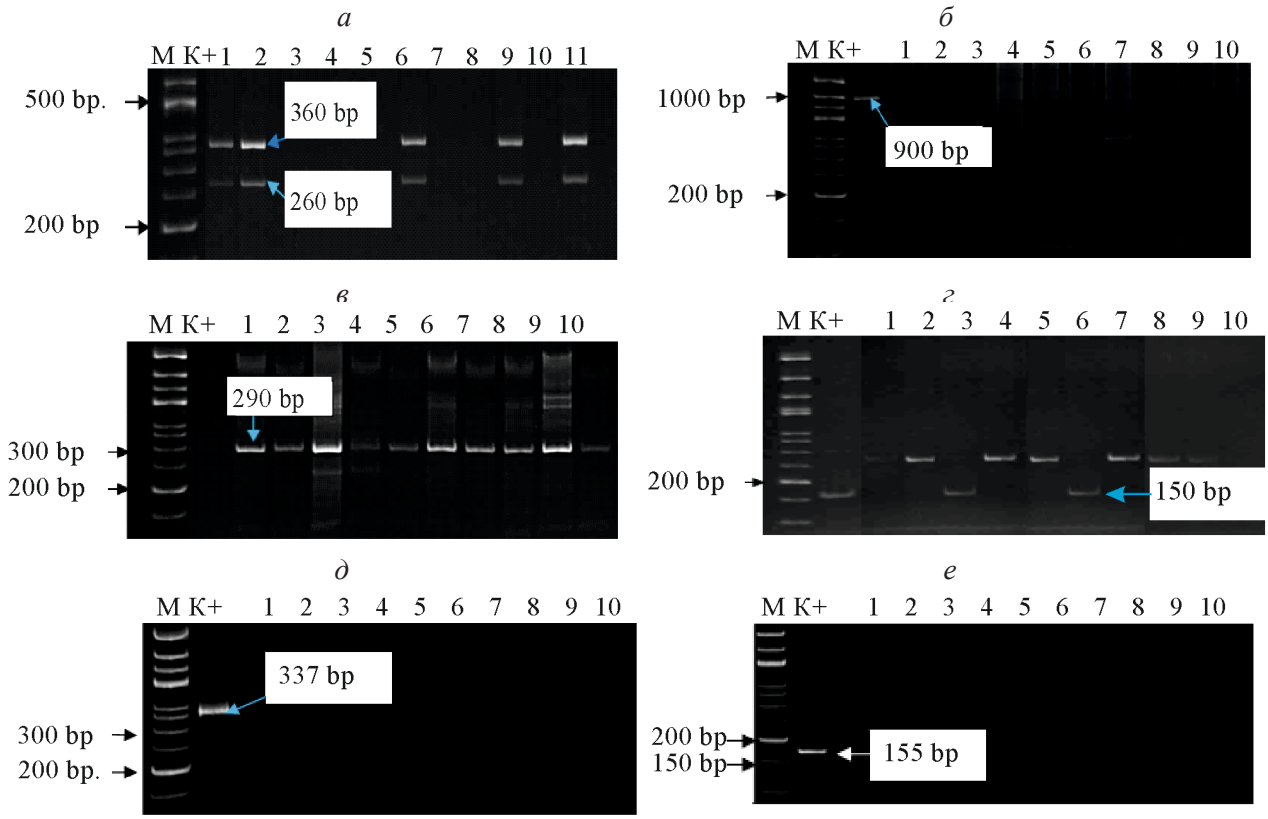

Рис. 2. Продукты амплификации ДНК-образцов интрогрессивных линий пшеницы и тритикале с использованием молекулярных маркеров:
а – идентификация генов *Lr26/Sr31/Yr9* с маркером P6M12; *б* – идентификация генов *Lr35/Sr39* с маркером *Sr39*; *в* – идентификация генов *Lr35/Sr39* с маркером BE500705; *г* – идентификация генов *Lr34/Yr18/Sr57* с маркером *csLV34*; *д* – идентификация гена *Sr2* с маркером CsSr2; *е* – идентификация гена *Sr36* с маркером *Xstm773-2*
М – маркер молекулярных весов Step50; К+ – положительный контроль; 1 – 1127-7; 2 – 1633-31; 3 – 1633-40; 4 – 1674-27; 5 – 1675-149; 6 – 1675-170; 7 – 1676; 8 – 1716-23; 9 – 1716-24; 10 – 1717-27; 11 – 1723-11

Fig. 2. Products of DNA amplification of samples of introgressive lines of wheat and triticale using molecular markers:
a – identification of the *Lr26*/*Sr31*/*Yr9* gene with the P6M12 marker; *б* – identification of the *Lr35*/*Sr39* genes with the *Sr39* marker; *в* – identification of the *Lr35*/*Sr39* genes with the BE500705 marker; *г* – identification of the *Lr34*/*Yr18*/*Sr57* genes with the *csLV34* marker; *д* – identification of the *Sr2* gene with the CsSr2 marker; *е* – identification of the *Sr36* gene with the marker *Xstm773-2*
M – molecular weight marker Step50; K+ – positive control; 1 – 1127-7; 2 – 1633-31; 3 – 1633-40; 4 – 1674-27; 5 – 1675-149; 6 – 1675-170; 7 – 1676; 8 – 1716-23; 9 – 1716-24; 10 – 1717-27; 11 – 1723-11

[23]URL: https://maswheat

markers, the conclusion about the absence of the sought-for alleles of *Lr35/Sr39* genes in the introgressive lines of wheat and triticale.

Lr34/Yr18/Sr57/Pm38 genes were identified using the csLV34 marker. The NIL-Thatcher-Lr34-PI58548 (RL6058) line was used as a positive control sample. The presence of valuable *Lr34/Yr18/Sr57/Pm38* genes was found in the following synthetic wheat samples: 1633-40, 1675-170, 1723-11, 2041-7 (see Fig. 2, *г*; the Table).

To identify the carriers of the *Sr2* gene, the stem rust resistance gene, the CAPS marker CsSr2 was used, which was developed based on the *Sr2* locus and detects three different *Sr2* alleles with high accuracy. *Pavon 76* line was used as a positive control. PCR analysis using the indicated marker showed that there was no amplification in the 19 samples studied (see Fig. 2, *д*; table). According to R. Mago et al. [8], a "null allele" not related to *Sr2* was detected. In the control *Pavon 76* line, after amplification, the BspHI enzyme was cut and three fragments (172, 112, and 53 bp) associated with the presence of *Sr2* were recorded.

Identification of the stem rust resistance gene *Sr36* derived from *Triticum timopheevii* was performed using the marker *Xstm773-2*. An isogenic line with the *Sr36*W2691SrTt-1 gene C1 17385 was used as a positive control. In the process of PCR-analysis of all the samples studied, it was found that the fragment of 155 bp length was present only in the positive control sample (see Fig. 2, *е*).

Phytopathological assessment. A total of 17 introgressive winter wheat lines and two triticale lines were evaluated for resistance to fungal diseases, including brown and yellow rust. No manifestation of stem rust was recorded. According to the results of the field assessment, nine lines (1127-7, 1675-170, 1676, 2005-13, 2041-13, 2046-1, KZ231, T-409-1, T-989-1) were highly resistant to the leaf rust (no symptoms of the disease) (see the table). One line (1633-40) with a stable reaction type (R) was assigned to the resistant group (lesion up to 5%). Moderate resistance (lesion up to 10%, reaction type R) was characterized by two specimens - 1633-31 and 1717-27. Moderate susceptibility (15-30% lesion) was detected in five lines: 1674-27, 1675-149, 1723-11, 2041-7, Century (reaction type MR). The other two samples had medium susceptibility to leaf rust, their lesions were 40-50%, MS reaction type.

All lines except 1716-23 and Vek showed high resistance to yellow rust under field conditions (no symptoms of lesions).

Thus, as a result of screening for resistance to leaf rust and yellow rust, nine samples (1127-7, 1675-170, 1676, 2005-13, 2041-13, 2046-1, KZ231, T-409-1, T-989-1) were noted, which can be recommended for use in organic farming.

Grain and flour quality. Grain quality of the introgressive winter wheat and winter triticale lines was evaluated by grain nature, vitreousness, protein content, gluten, gluten quality and sedimentation.

Studies in 2020, 2021 showed that the samples under study had natures ranging from 690-825 g/l. Most of the collection is classified as strong wheat (not less than 750 g/l) (see the table). Specimens with high grain nature (≥ 800 g/l) are marked out: 1674-27 (825 g/l), 1675-149 (815 g/l), 1675-170 (807 g/l).

Grain vitreousness values ranged from 63-83%, therefore, all introgressive wheat lines were classified as strong wheat (≥ 60%) (see table).

The real value of grain largely depends on its protein content [10]. The total amount of protein in the grain of wheat (on average 12.0-14.0% of grain weight) and its wild relatives can vary within a very wide range - from 9.8 to 30.2% and more, depending on the genotype and growing conditions [11]. There are seven classes of soft wheat according to the protein content: strong (excellent - 16.0%; good - 15.0%; satisfactory - 14.0%), valuable (13.0%), filler (good - 12.0%; satisfactory - 11.0%) and weak (8.0%). Introgressive lines obtained by distant hybridization had very high protein content - in the range of 13.6-19.0%. Of these, nine meet the parameters of strong wheat of the 1st class - excellent improveers (≥ 16.0%): 2041-13 (19.0%), 2005-13 (17.3%), 2041-7 (17.3%), 1716-24 (17.0%), 1675-149 (16.4%), 1127-7 (16.3%), 1633-31 (16.3%), 1717-27 (16.1%), KZ231 (16.0%) (see the table).

Wheat flour gluten is a mass of protein, jelly, which can swell by absorbing water, increase in volume, turn into an elastic formation capable of stretching and springing like rubber. There are seven classes of wheat according to gluten content: strong - improving (excellent - 32.0%; good - 30.0%; satisfactory - 28.0%), valuable (25.0%), filler (good - 24.0%; satisfactory - 22.0%), weak (15.0%) [6].

The results of estimation of gluten content in flour of introgressive winter wheat and triticale lines showed that the average values are within 30,0-49,7% and all the lines under consideration correspond to strong wheat class - excellent improveers (≥ 32,0%) (see the table). The highest values were found in the following lines: 1633-31 (49.4%), 2005-13 (49.6%), 2041-13 (49.0%), 2041-7 (49.3%), 1675-149 (47.7%), 1716-24 (47.0%), 1633-40 (47.0%).

The accepted ranking (FDM units) was used to evaluate the quality of gluten: 45-75 - strong; 40 and 85 - valuable; 35-20 and 90-100 - filler; 105-120 - weak. The quality of gluten in the introgressive lines varies considerably: from 80 to 120 FDM units. There were lines 1127-7 (80,0 FDM units) and KZ231 (82,5 units) of the introgressive lines with indices corresponding to the class of valuable wheat. Lines 1675-170 (91,6 units), 1674-27 (92,5 units), 1717-27 (95,0 units), T-409-1 (95,0 units), 1716-24 (95,0 units) were referred to fillers (see table).

CONCLUSION

PCR-identification of the 17 introgressive winter wheat lines and varieties and 2 triticale lines for the detection of 35S and NOS regulatory elements, which are most commonly used in genetically engineered constructs, showed their absence.

According to the results of the DNA identification for the detection of effective genes of leaf (*Lr-*), stem (*Sr-*) and yellow rust (*Yr-*) (*Lr9, Lr26/Sr31/Yr9*, *Lr34/Yr18/Sr57*, *Lr35/Sr39, Lr34, Sr2, Sr36*) from 19 introgressive winter wheat and triticale lines, four samples (1633-40, 1675-170, 1723-11, 2041-7) with valuable genes *Lr34/ Yr18/Sr57/Pm38* and one sample (1127-7) which is a carrier of *Lr26/Sr31/ Yr9/ Pm8* genes were identified.

On the basis of studying 21 specimens for resistance to rust on natural background, nine specimens resistant to two types of rust (leaf rust and yellow rust) with reaction type R and absence of lesions (1127-7, 1675-170, 1676, 2005-13, 2041-13, 2046-1, KZ231, T-409-1, T-989-1) were noted.

Evaluation of winter wheat and winter triticale introgressive lines in terms of grain quality parameters (naturalness, vitreousness, protein content, gluten content, gluten quality and sedimentation) allowed selecting two specimens (1127-7, KZ231) with high indices corresponding to strong wheat, and in terms of gluten quality - valuable wheat.

According to the results of the complex evaluation, two lines (1127-7, KZ231), distinguished by significant resistance to the two types of rust and high-quality grain. These lines are recommended for use in organic farming.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

(✉)**Ержебаева Р.С.,** кандидат биологических наук, заведующая лабораторией; **адрес для переписки:** Республика Казахстан, 040909, Алматинская область, пос. Алмалыбак, ул. Ерлепесова, 1; e-mail: raushan_2008@mail.ru

Абекова А.М., кандидат сельскохозяйственных наук, ведущий научный сотрудник

Базылова Т.А., научный сотрудник

Масимгазиева А.С., магистр естественных наук, научный сотрудник

Мереева Т.Д., старший лаборант

Кожахметов К., доктор биологических наук, ведущий научный сотрудник

Бастаубаева Ш.О., председатель Правления

Слямова Н.Д., кандидат сельскохозяйственных наук, заведующая лабораторией

AUTHOR INFORMATION

(✉)**Raushan S. Yerzhebayeva,** Candidate of Science in Biology, Laboratory Head; **address:** 1, Yerlepesov St., Almalybak, Almaty region, 040909, Republic of Kazakhstan; e-mail: raushan_2008@mail.ru

Alfiya M. Abekova, Candidate of Science in Agriculture, Lead Researcher

Tamara A. Bazylova, Researcher

Aigerim S. Massimgaziyeva, Master of Science, Researcher

Tolkyn D. Mereyeva, Senior Assistant

Kenebay Kozhakhmetov, Doctor of Science in Biology, Lead Researcher

Sholpan O. Bastaubayeva, Board Chairman

Nazira D. Slyamova, Candidate of Science in Agriculture, Laboratory Head

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ПРАВИЛА ДЛЯ АВТОРОВ

Правила для авторов составлены на основе этических принципов, общих для членов научного сообщества, и правил публикации в международных и отечественных научных периодических изданиях, а также в соответствии с требованиями ВАК для периодических изданий, включенных в Перечень российских рецензируемых научных журналов, в которых должны быть опубликованы основные научные результаты диссертаций на соискание ученой степени доктора и кандидата наук.

Журнал публикует оригинальные статьи по фундаментальным и прикладным проблемам по направлениям:

- общее земледелие и растениеводство;
- селекция, семеноводство и биотехнология растений;
- агрохимия, агропочвоведение, защита и карантин растений;
- кормопроизводство;
- инфекционные болезни и иммунология животных;
- частная зоотехния, кормление, технологии приготовления кормов и производства продукции животноводства;
- разведение, селекция, генетика и биотехнология животных;
- технологии, машины и оборудование для агропромышленного комплекса;
- пищевые системы.

Статья, направляемая в редакцию, должна соответствовать тематическим разделам журнала «Сибирский вестник сельскохозяйственной науки»:

Наименование рубрики	**Шифр и наименование научной специальности в соответствии с Номенклатурой научных специальностей, по которым присуждаются ученые степени**
Земледелие и химизация	4.1.1. Общее земледелие и растениеводство 4.1.3. Агрохимия, агропочвоведение, защита и карантин растений
Растениеводство и селекция	4.1.1. Общее земледелие и растениеводство 4.1.2. Селекция, семеноводство и биотехнология растений
Защита растений	4.1.3. Агрохимия, агропочвоведение, защита и карантин растений
Кормопроизводство	4.1.1. Общее земледелие и растениеводство 4.1.2. Селекция, семеноводство и биотехнология растений 4.1.3. Агрохимия, агропочвоведение, защита и карантин растений
Зоотехния и ветеринария	4.2.3. Инфекционные болезни и иммунология животных 4.2.4. Частная зоотехния, кормление, технологии приготовления кормов и производства продукции животноводства 4.2.5. Разведение, селекция, генетика и биотехнология животных
Механизация, автоматизация, моделирование и информационное обеспечение	4.3.1. Технологии, машины и оборудование для агропромышленного комплекса
Переработка сельскохозяйственной продукции	4.3.3. Пищевые системы
Проблемы. Суждения Научные связи Из истории сельскохозяйственной науки Краткие сообщения Из диссертационных работ	4.1.1. Общее земледелие и растениеводство 4.1.2. Селекция, семеноводство и биотехнология растений 4.1.3. Агрохимия, агропочвоведение, защита и карантин растений 4.2.3. Инфекционные болезни и иммунология животных 4.2.4. Частная зоотехния, кормление, технологии приготовления кормов и производства продукции животноводства 4.2.5. Разведение, селекция, генетика и биотехнология животных 4.3.1. Технологии, машины и оборудование для агропромышленного комплекса 4.3.3. Пищевые системы

В журнале также публикуются обзоры, краткие сообщения, хроника, рецензии, книжные обозрения, материалы по истории сельскохозяйственной науки и деятельности учреждений и ученых.

Число публикаций одного автора в номере журнала не должно превышать двух, при этом вторая статья допустима лишь в соавторстве.

К рассмотрению принимаются материалы от различных категорий исследователей, аспирантов, докторантов, специалистов и экспертов в соответствующих областях знаний.

Все статьи рецензируются и имеют зарегистрированный в системе CrossRef индекс DOI.

Публикации для авторов **бесплатны**.

При направлении статьи в редакцию журнала «Сибирский вестник сельскохозяйственной науки» рекомендуем руководствоваться следующими правилами.

РЕКОМЕНДАЦИИ АВТОРУ ДО ПОДАЧИ СТАТЬИ

Представление статьи в журнал «Сибирский вестник сельскохозяйственной науки» подразумевает, что:

- статья ранее не была опубликована в другом журнале;
- статья не находится на рассмотрении в другом журнале;
- все соавторы согласны с публикацией текущей версии статьи.

Перед отправкой статьи на рассмотрение необходимо убедиться, что в файле (файлах) содержится вся необходимая информация на русском и английском языках, указаны источники информации, размещенной на рисунках и в таблицах, все ссылки оформлены корректно.

ПОРЯДОК НАПРАВЛЕНИЯ РУКОПИСЕЙ СТАТЕЙ

1. Отправка статьи осуществляется через электронную редакцию на сайте журнала **https://sibvest.elpub.ru/jour/index.** После предварительной регистрации автора, в правом верхнем углу страницы выбрать опцию «Отправить рукопись». Затем загрузить рукопись статьи (в формате *.doc или *.docx) и сопроводительные документы к ней. После завершения загрузки материалов обязательно выбрать опцию «Отправить письмо», в этом случае редакция автоматически будет уведомлена о получении новой рукописи.

Сопроводительные документы к рукописи статьи:

- скан-копия письма от организации с подтверждением авторства и разрешением на публикацию (образец на **http://sibvest.elpub.ru/**);
- скан-копия авторской справки по представленной форме (образец на **http://sibvest.elpub.ru/**), в которой должно быть выражено согласие на открытое опубликование статьи в печатном варианте журнала и его электронной копии в сети Интернет;
- скан-копия рукописи с подписями авторов. Автор, подписывая рукопись и направляя ее в редакцию, тем самым передает авторские права на издание этой статьи СФНЦА РАН;
- анкеты авторов на русском и английском языках (образец на **http://sibvest.elpub.ru/**);
- скан-копия справки из аспирантуры (для очных аспирантов).

2. Все поступающие в редакцию рукописи статей регистрируются через систему электронной редакции. В личном кабинете автора отражается текущий статус рукописи.

3. Нерецензируемые материалы (материалы научной хроники, рецензии, книжные обозрения, материалы по истории сельскохозяйственной науки и деятельности учреждений и ученых) направляются на e-mail: **sibvestnik@sfsca.ru** и регистрируются ответственным секретарем.

ПОРЯДОК ОФОРМЛЕНИЯ СТАТЬИ

Текст рукописи оформляется шрифтом Times New Roman, кеглем 14 с интервалом 1,5, все поля 2,0 см, нумерация страниц внизу. Объем статьи не более 15 страниц (включая таблицы, иллюстрации и библиографию); статей, размещаемых в рубриках «Из диссертационных работ» и «Краткие сообщения», – не более 7 страниц.

Структура оформления статьи:

1. **УДК**
2. **Заголовок статьи на русском и английском языках (не более 70 знаков).**
3. **Фамилии и инициалы авторов, полное официальное название научного учреждения, в котором проведены исследования на русском и английском языках.**

Если в подготовке статьи принимали участие авторы из разных учреждений, необходимо указать принадлежность каждого автора к конкретному учреждению с помощью надстрочного индекса.

4. **Реферат на русском и английском языках.** Объем реферата не менее 200–250 слов. Реферат является кратким и последовательным изложением материала статьи по основным разделам и должен отражать основное содержание, следовать логике изложения материала и описания результатов в статье с приведением конкретных данных. Не следует включать впервые введенные термины, аббревиатуры (за исключением общеизвестных), ссылки на литературу. В реферате не следует подчеркивать новизну, актуальность и личный вклад автора; место исследования необходимо указывать до области (края), не упоминать конкретные организации.
5. **Ключевые слова на русском и английском языках.** 5–7 слов по теме статьи. Желательно, чтобы ключевые слова дополняли реферат и название статьи.
6. **Информация о конфликте интересов либо его отсутствии.** Автор обязан уведомить редактора о реальном или потенциальном конфликте интересов, включив информацию о конфликте интересов в соответствующий раздел статьи. Если конфликта интересов нет, автор должен также сообщить об этом.

Пример формулировки: «Автор заявляет об отсутствии конфликта интересов».

7. **Благодарности на русском и английском языках.** В этом разделе указываются все источники финансирования исследования, а также благодарности людям, которые участвовали в работе над статьей, но не являются ее авторами.
8. **Основной текст статьи.** При изложении оригинальных экспериментальных данных рекомендуется использовать подзаголовки:

ВВЕДЕНИЕ (постановка проблемы, цели, задачи исследования)

МАТЕРИАЛ И МЕТОДЫ (условия, методы (методика) исследований, описание объекта, место и время проведения)

РЕЗУЛЬТАТЫ И ОБСУЖДЕНИЕ

ЗАКЛЮЧЕНИЕ или **ВЫВОДЫ**

СПИСОК ЛИТЕРАТУРЫ. Количество источников не менее 15. В список литературы включаются только рецензируемые источники: статьи из научных журналов и монографии. Самоцитирование не более 10% от общего количества. Библиографический список должен быть оформлен в виде общего списка в порядке упоминания в тексте, желательны ссылки на источники 2–3-летнего срока давности. Правила оформления списка литературы – в соответствии с ГОСТ Р 7.05–2008 (требования и правила составления библиографической ссылки). В тексте ссылка на источник отмечается порядковой цифрой в квадратных скобках, например [1]. Литература в списке дается на тех языках, на которых она издана. В библиографическое описание публикации необходимо вносить всех авторов, не сокращая их одним, тремя и т.п. Недопустимо сокращение названий статей, журналов, издательств.

Если необходимо сослаться на авторефераты, диссертации, сборники статей, учебники, рекомендации, учебные пособия, ГОСТы, информацию с сайтов, статистические отчеты, статьи в общественно-политических газетах и прочее, то такую информацию следует оформить в *сноску* в конце страницы. Сноски нумеруются арабскими цифрами, размещаются постранично сквозной нумерацией.

Внимание! Теоретические, обзорные и проблемные статьи могут иметь произвольную структуру, но обязательно должны содержать реферат, ключевые слова, список литературы.

ПРИМЕРЫ ОФОРМЛЕНИЯ СПИСКА ЛИТЕРАТУРЫ, REFERENCES И СНОСОК

СПИСОК ЛИТЕРАТУРЫ:

Монография

Климова Э.В. Полевые культуры Забайкалья: монография. Чита: Поиск, 2001. 392 с.

Часть книги

Холмов В.Г. Минимальная обработка кулисного пара под яровую пшеницу при интенсификации земледелия в южной лесостепи Западной Сибири // Ресурсосберегающие системы обработки почвы. М.: Агропромиздат, 1990. С. 230–235.

Периодическое издание

Пакуль А.Л., Лапшинов Н.А., Божанова Г.В., Пакуль В.Н. Технологические качества зерна мягкой яровой пшеницы в зависимости от системы обработки почвы // Сибирский вестник сельскохозяйственной науки. 2018. Т. 48. № 4. С. 27–35. DOI: 10.26898/0370-8799-2018-4-4.

REFERENCES:

Составляется в том же порядке, что и русскоязычный вариант, по следующим правилам:

Фамилии И.О. авторов в устоявшемся способе транслитерации, англоязычное название статьи, *транслитерация названия русскоязычного источника (например через сайт: https://antropophob.ru/translit-bsi) = англоязычное название источника.* Далее оформление для монографии: город, англоязычное название издательства, год, количество страниц; для журнала: год, номер, страницы). (In Russian).

Пример: Avtor A.A., Avtor B.B., Avtor C.C. Title of article.

Транслитерация авторов. Англоязычное название статьи

Zaglavie jurnala = Title of Journal, 2012, vol. 10, no. 2, pp. 49–54.

Транслитерация источника = Англоязычное название источника

Монография

Klimova E.V. *Field crops of Zabaikalya*. Chita, Poisk Publ., 2001, 392 p. (In Russian).

Часть книги

Kholmov V.G. Minimum tillage of coulisse-strip fallow for spring wheat with intensification of arable agriculture in southern forest-steppe of Western Siberia. *Resource-saving tillage systems*, Moscow, Agropromizdat Publ., 1990, pp. 230–235. (In Russian).

Периодическое издание

Pakul A.L., Lapshinov N.A., Bozhanova G.V., Pakul V.N. Technological grain qualities of spring common wheat depending on the system of soil tillage. *Sibirskii vestnik sel'skokhozyaistvennoi nauki = Siberian Herald of Agricultural Science*, 2018, vol. 48, no. 4, pp. 27–35. (In Russian). DOI: 10.26898/0370-8799-2018-4-4.

СНОСКИ:

Цитируемый текст[1].

[1]*Климова Э.В., Андреева О.Т., Темникова Г.П.* Пути стабилизации кормопроизводства Забайкалья // Проблемы и перспективы совершенствования зональных систем земледелия в современных условиях: материалы науч.-практ. конф. (Чита, 16–17 октября 2008 г.). Чита, 2009. С. 36–39.

Цифровой идентификатор Digital Object Identifier – DOI (когда он есть у цитируемого материала) необходимо указывать в конце библиографической ссылки.

Пример:

Chu T., Starek M.J., Brewer M.J., Murray S.C., Pruter L.S. Assessing lodging severity over an experimental maize (Zea mays L.) field using UAS images // Remote Sensing. 2017. Vol. 9. P. 923. DOI: 10.3390/rs9090923.

Наличие DOI статьи следует проверять на сайте http://search.crossref.org/ или https://www.citethisforme.com. Для этого нужно ввести в поисковую строку название статьи на английском языке.

РИСУНКИ, ТАБЛИЦЫ, СКРИНШОТЫ И ФОТОГРАФИИ

Рисунки должны быть хорошего качества, пригодные для печати. Все рисунки должны иметь подрисуночные подписи. Подрисуночную подпись необходимо перевести на английский язык. Рисунки нумеруются арабскими цифрами по порядку следования в тексте. Если рисунок в тексте один, то он не нумеруется. Отсылки на рисунки оформляются следующим образом: «На рис. 3 указано, что …» или «Указано, что … (см. рис. 3)». Подрисуночная

подпись включает порядковый номер рисунка и его название. «Рис. 2. Описание жизненно важных процессов». Перевод подрисуночной подписи следует располагать после подрисуночной подписи на русском языке.

Таблицы должны быть хорошего качества, пригодные для печати. Предпочтительны таблицы, пригодные для редактирования, а не отсканированные или в виде рисунков. Все таблицы должны иметь заголовки. Название таблицы должно быть переведено на английский язык. Таблицы нумеруются арабскими цифрами по порядку следования в тексте. Если таблица в тексте одна, то она не нумеруется. Отсылки на таблицы оформляются следующим образом: «В табл. 3 указано, что …» или «Указано, что … (см. табл. 3)». Заголовок таблицы включает порядковый номер таблицы и ее название: «Табл. 2. Описание жизненно важных процессов». Перевод заголовка таблицы следует располагать после заголовка таблицы на русском языке.

Фотографии, скриншоты и другие нерисованные иллюстрации необходимо загружать отдельно в виде файлов формата *.jpeg (*.doc и *.docx – в случае, если на изображение нанесены дополнительные пометки). Разрешение изображения должно быть >300 dpi. Файлам изображений необходимо присвоить название, соответствующее номеру рисунка в тексте. В описании файла следует отдельно привести подрисуночную подпись, которая должна соответствовать названию фотографии, помещаемой в текст.

Следует обратить внимание на написание формул в статье. Во избежание путаницы необходимо греческие (α, β, π и др.), русские (А, а, Б, б и др.) буквы и цифры писать прямым шрифтом, латинские – курсивным (*W*, *Z*, *m*, *n* и др.). Математические знаки и символы нужно писать также прямым шрифтом. Необходимо четко указывать верхние и нижние надстрочные символы (W^1, F_1 и др.).

ВЗАИМОДЕЙСТВИЕ МЕЖДУ ЖУРНАЛОМ И АВТОРОМ

Редакция просит авторов при подготовке статей руководствоваться изложенными выше правилами.

Все поступающие в журнал «Сибирский вестник сельскохозяйственной науки» статьи проходят предварительную проверку на соответствие формальным требованиям. На этом этапе редакция оставляет за собой право:

- принять статью к рассмотрению;
- вернуть статью автору (авторам) на доработку с просьбой устранить ошибки или добавить недостающие данные;
- вернуть статью автору (авторам) без рассмотрения, оформленную не по требованиям журнала;
- отклонить статью из-за несоответствия ее целям журнала, отсутствия оригинальности, малой научной ценности.

Переписка с авторами рукописи ведется через контактное лицо, указанное в рукописи.

Все научные статьи, поступившие в редакцию журнала «Сибирский вестник сельскохозяйственной науки», проходят обязательное двухстороннее «слепое» рецензирование (double-blind – автор и рецензент не знают друг о друге). Рукописи направляются по профилю научного исследования на рецензию членам редакционной коллегии.

В спорных случаях редактор может привлечь к процессу рецензирования нескольких специалистов, а также главного редактора. При положительном заключении рецензента статья передается редактору для подготовки к печати.

При принятии решения о доработке статьи замечания и комментарии рецензента передаются автору. Автору дается 2 месяца на устранения замечаний. Если в течение этого срока автор не уведомил редакцию о планируемых действиях, статья снимается с очереди публикации.

При принятии решения об отказе в публикации статьи автору отправляется соответствующее решение редакции.

Ответственному (контактному) автору принятой к публикации статьи направляется финальная версия верстки, которую он обязан проверить.

ПОРЯДОК ПЕРЕСМОТРА РЕШЕНИЙ РЕДАКТОРА/РЕЦЕНЗЕНТА

Если автор не согласен с заключением рецензента и/или редактора или отдельными замечаниями, он может оспорить принятое решение. Для этого автору необходимо:

- исправить рукопись статьи согласно обоснованным комментариям рецензентов и редакторов;
- ясно изложить свою позицию по рассматриваемому вопросу.

Редакторы содействуют повторной подаче рукописей, которые потенциально могли бы быть приняты, однако были отклонены из-за необходимости внесения существенных изменений или сбора дополнительных данных, и готовы подробно объяснить, что требуется исправить в рукописи для того, чтобы она была принята к публикации.

ДЕЙСТВИЯ РЕДАКЦИИ В СЛУЧАЕ ОБНАРУЖЕНИЯ ПЛАГИАТА, ФАБРИКАЦИИ ИЛИ ФАЛЬСИФИКАЦИИ ДАННЫХ

Редакция научного журнала «Сибирский вестник сельскохозяйственной науки» в своей работе руководствуется традиционными этическими принципами научной периодики и сводом принципов «Кодекса этики научных публикаций», разработанным и утвержденным Комитетом по этике научных публикаций, требуя соблюдения этих правил от всех участников издательского процесса.

ИСПРАВЛЕНИЕ ОШИБОК И ОТЗЫВ СТАТЬИ

В случае обнаружения в тексте статьи ошибок, влияющих на ее восприятие, но не искажающих изложенные результаты исследования, они могут быть исправлены путем замены pdf-файла статьи. В случае обнаружения в тексте статьи ошибок, искажающих результаты исследования, либо в случае плагиата, обнаружения недобросовестного поведения автора (авторов), связанного с фальсификацией и/или фабрикацией данных, статья может быть отозвана. Инициатором отзыва статьи может быть редакция, автор, организация, частное лицо. Отозванная статья помечается знаком «Статья отозвана», на странице статьи размещается информация о причине отзыва статьи. Информация об отзыве статьи направляется в базы данных, в которых индексируется журнал.

УВАЖАЕМЫЕ ПОДПИСЧИКИ!

Подписку на журнал «Сибирский вестник сельскохозяйственной науки»
(как на годовой комплект, так и на отдельные номера)
можно оформить одним из следующих способов:

– в агентстве подписки ГК «Урал-Пресс» по индексу 014973. Ссылка на издание https://www.ural-press.ru/catalog/97210/8707659/?sphrase_id=392975. В разделе контакты зайти по ссылке http://ural-press.ru/contact/, где можно выбрать филиал по месту жительства;
– в редакции журнала (телефон 7-383-348-37-62; e-mail: sibvestnik@sfsca.ru).

Полнотекстовая версия журнала
«Сибирский вестник сельскохозяйственной науки»
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