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LEAF-SPOTTING DISEASES AS A MATTER OF DAMAGE OF ALFALFA BREEDING POPULATIONS IN AN EVIDENT MULTIFOLIATE PHASE IN DIFFERENT CYCLES OF PHENOTYPIC RECURRENT SELECTION

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Abstract. Alfalfa (Medicago varia Mart.) is the most important forage crop for considerable amount of regions of the Russian Federation and throughout the entire world. Assessment of the stability and resistance of alfalfa varieties to a complex of leaf-spotting diseases under various environmental conditions is important for breeding programs as a tool for selecting the best varieties and breeding samples. In the course of a three-year experiment (2016-2018) there were tested the productivity and resistance to a complex of leaf spotting diseases of a commercial variety Krasnoyaruzhskaya 1 and breeding populations with high multifoliate expression (evident multi-leaf phase – mf - multi-leaf mutations) obtained on its basis in several cycles (I-III) of periodic (recurrent) selection. Six alfalfa-breeding populations were tested in two ecotopes on two soil differences: plot 1 - ecotope of field crop rotation; plot 2 – ecotope of a plowed meadow in the floodplain of the river Oskol in Chernyansky district, Belgorod region (meadow ecotope). The data obtained were analyzed by the method of two-way analysis of variance (ANOVA) according to a 2x4 scheme with the calculation of LSD 0.05. There were evaluated organized factors: factor A — selection feature: A1 (G) – high multifoliate expression (mf-mutation) without the manifestation of anthocyanin color of the stem (green color of the stem); A2 (AN) – high multifoliate expression (mf-mutation) with an evident anthocyanin color of the stem (purple color of the stem); factor B – a recurrence selection cycle: C-0, C-1, C-2, C-3. Pearson's correlation coefficient was used to identify the close relationships between the studied indicators. It has been established that three cycles of recurrent selection are effective for increasing multifoliate expression in alfalfa breeding populations without increasing inbreeding depression based on seed productivity. Multifoliate forms are resistant to the development of leaf-spotting diseases and their prevalence. The most stable are the forms in which selection for the expression of the mf-mutation was carried out simultaneously with the increase in the index of the anthocyanin color of the stem. For a more effective assessment of the resistance of alfalfa breeding populations to leaf-spotting in Central Russia, comparative testing must be carried out simultaneously under conditions of favorable ecotopes of field crop rotation with fertile black soil rich in humus (chernozem) and in adverse conditions of meadow ecotopes.
Keywords: multifoliate expression, anthocyanin color, green coloration, ecotopes, disease prevalence, degree of disease development, seed yield.

1. Introduction
Alfalfa is one of the most important forage crops cultivated throughout the entire world. It is an almost ideal crop for milk production and livestock breeding because it has high value and biomass yield [Béguier et al., 2018; Dumacheva et al., 2018, Chernyavskikh et al., 2019 a].

The most difficult is to obtain seeds out of this culture, since it is, firstly, a cross-pollinated autotetraploid, and secondly, when cultivated that plant's seeds can be affected by a large number of diseases [Kimbeng et al., 2000; Abbasi et al., 2018; Chernyavskikh et al., 2018; Leyronas et al., 2018].

Leaf spotting is particularly harmful – they are necrotic leaf lesions visually similar to each other, but caused by various pathogens, such as Uromyces striatus Schrot., Pseudopeziza medicaginis (Lib.) Sacc., P. jonesii Nannf., Peronospora aestivalis Syd., Phoma medicaginis Malbr. & Roum. var. medicaginis, Erysiphe pisi DC., Colletotrichum destructivum, C. coccodes, C. trifolii, C. dematium, C. truncatum, C. linicola, C. americae-borealis [Gui Zhi et al., 2016; Li et al., 2018; Salunkhe et al., 2018; Wang et al., 2018].

Most modern varieties are synthetic populations obtained by crossing different genotypes. At present studies are being conducted to the reaction of varieties and breeding lines to resistance to the main types of diseases, the development of methods for such resistance, and the reactions of M. varia populations to a decrease in the genetic basis associated with breeding programs in various areas: protein increase, foliage increase, increase of digestibility, adaptation to certain conditions, etc. [Nutter et al., 2002; O'Neill et al., 2003; Samac, 2008; Laxman Adhikari et al., 2019].

A worldwide trend is the individualization of varieties according to their adaptability to various ecotope conditions, reactions to salinization, nutrients, irrigation, drought, etc. [Shao, 2018; Moghaddam et al., 2010; Gui Zhi et al., 2016; Shi Shangli et al., 2017; Meng Kong et al., 2020].

One of the directions of alfalfa selection is the creation of multifoliate varieties, which are associated with the possibility of improving the quality of alfalfa forage mass, foliage, and other important fodder indicators [Bingham, Murphy, 1965; Dumacheva et al., 2018].

The multi-leaf mutation is a recessive one, controlled by the recessive gene (mj) and two other genes that influence its manifestation. Along with the indisputable advantages of alfalfa, it is feared that as a result of its use, there may be problems with reducing the genetic diversity of varieties, reducing their resistance to pests and diseases, inbreeding depression, loss of adaptability to various conditions, etc. Along with the breeding programs for creating multifoliate (multi-leaf) varieties, open up new useful prospects for the use of this mutation. Phenotypic recurrent selection has a definite perspective [Petkova 2010; Odorizzi et al., 2015, 2018; Popescu et al., 2016; Chernyavskikh et al., 2019 b].

The purpose of this study was to test the seed productivity and the resistance to a complex of leaf-spotting diseases of alfalfa populations obtained during three cycles of recurrent selection of forms with high expression of the mf-mutation in comparison with the original commercial variety Krasnoyaruzhskaya 1.

2. Methods and materials
The productivity and resistance to the complex of leaf- spotting diseases were tested for the commercial variety Krasnoyruzhskaya 1 and breeding populations with high multifoliate expression (mf-mutations) obtained on its basis during several (I-III) cycles of periodic (recurrent) selection.

The choice of the commercial variety Krasnoyruzhskaya 1 as the initial one for recurrent selection is due to the fact that it is one of the few Russian varieties with multifoliate expression. The variety is characterized by aligned grass stand, high occurrence of multi-leafed forms, and fast regrowth after mowing, high winter hardiness, high disease resistance, very low occurrence of dark-colored and variegated flowers, dark green with an evident bluish tint of leaves [Krasnoyruzhskaya 1, 2016].

Individual selection of forms with high multifoliate expression (mf-mutations) was carried out by the method of vegetative propagation in two main directions:

- selection of forms with an index of multifoliate expression (mf–mutation) not lower than 4.5, and an index of brightness of anthocyanin color of the stem not higher than 0.2 (green color of the stem);

- selection of forms with an index of multifoliate expression (mf–mutation) of at least 4.5, and an index of brightness of anthocyanin color of the stem at least 4.5 (purple color of the stem).

The expression index (mf-mutation) of multi-leaf is calculated as the quotient of the product of the number of shoots in each mf category and the total number of stems examined. Mf categories: 0 – no mutation, 1 – 1 mf-leaf per 1 stem, 2 – 2-3 mf-leaves per 1 stem, 3 – 4-5 mf-leaves per 1 stem, 4 – 6-7 mf-leaves per 1 stem, 5 – more than 8 mf leaves per 1 stem according to the standard method [Sheaffer et al., 1995].

The manifestation index of anthocyanin color of the stem (the index of brightness of anthocyanin color) was determined according to a scale, where 0 - is absence of coloration, 1 – coloration of the base of the petiole, 2 – coloration of the base of the stem, 3 – coloration of the stem except for petioles, 4 – coloration all over the stem, 5 – coloration of the stem and the petiole.

Breeding populations were created for further pollination based on the selected forms. In total, three cycles of recurrent selection were carried out according to two main breeding characters.

Basic information about the tested samples is presented in table 1.

Table 1. Features of the tested breeding populations of alfalfa (*M. varia*)

№	Selection number	Recursion selection cycle	Code	Selection feature
1	Krasnoyruzhskaya 1 (ST)	Original	C-0	Low multifoliate expression (mf-mutation) with a medium evident anthocyanin color of the stem (green-purple color of the stem (G-AN))
2	K-1/10mf (1 G)	I	C-1 G	High multifoliate expression (mf-mutation) without manifestation of the anthocyanin color of the stem (green color of the stem (G))
3	K-1/23mf (2 G)	II	C-2 G	
4	K-1/06 mf (3 G)	III	C-3 G	
5	K-1/17mf (1AN)	I	C-1 AN	High multifoliate expression (mf-mutation) with an evident anthocyanin color of the stem (purple color of the stem (AN))
6	K-1/41mf (2 AN)	II	C-2 AN	
7	K-1/15 mf (3 AN)	III	C-3 AN	

Alfalfa breeding populations were tested in two ecotopes on two soil differences:

- plot 1 – ecotope of field crop rotation;
- plot 2 – ecotope of a plowed meadow in the floodplain of the river Oskol in Chernyansky district, Belgorod region (meadow ecotope).

The geographical and environmental features of the research sites are shown in table 2.

Table 2. Features of the research sites

Features	Plot 1	Plot 2
Coordinates	50°59'04.1"N, 37°43'49.2"E	50°57'42.4"N, 37°47'40.1"E
Soil	Typical heavy loamy black soil rich in humus (chernozem) in field crop rotation (TBS)*	Meadow-gley light-loamy, on a plowed meadow in the floodplain of the river Oskol (GL)**

P_2O_5, mg*kg^{-1}	235	88
K_2O, mg*kg^{-1}	292	81
pH_{sol}	6.5	5.4
Humus, %	5.1	2.9
Rainfall mm / year		
2016	741 (ratio to the long-term average norm of 127.3 %)	
2017	520 (ratio to the long-term average rate of 89.3 %)	
2018	697(ratio to the long-term average norm of 119.7 %)	
Monthly average air temperature, °C		
2016	7.6 (deviation from mean annual norm +0.2)	
2017	8.4 (deviation from the mean annual norm +0.9)	
2018	7.7 (ratio to the long-term average norm of +0.2)	

TBS – typical black soil
**GL –gleysol

The amount of precipitation and average monthly air temperature are presented according to the weather station in town of Novy Oskol.

The content of mobile compounds of phosphorus (P_2O_5) and potassium (K2O) in the soil was determined by extracting them from the soil with a solution of acetic acid at a concentration of 0.5 mol * $(dm^3)^{-1}$ with a soil to solution ratio of 1:25 and subsequent determination of phosphorus in the form of blue phosphorus-molybdenum complex on a photoelectrocolorimeter and potassium – on a flame photometer (Soils. Determination of mobile compounds of phosphorus and potassium by Chiricov method modified by CINAO (GOST 26204-91)).

Determination of pH_{sol} was carried out by extracting exchange cations, nitrates and mobile sulfur from the soil with a solution of potassium chloride with a concentration of 1 mol * $(dm^3)^{-1}$ (1 N) with a soil to solution ratio of 1:25 with potentiometric determination of pH_{sol} using a glass electrode.

Determination of the humus content in the soil was carried out by the method of oxidation of organic matter with a solution of potassium dichromate in sulfuric acid and the subsequent determination of trivalent chromium, equivalent to the content of organic matter in a photoelectric colorimeter (Soils. Methods for determination of organic matter GOST 26213-91).

The studies were conducted in the years 2015-2018 in the pilot areas of the Krasnoyruzhskaya Grain Company JSC in the Belgorod Region. Experience is laid in a standard way. The area of the accounting plot is 2 m^2. Four repetition. Two-row plots. The row spacing in the plot is 25 cm, between the plots 45 cm. The standard is laid out after 4 plots. Seeds were sown at the rate of 100 pieces per 1 linear meter.

For seed purposes, the second mowing was used. Assessment of seed productivity was carried out on each plot by continuous mowing. Harvest dates: third decade of August – first decade of September. Plants were dried to an air-dry state and threshed in a laboratory sheaf thresher. Bringing to sowing conditions was carried out on manual laboratory sieves.

The degree of development of diseases (average leaf affection) (DDD) and the spread of diseases (the number of stems affected by spotting) (DS – disease spreading) were determined during the period of complete fruit formation in each plot by examining 200 stems.

Assessment of the degree of development was carried out on a 4-point scale: 0 – no disease; 1 – up to 10 % of the leaf surface is stained; 2 – up to 25 %; 3 – up to 50 %; 4 – more than 50 %. The average score for each plot was calculated as the arithmetic average of the weighted lesions by plant leaf diseases in the samples according to the formula:

$DDD = \sum (a * b) / N$, where

DDD – the degree of development of diseases;

∑ (a * b) - the sum of the products of the number of stems affected by the corresponding leaf damage score;

N – the total number of analyzed stems.

Disease spreading was calculated as the percentage of stems affected in the total sample according to the formula:

DS = n * 100 / N

DS – disease spreading;

N – the total number of plants in the samples;

n – the number of affected plants

The data obtained were analyzed by the method of two-way analysis of variance (ANOVA) according to a 2x4 scheme with the calculation of LSD 0.05.

Organized factors were evaluated: factor A - breeding feature:

A1 – G – high multifoliate expression (mf-mutation) without manifestation of the anthocyanin color of the stem (green color of the stem);

A2 – AN – high multifoliate expression (mf-mutation) with an evident anthocyanin color of the stem (purple color of the stem).

Factor B – recurrence selection cycle: C-0, C-1, C-2, C-3.

Pearson's correlation coefficient was used to identify the close relationships between the studied indicators. [Dospekhov, 2012].

3. Results and discussion

At the first stage of research, we compared the effects of two habitats (field crop rotation with chernozem and a plowed meadow with meadow gley soil) on the main productive traits. It was established that the influence of habitats in the total variance of the studied productive traits in the studied alfalfa selection samples occupies the most significant share compared to the influence of the year conditions: for SY – 90.4% (Ff / F st 0.05 = 130.9); for DDD – 59.5% (Ff / F st 0.05 = 70.1); for DS – 76.4% (Ff / F st 0.05 = 474.3).

The influence of the conditions of the year on the main productive indicators ranged from 0.1 to 8.18 % and was insignificant ($p>0.05$).

Thus, it is clear that it was necessary to evaluate the studied breeding samples in various environmental conditions in order to learn data that are more objective.

Under favorable conditions of chernozem soils, regularities were established for the manifestation of the main characteristics of breeding populations in three cycles of recurrent selection in comparison with the original variety (Table 3).

Table 3. Comparison of the average of various indicators for breeding populations of alfalfa (M. *varia*) with multifoliate expression between selection cycles (from C-1 to C-3) and the original variety (C-0). The studies were conducted in the years 2016-2018 in the ecotope of field crop rotation with fertile black soil rich in humus (chernozem) or typical black soil (TBS) (Chernyansky district, Belgorod region, Russia)

Indicators	Breeding feature	Cycle of recurrent selection				$LSD_{0.05}$
		C-0	C-1	C-2	C-3	
SY, $g*(m^2)^{-1}$	G	47.8	63.7	59.1	55.8	10.9
	AN		60.7	55.2	54.7	
DDD, %	G	4.2	3.3	3.9	3.7	1.2
	AN		3.0	2.7	2.3	
DS, %	G	8.5	7.8	7.6	7.1	1.0
	AN		6.6	6.2	6.8	
EI mf	G	0.7	1.5	1.9	2.2	0.4

	AN		1.8	2.7	3.0	
ASI	G	1.8	1.7	1.0	0.8	0.1
	AN		3.0	3.7	4.1	

Note. *SY – seed yield; DDD – the degree of development of diseases; DS – disease spreading; EI mf – mf-mutation expression index; ASI – anthocyanin stain index; G – high multifoliate expression (mf-mutation) without the manifestation of anthocyanin color of the stem (green color of the stem); AN – high multifoliate expression (mf-mutation) with an evident anthocyanin color of the stem (purple color of the stem).*

A tendency has been established for a significant increase in seed productivity of alfalfa breeding populations in the first selection cycle during selection for both G and AN and stabilization in further cycles at the level of the initial variety. This fact may indicate the absence of inbreeding depression on the basis of SY (seed yield) during selection for high multifoliate expression.

In the process of recurrent selection, a decrease in DDD was found in alfalfa breeding populations with the AN feature in all selection cycles compared to the initial variety ($p < 0.05$).

A significant decrease in DDD was revealed in populations breeding on the basis of AN in the selection cycles C-2 and C-3 compared with populations breeding on the basis of G ($p < 0.05$).

A more intense decrease in DS was found in populations selected by G.

Significantly in all cycles of recurrent selection, an increase in EI mf was found, which dynamically increases from C-1 to C-3 compared with the original variety.

The results of two-way analysis of variance for all the studied characteristics when cultivating alfalfa in the ecotope of field crop rotation with chernozem soil are presented in table 4.

Table 4. The results of two-way analysis of variance for all the studied characteristics when cultivating selection populations of alfalfa (M. *varia*) in the ecotope of field crop rotation with black soil (TBS - typical black soil) (Chernyansky district, Belgorod region, Russia), 2016-2018

Effective sign	Source of variation	D	n-1	s^2	F_f	$F_{st\ 0.05}$	h^2_x
SY, $g*(m^2)^{-1}$	Common factors	1559.9	23				100
	Conditions of the year	286.4	2				18.4
	Random factors	576.5	14	41.1			36.9
	Factor A	25.0	1	25.1	0.6	8.7	1.6
	Factor B	658.1	3	219.4	5.3	3.3	42.2
	AB factor	13.9	3	4.7	0.1	8.7	0.9
DDD, %	Common factors	36.0	23				100.0
	Conditions of the year	17.7	2				49.3
	Random factors	7.5	14	0.5			20.9
	Factor A	3.0	1	3.0	5.6	4.6	8.3
	Factor B	5.6	3	1.9	3.5	3.3	15.5
	AB factor	2.1	3	0.7	1.3	3.3	6.0
DS, %	Common factors	66.5	23				100.0
	Conditions of the year	47.2	2				71.0
	Random factors	5.2	14	0.4			7.9
	Factor A	3.0	1	3.0	8.0	4.6	4.5
	Factor B	9.1	3	3.0	8.2	3.3	13.7
	AB factor	1.9	3	0.6	1.7	3.3	2.9
EI mf	Common factors	21.3	23				100.0
	Conditions of the year	5.3	2				24.7
	Random factors	1.0	14	0.1			4.6

	Factor A	1.5	1	1.5	20.8	4.6	6.9
	Factor B	12.8	3	4.3	60.6	3.3	60.1
	AB factor	0.8	3	0.3	3.7	3.3	3.7
ASI	Common factors	31.1	23				100.0
	Conditions of the year	0.0	2				0.1
	Random factors	0.0	14	0.0			0.0
	Factor A	19.6	1	19.6	74746.2	4.6	63.1
	Factor B	1.7	3	0.6	2108.7	3.3	5.3
	AB factor	9.8	3	3.3	12410.5	3.3	31.5

Note. *Factor A – breeding feature (G, AN); factor B - recurrence selection cycle (C-0 - C-3); D is the sum of the squared deviations (deviant); s2 is the dispersion; n-1 is the number of degrees of freedom; h2x - force of influence on the effective attribute.*

It is shown that the cycle of recurrent selection has the greatest influence on the SY and EI. The DDD and DS indicators were most influenced by the conditions of the year.

Thus, under favorable conditions of the ecotope of field crop rotation with chernozem soil the conditions of the year exerted the maximum influence on the spread of leaf spot diseases in breeding alfalfa populations.

In our previous studies, it was shown that in the adaptive breeding system, it is necessary to assess the alfalfa breeding populations in different ecotopes. Provocative backgrounds with a possibly high level of damage have particular importance due to a complex of leaf-spotting diseases [Chernyavskikh et al., 2019 b].

We tested the studied breeding populations of alfalfa in the ecotope with meadow chernozem soil (GL – gleysol) in the floodplain of the river Oskol. River floodplains are often used for alfalfa crops. At the same time, they are characterized by a whole range of unfavorable conditions for its cultivation: proximity to groundwater, lack of nutrients, severe microclimatic conditions (frequent dew loss), etc. This contributes to a more intensive development of diseases.

Results of the test of breeding alfalfa populations in a meadow ecotope are shown in table 5.

Table 5. Comparison of the average of various indicators for breeding populations of alfalfa (M. *varia*) with multifoliate expression between selection cycles (from C-1 to C-3) and the original variety (C-0). The studies were conducted in years 2016-2018 in an ecotope with meadow chernozem soil (GL – gleysol) on a plowed meadow in the floodplain of the river Oskol (Chernyansky district, Belgorod region, Russia)

Indicators	Sign of selection	Recurrence selection cycle				LSD
		C-0	C-1	C-2	C-3	0.05
SY, g*(m^2)$^{-1}$	G	9,6	5.4	8.9	10.7	3.8
	AN		15.9	18.4	18.5	
DDD, %	G	12,5	10.8	9.7	8.6	2.1
	AN		8.4	5.9	5.1	
DS, %	G	60,0	62.0	56.1	54.9	5.2
	AN		38.4	32.1	28.3	
EI mf	G	0,5	0.9	1.4	1.7	0.2
	AN		1.2	1.8	2.1	
ASI	G	1,9	1.9	1.2	0.9	0.1
	AN		1.9	3.8	4.3	

Note. *SY – seed yield; DDD – the degree of development of diseases; DS – disease spreading; ASI – anthocyanin stain index; EI mf – mf mutation expression index; G – high multifoliate expression (mf-mutation) without the manifestation of anthocyanin color of the stem (green color of the stem); AN –*

high multifoliate expression (mf-mutation) with an evident anthocyanin color of the stem (purple color of the stem).

It was established that in the conditions of the meadow ecotope breeding populations in which selection was carried out on the basis of AN were more productive and had less evident DDD and DS.

The indicator of SY according to the selection criterion G was at the level of the initial variety. The productivity of breeding populations selected on the basis of AN exceeded the initial population after the first selection cycle.

Signs of diseases of DDD and DS in breeding populations selected on the basis of G was at the level of the initial population in all selection cycles.

In populations selected on the basis of AN the manifestation of the DDD feature dynamically decreased from C-1 to C-3 and reached significant differences from the original variety in the second and third selection cycles, showing high resistance to disease.

The manifestation of the DS features in populations selected by the AN trait, starting from the first selection cycle, decreased by almost 2 times in comparison with the initial variety.

The results of two-way analysis of variance for all the studied traits when cultivating breeding populations of alfalfa in the ecotope with meadow chernozem soil (GL – gleysol) on a plowed meadow in the Oskol floodplain are shown in Table 6.

Table 6. The results of two-way analysis of variance for all the studied characteristics when cultivating breeding populations of alfalfa (M. *varia*) in the ecotope with meadow chernozem soil (GL – gleysol) on a plowed meadow in the floodplain of the river Oskol (Chernyansky district, Belgorod region, Russia), 2016-2018

Effective sign	Source of variation	D	n-1	s^2	$F_{ф}$	$F_{st\,0.05}$	h^2_x
SY, $g*(m^2)^{-1}$	Common factors	848.3	23.0				100.0
	Conditions of the year	302.9	2.0				35.7
	Random factors	72.8	14.0	5.2			8.6
	Factor A	273.1	1.0	273.1	52.5	4.6	32.2
	Factor B	102.8	3.0	34.3	6.6	3.3	12.1
	AB factor	96.7	3.0	32.2	6.2	3.3	11.4
DDD, %	Common factors	230.2	23.0				100.0
	Conditions of the year	48.1	2.0				20.9
	Random factors	20.4	14.0	1.5			8.8
	Factor A	37.8	1.0	37.8	26.0	4.6	16.4
	Factor B	110.5	3.0	36.8	25.3	3.3	48.0
	AB factor	13.4	3.0	4.5	3.1	3.3	5.8
DS, %	Common factors	5360.6	23.0				100.0
	Repeated factor	218.1	2.0				4.1
	Random factors	127.6	14.0	9.1			2.4
	Factor A	2057.6	1.0	2057.6	225.7	4.6	38.4
	Factor B	2263.2	3.0	754.4	82.7	3.3	42.2
	AB factor	694.0	3.0	231.3	25.4	3.3	12.9
EI mf	Common factors	10.5	23.0				100.0
	Conditions of the year	2.3	2.0				21.9
	Random factors	0.3	14.0	0.0			2.8
	Factor A	0.5	1.0	0.5	25.7	4.6	5.2
	Factor B	7.1	3.0	2.4	112.8	3.3	68.3
	AB factor	0.2	3.0	0.1	3.0	3.3	1.8
ASI	Common factors	31.9	23.0				100.0

Conditions of the year	0.0	2.0				0.0
Random factors	0.0	14.0	0.0			0.0
Factor A	19.7	1.0	19.7	221377.9	4.6	61.7
Factor B	1.7	3.0	0.6	6245.7	3.3	5.2
AB factor	10.5	3.0	3.5	39551.8	3.3	33.1

Note. *Factor A – "breeding trait (G, AN)"; factor B - "recurrence selection cycle (C-0 - C-3)"; D is the sum of the squared deviations (deviant); s2 – the dispersion; n-1 is the number of degrees of freedom; h2x – force of influence on the effective attribute.*

It was established that when cultivating sectional alfalfa populations in the ecotopes of the meadow, differences in the influence of organized factors on the manifestation of the studied characteristics were noted compared with the ecotope of field crop rotation with black soil.

In contrast to the ecotope favorable for cultivating alfalfa with chernozem soils, the meadow ecotope has a stronger influence of the conditions of the year as well as the selection factor.

It was revealed that the factor of the year (h2x = 35.7 %) and factor A "breeding trait" (h2x = 32.2 %) have a greater influence on the SY index in the meadow ecotope.

The null hypothesis of the influence of factor B cannot be rejected in the "recurrent selection cycle" (Ff / F st 0.05 = 1.88), despite the rather low strength of its influence on the resultant attribute (h2x = 12.1 %).

Compared with the ecotope of the field crop rotation with chernozem soil, the influence of the year conditions on the severity of signs of DDD and DS in the meadow ecotope is much lower – h2x = 20.9 % and 4.1 %, respectively.

In the meadow ecotope the degree of severity of the manifestation of diseases (DDD and DS) was maximally influenced by factor A – the "selection trait" and factor B – the "recurrent selection cycle" and not the conditions of the year.

In fact, against the provocative background of the meadow ecotope the influence of genotypes of breeding alfalfa populations was most evident.

The results of the analysis of the tightness of the relationship between the studied parameters for breeding populations of alfalfa with multifoliate expression as a whole for all studied ecotopes and breeding samples are shown in Table 7.

Table 7. Correlation between the studied parameters for breeding populations of alfalfa (M. *varia*) with multifoliate expression in general for all studied ecotopes and breeding samples

Indicators	SY, г*(м^2)$^{-1}$	DDD, %	DS, %	EI mf	ASI
SY, g*(m^2)$^{-1}$	1.000				
DDD, %	0.378	1.000			
DS, %	-0.267	0.898^b	1.000		
EI mf	-0.209	-0,606^b	0.140	1.000	
ASI	-0.132	-0.463^a	-0.283^a	0.459^a	1.000

Note. a – the correlation is significant at p = 0.05; b – the correlation is significant at p = 0.01; SY – seed yield; DDD – the degree of development of diseases; DS – the prevalence of disease; ASI-anthocyanin stain index; EI mf — mf mutation expression index.

It was revealed that none of the studied indicators had a strong impact on the index of DDD.

Selection alfalfa populations with a high degree of expression of the mutation had a lower degree of DS, which is confirmed by a negative correlation of average strength.

A similar trend was manifested in breeding alfalfa populations with a high index of anthocyanin color of the stem.

Signs of EI mf and ASI are positively interconnected, which is confirmed by a reliable correlation of average strength ($p<0.05$).

4. Conclusion

1. Three cycles of recurrent selection were effective for increasing multifoliate expression in alfalfa breeding populations without increasing inbreeding depression based on seed productivity.
2. Multifoliate forms are resistant to the development of leaf- spotting diseases and their prevalence. The most stable are the forms in which selection for the expression of the mf-mutation was carried out simultaneously with an increase in the index of the anthocyanin color of the stem.
3. For a more effective assessment of the resistance of alfalfa breeding populations to leaf-spotting in Central Russia comparative testing must be carried out simultaneously under conditions of favorable ecotopes of field crop rotation with fertile chernozem soils and in adverse conditions of meadow ecotopes.

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