# Yield formation

UDC 633.11:575.21

doi: 10.15389/agrobiology.2021.1.78eng doi: 10.15389/agrobiology.2021.1.78rus

# PHENOTYPIC VARIABILITY OF COMMON WHEAT (*Triticum aestivum* L.) BREEDING LINES ON YIELD COMPONENTS UNDER ENVIRONMENTAL CONDITIONS OF WESTERN SIBERIA AND TATARSTAN

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Supported financially from the Russian Science Foundation, grant NF 16-16-00011. Reproduction of breeding lines was performed within the framework of the state assignment of the Federal Research Center ICG SB RAS  $\ge 0259-2021-0012$ . Field trials in the Republic of Tatarstan were carried out in accordance with the state assignment of the Federal Research Center of KazSC RAS AAAA-A18-118031390148-1. *Received June 15, 2020* 

#### Abstract

Spring wheat is one of the widely cultivated crops in the Russian Federation. Wheat breeding is aimed at creation of varieties characterized by high productivity and grain quality. When creating new varieties, the main attention is paid to ecological stability to environments, which negatively affects the agromomic traits and yield. Currently there is no enough information on the inheritance and manifestation of productivity traits in various agro-ecological conditions in advanced generations of the crosses between winter and spring wheat varieties. In this study, we analyzed the variability of the yield traits in wheat breeding lines (Triticum aestivum L.) of F6-7 generations, obtained from hybridization of winter wheat varieties with spring donors of leaf rust resistance genes with the aim to identify promising genotypes as  $\phi$  source of valuable agronomic traits. Field trials were performed in 2018 in three regions, the Novosibirsk and Omsk region and the Republic of Tatarstan. The following traits were studied: tiller number per plant, the grain number per ear, the grain weight per ear, 1000 grain weight, and the grain weight per plant. Ecological plasticity of the lines was studied using indexes of the intensity and stability. Analysis of variance based on the results of field evaluation indicates a significant influence of the genotype, the environment and their interaction in the phenotypic manifestation of all the studied characters. The highest contribution of the genotype is shown for the grain number per ear (42.8 %) and the 1000 grain weight (57.0 %). A high contribution of environmental factors was found for tiller number (41.8 %) and grain weight per ear (40.3 %). The genotype  $\times$  environment interaction had a significant effect on all traits, its contribution varied from 25.9 % (1000 grain weight) to 41.0 % (grain weight per ear), which indicates a significant response of genotypes to changing climatic factors. The results of field tests showed that there was a high variability of all characters in all three climatic zones, but the degree of variation differed. The averaged indicators of the studied traits were lower in the field conditions of the Omsk zone as compared to the Novosibirsk region and Tatarstan. Higher fluctuations were noted for the grain number per ear (13.0-69.0), the grain weight per ear (0.35-2.65 g), and the grain weight per plant (0.15-6.95) in Tatarstan's environments in comparison with other regions. The estimation of stability and intensity indices showed that 16 of 55 genotypes have intensive type, 35 were semi-intensive and 4 were extensive. The grouping of samples by the principal coordinate analysis method divided the genotypes into four main clusters according to the stability and intensity parameters. Molecular analysis for the presence of leaf rust resistance genes Lr6Ai#2, LrAsp5, and LrTt2 introduced from Thinopyrum intermedium (Host) Barkworth & D.R. Dewey, Aegilops speltoides Tausch and *Triticum timopheevii* Zhuk., respectively, showed that 10 out of 55 lines do not contain alien genetic material. The results of marker analysis for the presence of resistance genes did not correlate with the clustering of samples by intensity and stability types. This fact suggests that the presence of alien genomes does not influence on the stability of breeding lines. Based on the obtained results, genotypes with valuable characters were selected as sources of productivity.

Keywords: Triticum aestivum, common wheat, ecological plastisity, agronomic traits, yield, leaf rust

Soft wheat (*Triticum aestivum* L.) is of great importance throughout the world as a source of food for the population and fodder grain for farm animals. The Russian Federation currently occupies a leading position in the production of winter and spring wheat [1]. Over the past decade, there has been an increase in the sown area occupied by this crop in the country, an increase in the gross grain harvest, and an increase in productivity [2, 3].

The main producers of spring soft wheat in Russia are the Volga regions, the Ural and West Siberian regions, while up to 40% of the sown area of the crop falls on the Siberian region. However, despite the tendency to increase the yield of spring wheat, there is a significant variation from year to year. According to Silaeva and Barinova [3], the yield of spring wheat in 2007-2017 as a whole across the country varied from 9.5 to 18.5 c/ha. A significant fluctuation in yield is shown for both old and modern spring wheat varieties cultivated in Western Siberia [4-6].

The genetic basis of the variety is the main factor that determines the yield. In recent years, with the emergence of saturated molecular maps and the development of technologies for high-throughput geno- and phenotyping, significant progress has been made in the study of the genetic architecture of yield and its components in common wheat. Major and minor loci have been identified that contribute to the phenotypic manifestation of productive tillering, the number of grains and grain weight per spike, grain weight per plant, parameters of the root system, etc. [7-9]. Quantitative trait loci and candidate genes that determine yield under abiotic stress factors have been identified [10-13]. However, the implementation of the genetic potential of wheat largely depends on environmental factors. Therefore, at certain stages of obtaining new forms, it is necessary to study their phenotypic plasticity and adaptability in various soil and climatic conditions.

Winter wheat differs from spring wheat in higher productive tillering, grain weight per ear, and 1000 grain weight, which make the main contribution to the yield of varieties. However, in contrast to winter wheat, spring wheat is characterized by high indicators of grain and gluten quality and is more drought-resistant [14-16]. Since the middle of the 20th century, a strategy of combining the genetic pools of these crops has been used to obtain new spring and winter wheat varieties with high yield and disease resistance. Despite the fact that in most winter wheat varieties the combining ability when crossed with spring wheat is characterized as medium or weak, it has been shown that in some cases hybridization can give the desired genotypes [17, 18]. The general combining ability (GCA) of populations from crossing winter and spring samples was studied mainly at the F1 stage to reveal the effect of heterosis and select the desired genotypes with higher indices for agronomically important traits [19, 20]. The GCA in the F<sub>1</sub>-F<sub>2</sub> generations was assessed mainly on hybrid populations obtained on the basis of hybridization of either winter or spring varieties. The authors of these studies argue that the  $F_1$ generation can be used for the initial stage of GCA assessment and donor selection. Thus, the study of the combining ability for the winter hardiness in 30 diallelic hybrids of the  $F_1$  generation obtained from crossing 6 winter wheat varieties made it possible to identify genotypes characterized by high GCA effects and the participation of dominant alleles in an increase in the trait [21]. Based on the results obtained, it was concluded that for the selection of unique genotypes with enhanced winter hardiness, it was necessary to test the hybrids of the F4-F6 generations. Studies for productivity traits performed using F1-F2 hybrids of spring and winter varieties indicate a significant contribution of genes with an additive type of action, which, according to the authors, makes it possible to start the selection of donors in early generations [22, 23]. As for the crosses of winter and spring wheat varieties, at present, there is not enough information on the manifestation and inheritance of productivity traits in advanced generations and in various agroecological conditions.

Earlier, the authors of this paper have created breeding lines based on hybridization of winter varieties and spring donors of resistance genes in order to expand the genetic diversity of common wheat for disease resistance genes [24, 25].

In the present study, these breeding lines were assessed according to the elements of the yield structure in different environmental conditions.

The aim of the work was to study the ecological plasticity of samples of advanced generations and to identify promising genotypes as sources of productivity traits for breeding.

*Materials and methods.* The studies were carried out on 55 lines of spring soft wheat of generations F6-7, obtained from crossing the winter varieties Novosibirskaya 3 (N3), Novosibirskaya 40 (N40), Filatovka, and Biyskaya winter with spring donors of genes for resistance to leaf rust – the varieties Tulaykovskaya 10 (T10) and lines 5366-180 and 21-4, obtained on the varieties Saratovskaya 29 and Novosibirskaya 29, respectively. The variety T10 contains the *Lr6Ai#2* gene from *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey, line 5366-180 – the *LrTt2* gene from *Triticum timopheevii* Zhuk., Line 21-4 – the *LrAsp5* gene from *Aegilops speltoides* Tausch. [24, 26]. The lines were selected according to the earing time and productivity parameters in the F<sub>3-5</sub> generations and propagated to the F6-7 generations.

DNA was isolated from the leaves of plants of the F<sub>2</sub> generation. A leaf fragment (2-3 cm) was placed in a 2 ml tube with Lysing Matrix Z (MP Biomedicals, USA) for grinding plant tissues, 700  $\mu$ L of lysis buffer (1 ml 1 M Tris-HCl, 1 ml 5 M NaCl, 1 ml 0.5 M EDTA, 0.625 ml 20% SDS, and 0.04 g Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>) was added, leaf fragment was grinded on a FastPrep-24 homogenizer (MP Biomedicals, USA) and incubated in a water bath at 60 °C for 30 min. Then, 700  $\mu$ L of a mixture of chloroform: isoamyl alcohol (24:1) was added and centrifuged for 25 min at 12,000 rpm. The upper fraction was placed in a 2 ml tube, 1.4 ml of 96% chilled ethanol (-20 °C) was added and centrifuged for 15 min at 4500 rpm. The supernatant was removed, the precipitate was air-dried and dissolved in 50  $\mu$ L of TE buffer.

To determine genotypes containing the *Lr6Ai#2* gene from *Th. intermedium*, the authors used primers MF2 (5'-GATGTCG-AGGAGCATTTTC-3'), MR1r2 (5'-GTGGTAGATTACTAGAGTTCAAGTG-3') and MR4 (5'-CGAA-TAGTTATACTAGGAGTAC-3') (Patent No. RU 2598275 C1, 2015). These primers are dominant, and two pairs, MF2/MR1r2 and MF2/MR4, must be used to identify plants homozygous and heterozygous for this gene. To identify introgressed fragments from *T. timopheevii* containing the *LrTt2* gene, the authors used the *Xbarc232* microsatellite marker (F – 5'-CGCATCCAACCATCCCCACCC-AACA-3', R – 5'-CGCAGTAGATCCACCACCCGCCAGA-3') [27]. Translocations from *Ae. speltoides* containing the *LrAsp5* gene were identified using a pair of primers P1/P5 (P1 – 5'-TACCCCTGCTACCAGTGCGC-3', P5 – 5'-GGCCAACCCTACACCCCAAG-3') (Patent No. RU 2535985 C1, 2013). Polymerase chain reaction (PCR) was carried out using a T-100 amplifier (Bio-Rad, USA) in a total volume of the reaction mixture of 20  $\mu$ l containing DNA (50-100 ng), 10× buffer for Taq polymerase (650 mM Tris-HCl, pH 8.9; 160 mM (NH4)2SO4, 25 mM MgCl<sub>2</sub>, 0.01% Tween 20), 0.25 mM each dNTP, 1 ng each primer, 1  $\mu$ l Taq polymerase (1 activity unit/ $\mu$ l), H<sub>2</sub>O (to the final volume). The PCR conditions for gene detection have been described previously [24]. PCR products were separated by electrophoresis in 1.5% agarose gel containing ethidium bromide.

Phenotyping was carried out in the fields of the Novosibirsk and Omsk regions and the Republic of Tatarstan (Kazan) in 2018. The soil of the experimental plots in the Novosibirsk Region was leached chernozem, in the Omsk Province – meadow-chernozemic low-humus medium loamy soil, in Tatarstan – well-cultivated gray forest soil. The field trials were laid in 2 replicates by a systematic method on plots 1 m wide, 60 grains in a row.

The harvest was collected in sheaves, after which the number of productive stems, the number of grains in an ear, the weight of grain from the ear and from the plant, and the weight of 1000 grains were assessed. Structural analysis was performed for 20 plants of each sample.

The ecological plasticity of the lines was assessed on the basis of the trait "grain weight per plant" using the indices of the intensity index (I), stability index (SI), and reliability of stability index (R) according to the methodological guidelines [28]. The value of I, showing the reaction of the lines to the environments, was determined as the ratio of the difference in the grain weight per plant for its two extreme values for each line to the average weight for all lines on all environments: I =  $(\overline{X}_{opt.} - \overline{X}_{lim.})/\overline{X} \times 100\%$ , where  $\overline{X}$  is the average value of the grain weight per plant for all lines on all environments,  $\overline{X}_{opt}$  and  $\overline{X}_{lim}$  – average values of grain weight per plant for a sample against optimal and limited environments. The optimal environment was considered the region where the grain weight per plant for a particular line was the highest, the limited one was the region in which the grain weight per plant was the smallest. In addition, to assign a line to a specific type of intensity, a generalized intensity indicator for a hypothetical line (I) was calculated based on the average characteristics of all lines on tested backgrounds and the least significant difference (LSD) of partial averages for residual variance after two-way ANOVA [29]. The classification of lines according to the degree of responsiveness to the agricultural background was carried out according to the ratios:  $I_{\text{line}} > I + LSD - \text{intensive}$ ,  $I - LSD \le I_{\text{line}} \le I + LSD - \text{semi-}$ intensive,  $I_{\text{line}} \leq I - \text{LSD}$  – extensive. The stability index (SI), which characterizes the manifestation of homeostatic reactions of lines under different environmental conditions, was estimated as the ratio of the square of the average grain weight per plant in a sample against a specific environment to the value of the standard deviation of this indicator under certain conditions:  $SI = X^2/S$ . The root-meansquare deviation was found by the formula  $S = \sqrt{\Sigma X^2/n} - (\Sigma X/n)^2$ , where X is the grain weight per plant in the line in each repetition against a certain environment (in the region), *n* is the number of repetitions in the conditions of this environment. The reliability index of the stability index (R), which characterizes the adaptive ability of the line, was determined by the formula:  $R = (1 - SI_{opt.} - SI_{lim.})/SI \times 100\%$ , where SI is the average value of the stability index in all experimental sites,  $SI_{opt}$  and SIlim – indices of stability of lines against optimal and limited backgrounds. The environment, against which the calculated stability index was the highest, was taken as optimal, and the lowest, as limited.

Statistical processing of the results was carried out using MS Excel 2016 and STATISTICA v. 10.0 (StatSoft, Inc., USA). The mean (M), minimum (min), and maximum (max) values of the traits, median (Me) and standard error of the

mean ( $\pm$ SEM) were estimated. The contribution of the factors was calculated based on the mean square of the deviations (*MS*). The level of significance p < 0.05 was taken as a significant one. Principal coordinate analysis was performed using PAST v. 3.15 (30).

**Results.** The West Siberian region belongs to the territories with risky farming and includes various climatic zones, as evidenced by the meteorological data for the Novosibirsk and Omsk Regions in 2018. The weather conditions in the Novosibirsk Region during the growing season differed from the average longterm, the average temperature in May was significantly lower (6.9 °C), May and June were characterized by abundant precipitation, exceeding the norm by almost 3 times. In the Omsk Region, the average temperature did not differ from the average annual temperature, 259 mm of precipitation fell in the period of May-September, while their greatest amount fell on the second half of the growing season. In May, on the contrary, there was a deficit of precipitation in Tatarstan, in the rest of the months it fell unevenly; in June the average daily air temperature was below the norm, in the rest of the period - 2-3 °C above the norm.

The characteristics of 55 studied lines by the origin and the yield components during environmental tests are presented in Table 1 (see http://www.agro-biology.ru).

To assess the ecological plasticity, one of the main indicators of productivity was used — the grain weight per plant, which, in fact, serves as an indicator of plant productivity. Calculation of indicators of ecological plasticity made it possible to divide the studied lines according to intensity and adaptability (Table 2).

Line Me	I, %	R, %		SI					
Line No.			Novosibirsk	Omsk	Kazan	51			
Winter variety Bivskava winter × spring variety Tulaykovskava 10									
1	130.27	-3.3	7.35	2.62	8.55	In/S			
2	92.95	48.1	7.72	4.74	5.71	In/S			
Winter variety Filatovka×spring line 5366-180									
3	108.84	-2.5	5.09	2.32	8.20	In/S			
4	71.00	51.3	5.59	6.48	8.38	P-In/S			
	Winter variety Filatovka $\times$ spring variety Tulaykovskaya 10								
5	99.52	-52.7	14.15	7.02	15.78	In/US			
6	22.78	84.4	6.36	5.46	5.52	P-In/S			
7	89.56	54.6	7.73	9.31	10.34	In/S			
8	231.49	-138.5	5.78	2.66	16.35	In/US			
9	109.09	4.1	6.93	3.88	9.38	In/S			
10	22.95	83.2	5.81	4.84	5.46	P-In/S			
11	75.54	-24.2	9.36	2.23	4.32	P-In/US			
12	97.12	31.1	8.21	4.30	4.26	In/S			
13	91.31	6.5	7.73	2.36	4.54	In/S			
14	41.58	30.5	4.03	4.71	8.02	P-In/S			
15	44.98	72.3	6.86	6.10	5.27	P-In/S			
16	60.83	11.7	7.05	1.98	4.93	P-In/S			
17	84.00	-36.4	7.72	3.17	11.00	In/US			
18	59.79	21.6	5.00	2.31	6.81	P-In/S			
19	30.71	74.0	5.26	5.82	4.32	P-In/S			
20	39.65	64.8	3.47	2.77	4.79	P-In/S			
21	117.18	13.6	6.65	3.03	7.99	In/S			
Winter variety Novosibirskaya 3 × spring line 21-4									
22	33.05	52.6	4.68	2.64	5.36	P-In/S			
23	72.26	30.9	5.58	3.76	7.73	P-In/S			
24	34.73	75.0	4.10	3.95	5.38	P-In/S			
25	31.93	85.4	5.14	4.77	4.30	P-In/S			

**2.** Parameters of ecological plasticity of 55 studied  $F_{6-7}$  wheat lines from different cross combinations based on phenotyping tests in three regions of Russia (for each sample n = 20, 2-fold biological replication, 2018)

						commuta rabit 2
	Winter	variety	Novosibirskaya	40 × spring	line 21-4	
26	42.53	67.5	6.34	7.09	5.22	P-In/S
27	19.85	44.3	6.64	3.44	6.04	P-In/S
28	22.78	53.6	6.17	7.60	4.94	P-In/S
29	50.62	91.0	5.81	5.62	6.14	P-In/S
30	13.53	68.4	4.36	2.55	4.20	E/S
31	36.58	66.8	4.10	2.23	4.14	P-In/S
32	55.58	44.5	4.40	3.32	6.51	P-In/S
33	66.84	46.0	6.21	3.76	6.87	P-In/S
34	63.17	-27.9	10.36	3.54	3.01	P-In/US
35	54.90	39.1	7.20	3.70	4.58	P-In/S
36	82.37	48.8	7.99	6.19	9.13	In/S
37	28.07	85.9	3.45	4.18	3.37	P-In/S
38	52.26	17.6	9.28	4.55	4.72	P-In/S
39	38.26	54.2	5.16	2.53	4.30	P-In/S
40	16.54	96.4	4.60	4.40	4.42	E/S
41	18.26	5.1	9.03	3.58	3.90	E/S
42	36.76	76.2	5.55	4.19	4.43	P-In/S
43	42.93	-18.2	11.19	4.41	5.88	P-In/US
44	88.26	-46.2	12.28	3.89	7.14	In/US
45	81.10	23.5	7.45	3.06	5.54	In/S
46	42.57	85.0	4.39	4.05	4.91	P-In/S
47	46.41	53.6	6.97	4.31	5.97	P-In/S
48	92.70	28.8	8.24	5.54	4.15	In/S
49	33.38	32.7	9.54	5.87	5.68	P-In/S
50	66.14	-11.6	10.26	3.86	3.90	P-In/S
51	54.85	-16.0	11.38	4.84	4.72	P-In/US
52	96.35	37.2	4.49	5.19	8.09	In/S
53	15.06	57.7	5.31	2.88	4.72	E/S
54	64.79	-15.8	9.56	3.90	2.91	P-In/US
55	45.89	89.2	5.77	5.15	5.55	P-In/S
Note. I – inte	nsity index, R –	reliability of	f stability index,	SI – stability in	ndex; In — intensi	ve, P-In – semi-
intensive, $E - explicitly = e^{-2}$	tensive; S – stat	ole, US – ui	nstable. For a des	scription, see the	e Materials and me	thods section.
				-		

Continued Table 2

ANOVA, carried out on the basis of the results of field trials, showed a significant effect (p < 0.001) of the genotype, environmental conditions, and their interaction on the phenotypic manifestation of traits (Table 3). The contribution of the "genotype" factor varied depending on the trait, with the highest contribution noted for the grain number per spike (42.8%) and the 1000 grain weight (57.0%). The influence of genotypic factors on the manifestation of phenotypic variability of productive tillering and grain weight per plant was less significant compared to environmental factors (20.4 and 22.1%, respectively). The interaction genotype × environment was characterized by a high contribution to the phenotypic variability of all studied traits, which indicates a significant reaction of genotypes to soil and weather conditions.

A significant contribution of the "genotype  $\times$  environment" factor has been described for the  $F_1$  generation obtained from crossing winter and spring wheat varieties [19]. However, the authors found that the effect of heterosis depended not so much on the growing conditions of the hybrids, but on the genotype of the varieties used for hybridization. According to many studies, fluctuations in weather conditions in test regions, waterlogging at the beginning of the growing season, and low temperatures lead to significant variations in the yield and its components [31, 32]. The obtained results are consistent with the literature data, which indicate the unequal contribution of the genotype and the environments to the formation of the yield in common wheat [6, 33, 34]. The contribution of the genotype in the phenotypic variability of the yield is significantly less compared to environmental and agrotechnological factors, as evidenced by the results of most publications [35-37]. A number of works have shown that the realization of the genetic potential of varieties depends not so much on the influence of climatic factors, but on agro-technological measures and the use of intensive technologies that reduce the negative effects of the environments, which leads to the prevailing

## influence of the genotype [38-40].

3. Analysis of variance based on the yield components in 55 studied wheat genotypes  $F_{6-7}$  from different cross combinations of winter wheats with spring wheats under environments in Novosibirsk, Omsk Regions, and the Republic of Tatarstan (for each sample n = 20, 2-fold biological replication, 2018)

Source of variation	Geneture	Pagion	Genetune X region	Error	
Trait, parameter	Genotype	Region	Genotype ~ legion		
Productive tillering:			•		
df	54	2	108		
MS	4.06	225.13	3.77	0.86	
F	4.7*	260.6*	4.4*		
contribution, %	20.4	41.8	35.9		
The number of grains per ear:					
df	54	2	108		
MS	774	10451	325	80	
F	9.7*	131.5*	4.1*		
contribution. %	42.8	21.4	35.9		
Grain weight per ear:					
df	54	2	108		
MS	1.22	23.11	0.72	0.16	
F	7.6*	144.7*	4.5*		
contribution. %	34.6	24.4	41.0		
1000 grain weight:					
df	54	2	108		
MS	422	3514	94	28	
F	15.3*	127.7*	3.4*		
contribution. %	57.0	17.6	25.4		
Grain weight per plant:					
df	54	2	108		
MS	9.33	458.70	7.94	1.43	
F	6.5*	320.2*	5.5*		
contribution, %	22.1	40.3	37.6		

N ot e. df — the number of degrees of freedom, MS — the mean square; F — Fisher's test. \* The contribution is statistically significant at p < 0.001.



Fig. 1. Statistical analysis of the variability of the yield structure elements in 55 studied wheat lines F6-7 without taking into account their origin. The trials were conducted in 2018 in Novosibirsk Province (1), Omsk Province (2), and Tatarstan (3): A – productive tillering, psc.; B – the number of grains per ear, psc.; C – grain weight per ear, g; D – 1000 grain weight, g; E – grain weight per plant, g. Whiskers denote the minimum (min) and maximum (max) values of the trait, the horizontal line in the box is the median (*Me*), the average value of the feature is marked with "×". The samples were obtained in combinations of crosses of winter varieties with spring forms. For each sample n = 20, 2-fold biological replication.

For a generalized assessment of the variability of yield traits in each of the three regions, we carried out statistical processing of field data for a total of 55 lines without taking into account their origin. As can be seen from Fig. 1, significant variability of all traits was observed when growing samples in all three climatic zones, but the degree of variation was different. For all signs, the obtained average value was lower in the field conditions of the Omsk Province. The most contrasting range of fluctuations was observed in the number of productive stems in the Novosibirsk Province (1-6 stems) compared to Omsk (1-2 stems) and Tatarstan

(1-4 stems). More significant fluctuations were observed for the ear grain number, ear grain weight, and grain weight per plant in Tatarstan as compared to other regions (see Fig. 1).

At present, various methods proposed by Eberhart and Russell, Tai, Udachin and Golovochenko, and others [28, 41, 42] are used to calculate the parameters of ecological plasticity. The most universal is the method of Eberhart and Russell [41], which is used by most researchers to assess the ecological plasticity and adaptability of cultivated varieties and at the final stages of breeding trials. However, the specified calculation method does not allow obtaining adequate and statistically reliable results in cases where the experimental samples are in the early hybrid stages or the number of field tests is limited. To assess the ecological plasticity of new breeding lines, the authors used the method of Udachin and Golovochenko [28], with the help of which it is possible to determine the tendency of plasticity formation at the initial breeding stages. The effectiveness of the method for obtaining preliminary information on the plasticity of varieties under conditions of a limited number of agricultural backgrounds and field seasons was confirmed in a number of works [6, 43, 44].

Based on the test results of 55 lines for each of the three climatic zones, stability indices (SI) were calculated, as well as intensity indices (I) and reliability of stability indices (R) (see Table 2). Intensity parameters allow determining the degree of responsiveness of genotypes to changes in plant cultivation conditions. The stability index shows how consistently a line is able to realize its potential in different environmental conditions. Lines with larger indices are more stable, that is, they are better adapted to changing conditions. The study found that 16 genotypes were included in the group of intensive type varieties, 35 in semi-intensive and 4 in extensive.



Fig. 2. Clustering by the method of principal coordinates of 55 studied wheat genotypes F6-7 from different cross combinations of winter wheats with spring wheats. Lines originating from different crossing combinations are indicated by the following symbols:  $\bullet$  — Biyskaya winter × Tulaykovskaya 10,  $\Box$  — Filatovka × line 5366-180,  $\blacksquare$  — Filatovka × Tulaykovskaya 10,  $\blacktriangle$  — Novosibirskaya 3 × line 21-4,  $\triangle$  — Novosibirskaya 40 × line 21-4. The analysis was carried out according to the results of evaluating stability indices (SI), reliability of stability indices (R) and intensity indices (I), calculated for three test regions (for each sample n = 20, 2-fold biological replication, Novosibirsk Province, Omsk Province, and Tatarstan, 2018).

Principal coordinate analysis carried out on the basis of the parameters of ecological plasticity, additionally divided the studied breeding lines into four main clusters (Fig. 2). The first cluster includes 9 lines, 8 of which are characterized by

a low stability coefficient (R), which indicates an unstable line type. The exception was line No. 50, which belongs to the stable type. Among the genotypes of this group, there are samples of both intensive and semi-intensive types. Clusters 2 and 3 include, respectively, 13 and 11 samples of the stable type, with all lines, except for No. 53, being semi-intense. A distinctive feature of cluster 3 is the higher stability indices of the stability index compared to the samples of cluster 2. Finally, cluster 4 unites 15 stable lines of predominantly intense type. Principal coordinate analysis also showed that clusters were formed from genotypes originating from different combinations of crosses. Seven lines (Nos. 8, 29, 30, 40, 41, 46, 55) were not assigned to any of the clusters, while line No. 8 significantly differed from all samples by a very low stability index (SI = -138.54) and high intensity factor (I = 231.49). Despite the fact that the combinations Biyskaya winter × Tulay-kovskaya 10 and Filatovka × 5366-180 are represented by only two lines each, the results of evaluating these hybrid forms do not affect the grouping.



Fig. 3. An example of identification of genotypes containing genes for resistance to leaf rust among 55 lines F6-7 from different cross combinations of winter wheats with spring wheats. Primers MF2/MR1r2 (A) and MF2/MR4 (B) reveal the *Lr6Ai#2* gene, primers P1/P5 (C) reveal the *LrAsp5* gene, the Xbarc232 marker (D) reveal the *LrTt2* gene; 5-21 - the lines derived from crossing Filatovka × Tulaykovskaya 10, F - variety Filatovka, T10 - variety Tulaykovskaya 10; 22-25 - the lines derived from crossing Novosibirskaya  $3 \times$  line 21-4, N3 - variety Novosibirskaya 3, 21-4 - line 21-4; 3, 4 - the lines derived from crossing Filatovka × line 5366-180. M - DNA 100 bp ladder (ZAO Biosan, Novosibirsk). Arrows point to diagnostic fragments 347 bp indicating the presence of translocations from *Thinopyrum intermedium*, and 328 bp indicating the absence of such translocations. The fragment 1100 bp shows the presence of translocations from *Aegilops speltoides*, and 968 bp - its absence. The fragment 240 bp indicates the presence of translocations from *Triticum timopheevii*, amplification of two fragments 200 and 240 bp indicates the absence of translocations.

Genotyping of the lines with *Lr6Ai#2*, *LrTt2*, and *LrAsp5* gene markers revealed the presence of amplification fragments with primers MF2/MR1r2 and MF2/MR4 in lines No. 11-21 (Filatovka  $\times$  Tulaykovskaya 10), suggesting that they

carry a *Th. intermedium* translocation (Fig. 3). We did not find alien translocations with resistance genes in lines No. 1 and No. 2 (Biyskaya winter × Tulaykovskaya 10), as well as in lines No. 5, 6, 7, 8, 9, and 10 (Filatovka × Tulaykovskaya 10). The presence of fragments of the *Ae. speltoides* genome was identified using primers P1/P5 in lines 22–55 obtained with the participation of Novosibirskaya 3 and Novosibirskaya 40 cultivars. PCR analysis of two lines participating in the study – no. 3 and no. 4 (Filatovka × line 5366-180) using the *Xbarc232* microsatellite marker showed the absence of introgression from *T. timopheevii* in these lines. The results of marker analysis for the presence of resistance genes did not correlate with the clustering of samples by the type of intensity and stability. The results obtained allow concluding that the presence of foreign genetic material in the genotypic environment of the recipient winter varieties does not affect the phenotypic manifestation of yield traits and the parameters of ecological plasticity.

So far, no unambiguous results have been obtained on the effect of alien translocations with resistance genes on the manifestation of productivity traits. It is known that foreign genetic material, which is part of extended introgressed fragments, can have both negative and positive effects on such traits as the number of grains per spike, the weight of grains per spike, and the 1000 grain weight [45, 46]. Similar effects are demonstrated by the example of wheat-rye translocations 1BL/1RS and 1AL/1RS, which are widely used in the world to create varieties with complex resistance to fungal diseases. Multidirectional effects on yield components during introgression of the genetic material of various species of wheat-grass *Thinopyrum* ssp., goat grass *Aegilops* ssp. and *T. timopheevii* in the common wheat genome are largely associated with the genotypic environment of the recipient cultivar, as evidenced by the literature data [47, 48].

Among the samples included in clusters 2 and 3, the authors selected 10 stable breeding lines of the semi-intensive type, distinguished by a higher grain weight per plant and a 1000 grain weight (Table 4). These lines will be involved in the next phase of testing to assess the prospects as sources of productivity.

Line No.	Yield structure component, $M\pm$ SEM						Desistance		
	number, psc.			Resistance genes					
	productive tillering	grains per ear	grains per ear	grains per plant	1000 grains	Lr6Ai#2	LrTt2	LrAsp5	
14	$1.50 \pm 0.09$	43.28±1.43	$1.60 \pm 0.06$	2.18±0.13	37.34±1.07	+	_	_	
15	$1.75 \pm 0.10$	37.63±0.96	$1.49 \pm 0.05$	2.23±0.13	38.81±0.57	+	-	-	
19	$1.85 \pm 0.12$	$38.00 \pm 1.59$	$1.50 \pm 0.07$	$2.18 \pm 0.14$	$37.62 \pm 0.85$	+	-	-	
20	$1.83 \pm 0.15$	$37.48 \pm 1.41$	$1.45 \pm 0.07$	2.27±0.19	37.71±0.62	+	-	-	
24	$2.02 \pm 0.13$	33.25±1.04	$1.22 \pm 0.04$	2.11±0.14	$35.86 \pm 0.96$	-	-	+	
26	$1.77 \pm 0.13$	41.13±1.11	$1.57 \pm 0.05$	$2.30 \pm 0.15$	$36.74 \pm 0.60$	-	-	+	
32	$1.60 \pm 0.10$	39.33±1.56	$1.52 \pm 0.07$	$2.09 \pm 1.52$	$37.78 \pm 0.75$	-	-	+	
42	$2.22 \pm 0.14$	34.85±1.13	$1.30 \pm 0.05$	2.34±0.16	$35.57 \pm 0.64$	-	-	+	
47	$2.12 \pm 0.13$	$39.40 \pm 0.98$	$1.50 \pm 0.05$	$2.62 \pm 0.17$	$35.26 \pm 0.62$	-	-	+	
49	$2.05 \pm 0.12$	$39.88 {\pm} 0.97$	$1.56 \pm 0.04$	$2.62 \pm 0.14$	37.16±0.64	-	-	+	

4. Characteristics of stable breeding lines of semi-intensive type from different cross combinations of winter forms with spring ones, selected according to the results of field tests (Novosibirsk Province, Omsk Province, and Tatarstan, 2018)

Thus, as a result of studying a set of common wheat breeding lines of  $F_{6-7}$  generations in three ecological-geographical regions, it was found that the contribution of the genotype and environments to phenotypic variability depended on the trait under study. A high influence of genotype × environment interaction (25.9-41.0%) on all components of productivity was noted. The analysis of ecological plasticity showed that the lines were grouped into four main clusters according to the type of stability and intensity, while the clustering of samples was not affected by the origin of genotypes from crossing combinations and the

presence of fragments of alien material from *Thinopyrum intermedium* and *Aegilops speltoides*. Based on the assessment of ecological plasticity, stable genotypes of the semi-intensive type (Nos. 14, 15, 19, 20, 24, 26, 32, 42, 47, and 49) were selected for further testing with different cultivation technologies in the same region.

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