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PLANT GENETICS

Chromosomal Location of the *b-Amy-A1* Gene and Distribution of Its Alleles in a Winter Common Wheat Culture

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Abstract—The chromosomal location of the *b-Amy-A1* gene, which controls the synthesis of wheat beta-amylase isoenzymes, was analyzed by assessing the linkage with the *B1* (Awnless) marker gene located in the long arm of chromosome 5A. The results are based on an F_2 and $F_{\rightarrow\infty}$ Delta × Selection 2092 study. The *b-Amy-A1* locus showed 6.55 ± 2.10% linkage to the *B1* gene. In parallel, alleles of the *Rht8* locus, which causes dwarfing of plants, were identified and its linkage to the *C* gene (Compact spike) was established in 22.59 ± 6.28% (F_2) and 24.22 ± 1.18% ($F_{\rightarrow\infty}$) of recombination in chromosome 2D. The Delta cultivar carries the *Rht8a* allele, and the Selection 2092 accession carries the *Rht8c* allele. A collection of winter common wheat cultivars created and released in the Russian Federation was studied. The selected cultivars carried identical *b-Amy-B1a* and *b-Amy-D1a* alleles of the 4B and 4D chromosome loci. At the same time, the most common *b-Amy-A1a* and *b-Amy-A1b* alleles of chromosome 5A differed. It was found that in the direction from south to north in European Russia, the frequency of the *b-Amy-A1a* allele increases from 24.2% (Northern Caucasus) to 72.50% (Moscow–Ulyanovsk oblasts). Accordingly, the frequency of the alternative allele *b-Amy-A1b* decreased from 75.8% (North Caucasus) to 25.0% (Moscow–Ulyanovsk oblasts). In Rostov oblast, the ratio of the occurrence of alleles *b-Amy-A1a* to *b-Amy-A1b* was 42.5–57.5%. At the same time, already in the Central Chernozem region, significant dominance of the *b-Amy-A1a* allele over *b-Amy-A1b* was manifested. Using the collection of cultivars as a population of $F_{\rightarrow\infty}$ demonstrated the linkage of *b-Amy-A1* with *B1* value in 5.56 ± 1.90% recombination.

Keywords: common wheat, beta-amylase, isoenzymes, genetic control, chromosomal location, marker genes

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INTRODUCTION

Isoenzymes A and B of beta-amylase are the most frequent in winter common wheat cultivars [1]. The isoenzymes of beta-amylase are controlled by three loci: *b-Amy-A1*, *b-Amy-B1,* and *b-Amy-D1*, which are located on chromosomes 5AL, 4BL, and 4DL, respectively [2–5]. Differences in isoenzymes A and B were monogenic [1]. Later it was shown that differences in the slow-migrating band on beta-amylase zymogram patterns occur due to alleles of the *b-Amy-A1* gene [6]. Therefore, we first aimed to establish the chromosomal control that determines the differences between zymotypes A and B of beta-amylase. Another goal was to identify patterns in the distribution of alleles controlling variants A and B of this enzyme in the varieties of European part—in Russia.

MATERIALS AND METHODS

The *B1/b1* genes (awnless/awn) of chromosome 5A were used as marker genes in order to identify the chromosome responsible for the synthesis of A and B beta-amylase isoenzymes. Awnlessness is controlled by the dominant *B1* gene located on the long arm of chromosome 5A [7, 8]. Winter wheat cultivars Delta and Selection 2092 were used as the parents. The maternal form Delta is 92 cm in height and has a loose spike with awns. The paternal parent Selection 2092 is 85 cm in height and has a compact spike without awns. According to the isoenzyme composition of beta-amylase, the parents differ as shown in Fig. 1. Accordingly, Delta has isoenzyme A, and Selection 2092 has isoenzyme B.

Since the parental forms differed in other qualitative characteristics, their genetic control was also evaluated. It is known that the spike density in wheat is controlled by the dominant *C* gene, which is localized on the long arm of chromosome 2D [9, 10]. *Rht* genes control reduced height in wheat; dwarfing alleles include gibberellin-insensitive *Rht-B1*, *Rht-D1 and* gibberellin-sensitive *Rht4–Rht22* loci [2].

Beta-amylase was extracted from mature grains that were preliminary crushed with pliers. 250 μL of 3% Na_2SO_3 solution was added to test tubes with

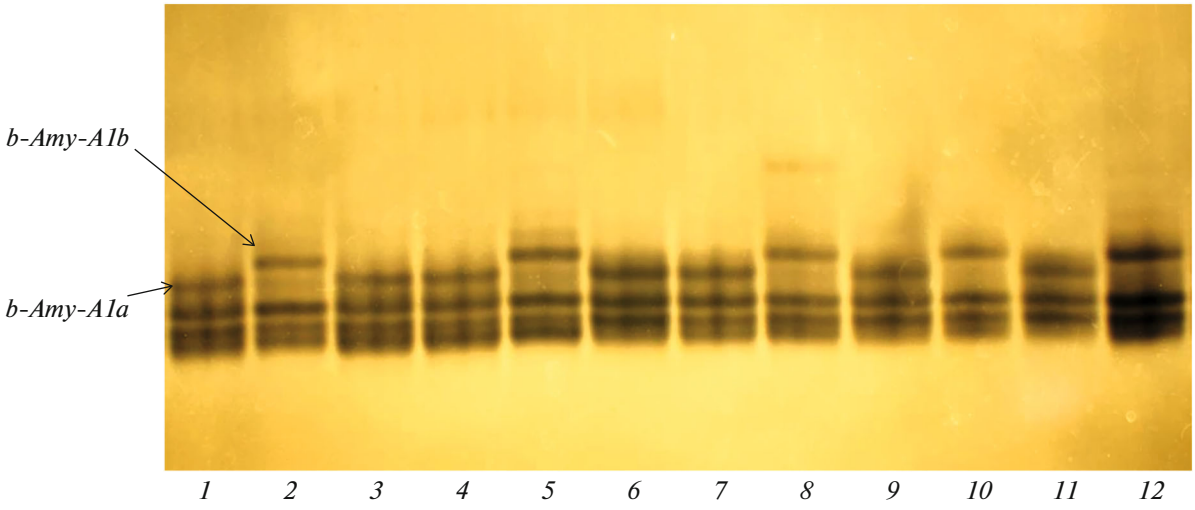

Fig. 1. Types of beta-amylase zymograms for parents Delta (*1*) and Selection 2092 (*2*), homozygous generation $F_{\rightarrow\infty}$ obtained from cross of the parents (*3–12*). The arrows indicate beta-amylase isoenzymes that are controlled by chromosome 5A. Alleles *b-Amy-A1b* (Selection 2092) and *b-Amy-A1a* (Delta).

crushed grains and left overnight. After grinding the grains in test tubes with a stainless steel rod, the resulting suspension was centrifuged for 4 min at 10000 rpm. Then, 10 μL of the supernatant fluid were taken into clean test tubes, and 10 μL of a solution containing 2% β-mercaptoethanol, 40% sucrose, and 0.03% bromophenol blue were added. 5 μL of extract were brought to the starting line of the zymography gel. The conditions of electrophoresis and the composition of the gel components are similar to those previously described for barley [11]. The separating gel contained acrylamide 4.88 g, methylenebisacrylamide 130 mg, tris 162.5 mg, glycine 0.98 g, ammonium persulfate 41 mg, distilled water—up to 65 mL, and TEMED 24 μL. The running buffer (1 L, pH 8.3) contained 1.2 g of tris and 5 g of glycine. The applied voltage for electrophoresis was 300 V. The electrophoretic separation was stopped—after the release of bromophenol blue 1.5 times (1.5 h). Amylase was incubated in an acetate buffer, pH 5.7. The acetate buffer contained 2.7 g of sodium acetate, 50.3 mL of 0.2 M acetic acid and was brought to 300 mL with water. 3–5 g of hydrolyzed potato starch was added to this solution, and the suspension was brought to a boil with constant stirring. Incubation time was 20–25 min. All the procedures were performed at room temperature. At the end of incubation, the gels were washed with running water and stained with iodine-potassium iodide solution containing KI, 2.5 g; I (crystalline), 1.3 g; TCA, 25.2 g; and water, up to 500 mL.

Linkage was estimated using the χ^2 test [12, 13]. The recombination fraction in a segregating population was estimated by the maximum likelihood method [14]. Linkage within homozygous populations of $F_{\rightarrow\infty}$ generations was estimated using the formulas presented previously [15, 16].

The frequencies of alleles were estimated as follows: 1 for a cultivar that has two identical alleles, and if a cultivar is heterozygous and the alleles are different, then quantity of each allele is 0.5. Errors in allele frequency estimates were calculated for diallelic distribution [12]. Difference significance was estimated using Student's test [12].

RESULTS AND DISCUSSION

Table 1 shows the results of genetic analysis for some morphological traits in F_2 Delta × Selection 2092.

It is seen that plant height (*Rht/rht*) was inherited independently of the trait that determines awned and awnless spike (*B1/b1*). Similarly, the traits of compact or loose spike (*C/c*) were also inherited independently. Fig. 2 illustrates the manifestation of these traits.

Meanwhile, the traits that determine plant height and spike density revealed genetic linkage. Hence, the *rht* locus is located on chromosome 2D at a distance of 22.59 ± 6.28% recombination from the *C* gene (compact spike). Based on a list of the genes that determine wheat height [2], this segregation is associated with differences in the alleles of the *Rht8* gene located on the short arm of chromosome 2D [17, 18]. Taking into account the phenotypic manifestation of plant height based on the results of genetic analysis, it is concluded that the Delta cultivar carries the *Rht8a* gene in chromosome 2D. The Selection 2092 parent carries the *Rht8c* allele, which reduces wheat height by approximately 7 cm [19].

This combination of cross was also investigated in subsequent generations after self-pollination ($F_{\rightarrow\infty}$). The results are shown in Table 2.

Table 1. Morphological features of F_2 Delta × Selection 2092 cross

Alleles	Phenotypical classes in F_2 (study of F_3)				Sample size	χ^2_L	Recombination, %
$\frac{A}{a} \times \frac{B}{b}$		BB	Bb	bb			
$\frac{Rht}{rht} \times \frac{B1}{b1}$	A- aa	10 3	19 4	13 7	56	2.13	Independent
$\frac{Rht}{rht} \times \frac{C}{c}$	A- aa	6 9	22 4	14 1	56	13.81	22.59 ± 6.28
$\frac{B1}{b1} \times \frac{C}{c}$	AA Aa aa	4 5 6	7 10 9	2 8 5	56	1.75	Independent

Self-pollinating populations are more convenient because they are homozygous, and the lack of heterozygotes eliminates possible errors in the classification of phenotypes. Furthermore, the lack of heterozygotes improves informativity of such samples. This genetic analysis confirmed linkage between the *C* and *Rht8* genes. The linkage value between the *C* and *Rht8* genes (24.22 ± 1.18%) is close to the linkage in the F_2 analysis (Table 1), i.e., 22.59 ± 6.28%. The population $F_{\rightarrow\infty}$ Delta × Selection 2092 confirmed that the *B1* and *Rht8a* genes as well as *B1* and *C* are inherited independently (Table 2).

Since beta-amylase isoenzymes differ in the parental forms Delta and Selection 2092 (Fig. 1), we analyzed the inheritance of these enzyme variants. The allele that is responsible for a component of beta-amy-

Fig. 2. Segregation according to spike types in $F_{\rightarrow\infty}$ Delta × Selection 2092 (*1*–*4*): *1*, type of spike specific for Selection 2092; *4*, type of spike specific for Delta cultivar.

Table 2. Genetic analysis of $F_{\to\infty}$ Delta × Selection 2092 generation

Alleles	Phenotypical classes in F_4			Sample size	χ^2_L	Recombination, %
$\frac{A}{a}\times\frac{B}{b}$		BB	bb			
$\frac{C}{c}\times\frac{Rht8a}{Rht8c}$	AA aa	35 20	11 29	95	11.47	24.22 ± 1.18
$\frac{B1}{b1}\times\frac{Rht8a}{Rht8c}$	AA aa	25 30	17 23	95	0.01	Independent
$\frac{B1}{b1}\times\frac{C}{c}$	AA aa	19 27	23 26	95	0.27	Independent
$\frac{B1}{b1}\times\frac{b\text{-}Amy\text{-}A1a}{b\text{-}Amy\text{-}A1b}$	AA aa	38 7	4 46	95	56.10	6.55 ± 2.10

lase, which migrates faster in the slow-migrating band of the zymogram, was designated as *b-Amy-A1a*, and the slower migrating component in this band was designated as *b-Amy-A1b*. The ratio of homozygous phenotypes in $F_{\to\infty}$ by the *b-Amy-A1a* and *b-Amy-A1b* alleles was 45 : 50 (Table 2), which corresponds to monogenic inheritance ($\chi^2_{1:1}$ = 0.16; $P > 0.50$). These zymotypes revealed linkage to the *B1* gene. Thus, phenotypes carrying the *b-Amy-A1b* allele were usually awnless; in contrast, carriers of the *b-Amy-A1a* allele had awns more frequently. The linkage value of the *b-Amy-A1* and *B1* loci was 6.55 ± 2.10% (Table 2).

Therefore, the differences in beta-amylase isoenzymes carrying zymotypes A and B are associated with the *b-Amy-A1a* and *b-Amy-A1b* alleles of chromosome 5A, which controls the synthesis of this enzyme.

In winter common wheat, the *b-Amy-A1a* and *b-Amy-A1b* alleles are the most common. The cultivars were less diverse by the remaining loci controlling the synthesis of beta-amylase. Typically, carriers of the *b-Amy-A1a* and *b-Amy-A1b* alleles carried the same alleles at the remaining loci designated here as *b-Amy-B1a* and *b-Amy-D1a*. Therefore, it is possible to evaluate the zonal distribution of the *b-Amy-A1a* and *b-Amy-A1b* alleles of chromosome 5A in carriers of the *b-Amy-B1a* and *b-Amy-D1a* alleles of chromosomes 4B and 4D.

To establish the features of the zonal distribution of the *b-Amy-A1a* and *b-Amy-A1b* alleles when the *b-Amy-* genes on chromosome 4B and 4D are the same, we investigated genotypes of winter common wheat cultivars used as crops in agricultural production [20] that carry only the indicated alleles (Table 3). Therefore, this combination of cultivars was selected twice by the environmental conditions of the respective regions. First, selection in regional breeding centers and, secondly, selection as a result of official trials conducted in these regions. Among this combination of cultivars, several contained a new allele designated as *b-Amy-A1c* that controls the synthesis of the least mobile isoenzyme in the slow band of the beta-amylase zymogram. The remaining components of the zymogram of this enzyme were identical to the carriers of zymotypes A and B (Fig. 3).

Winter common wheat cultivars were sorted out into the groups created in the regions of the European part of Russia from south to north. The estimates for the *b-Amy-A1a* and *b-Amy-A1b* allele frequencies in these groups are shown in Table 4.

It is seen that in the south of European Russia (region I, North Caucasus), the *b-Amy-A1b* allele that

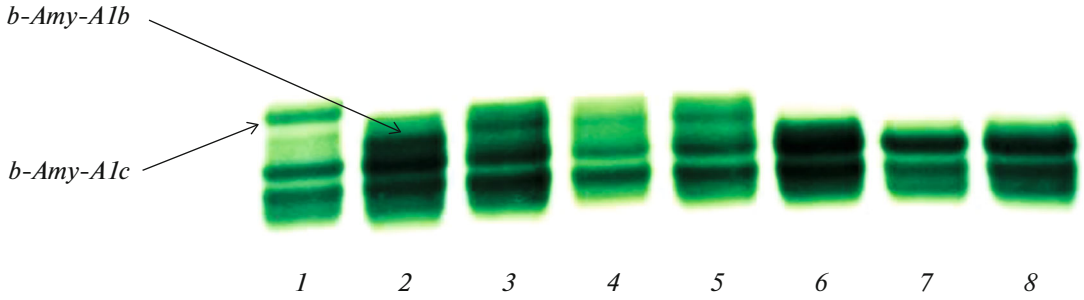

Fig. 3. Zymograms of some winter common wheat cultivars (*1*, *2*, Dar Zernograda, *3–5*, Donskoi mayak, *6–8*, Stanichnaya) that carry alleles of the *b-Amy-A1* locus: *1*, *A1c*, *2–5*, *A1b*, *6–8*, *A1a*.

Table 3. Differentiation of winter common wheat based on the *B1/b1* genes and beta-amylase isoenzymes associated with the *b-Amy-A1* locus

No.	Cultivar	Phenotypes according to genes		Originator
		B1/b1	*b-Amy-A1*	
1	Aksin'ya	b1b1	A1a	Donskoy, Agricultural Research Center, Zernograd
2	Al'meraa	b1b1	A1a	Shestopalov I.O., Belgorod
3	Armada	b1b1	A1a	Federal Research Agricultural Center, Stavropol, 53
4	Ariadna	b1b1	A1a + **A1b**	Federal Agricultural Center, Belgorod, 5829
5	Akapello	b1b1	**A1b**	Federal Rostov Agricultural Center, Rostov, 223
6	Asket	b1b1	A1a + **A1b**	Donskoy, Agricultural Research Center, Zernograd
7	Belgorodskaya 16	b1b1	A1a	Belgorod State Agricultural University, Belgorod, 133
8	Bogdanka	b1b1	A1a*	Federal Agricultural Center, Belgorod, 5829
9	Vezelka	b1b1	A1a	Federal Agricultural Center, Belgorod, 5829
10	Viktoria	b1b1	**A1b**	Federal Research Agricultural Center, Stavropol, 53
11	Volzhskaya K	b1b1	A1a	Selektsiya, Research and Production Center, Ulyanovsk
12	Volzhskaya S3	b1b1	A1a	Selektsiya, Research and Production Center, Ulyanovsk
13	Volzhskaya 100	b1b1	**A1b**	Selektsiya, Research and Production Center, Ulyanovsk
14	Vol'nitsa	b1b1	A1a	Donskoy, Agricultural Research Center, Zernograd, 5917
15	Gubernator Dona	b1b1	A1a + **A1b**	Federal Rostov Agricultural Center, Rostov, 223
16	Donskaya step'	b1b1	A1a	Donskoy, Agricultural Research Center, Zernograd
17	Don 107	b1b1	A1a	Donskoy, Agricultural Research Center, Zernograd
18	Donera	b1b1	A1a	Federal Rostov Agricultural Center, Rostov, 223
19	Ermak	b1b1	A1a*	Donskoy, Agricultural Research Center, Zernograd
20	Zhavoronok	b1b1	A1a	Donskoy, Agricultural Research Center, Zernograd
21	Zarnitsa	b1b1	A1a	Donskoy, Agricultural Research Center, Zernograd 5917
22	Zvonnitsa	b1b1	A1a	Shestopalov I.O., Belgorod
23	Kapitan	b1b1	A1a	Donskoy, Agricultural Research Center, Zernograd
24	Korochanka	b1b1	A1a	Federal Agricultural Center, Belgorod, 5829
25	Korotyshka	b1b1	A1a	Federal Agricultural Center, Belgorod, 5829
26	Larisa	b1b1	A1a	Federal Agricultural Center, Belgorod, 5829
27	Levoberezhnaya 3	b1b1	A1c	Federal Center of Agriculture Research of the SoutheastRegion, Saratov
28	L'govskaya 8	b1b1	A1a	Experimental Breeding Station, L'gov, 95
29	Magia	b1b1	A1a	Federal Rostov Agricultural Center, Rostov, 223
30	Maiskaya yubileinaya	b1b1	A1a	Belgorod State Agricultural University, Belgorod 133
31	Moskovskaya 56	b1b1	A1a	Nemchinovka, Federal Research Center, Moscow, 168
32	Moskovskaya 40	b1b1	A1a	Nemchinovka, Federal Research Center, Moscow, 168
33	Nemchinovskaya 17	b1b1	A1a	Nemchinovka, Federal Research Center, Moscow, 168
34	Nemchinovskaya 57	b1b1	A1a	Nemchinovka, Federal Research Center, Moscow, 168
35	Oksana	b1b1	**A1b**	Breeding and Genetics Institute, Odessa
36	Partner	b1b1	A1a	Federal Research Agricultural Center, Stavropol, 53
37	Pionerskaya 32	b1b1	**A1b**	Orenburg State Agrarian University
38	Praskov'ya	b1b1	A1a	National Grain Center, Krasnodar, 25
39	Rostovchanka 3	b1b1	A1a	Donskoy, Agricultural Research Center, Zernograd
40	Rostovchanka 7	b1b1	A1a	Donskoy, Agricultural Research Center, Zernograd
41	Sirena	b1b1	A1a	Federal Agricultural Center, Belgorod, 5829
42	Sintetik	b1b1	A1a + **A1b**	Federal Agricultural Center, Belgorod, 5829

Table 3. (Contd.)

No.	Cultivar	Phenotypes according to genes		Originator
		B1/b1	*b-Amy-A1*	
43	Severo-donetskaya yubileinaya	b1b1	A1a	Federal Rostov Agricultural Center, Rostov, 223
44	Sofiika	b1b1	A1a	Breeding and Genetics Institute, Odessa
45	Stanichnaya	b1b1	A1a	Donskoy, Agricultural Research Center, Zernograd
46	Surava	b1b1	A1a	Shestopalov I.O., Belgorod
47	Tarasovskaya 70	b1b1	A1a	Federal Rostov Agricultural Center, Rostov, 223
48	Shef	b1b1	A1a	Donskoy, Agricultural Research Center, Zernograd
49	Chernobrova	b1b1	**A1b** + A1a	Breeding and Genetics Institute, Odessa
50	Avesta	B1B1	A1b	Federal Rostov Agricultural Center, Rostov, 223
51	Agra	B1B1	A1b	Federal Rostov Agricultural Center, Rostov, 223
52	Alekseich	B1B1	A1b	National Grain Center, Krasnodar, 25
53	Akhmat	B1B1	A1b + **A1a**	National Grain Center, Krasnodar, 25
54	Bezenchukskaya 380	B1B1	A1b	Samara Federal Research Center, Russian Academy of Sciences, Samara, 21181
55	Bezostaya 100	B1B1	A1c	National Grain Center, Krasnodar, 25
56	Brigada	B1B1	A1b	National Grain Center, Krasnodar, 25
57	Viktoria 95	B1B1	A1b	Federal Center of Agriculture Research of the SoutheastRegion, Saratov
58	Vol'nyi Don	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd, 5917
59	Grom	B1B1	A1b	National Grain Center, Krasnodar, 25
60	Gomer	B1B1	A1b	National Grain Center, Krasnodar, 25
61	Dar Zernograda	B1B1	A1b + A1c	Donskoy, Agricultural Research Center, Zernograd
62	Deviz	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd
63	Dolya	B1B1	**A1a** + A1c	National Grain Center, Krasnodar, 25
64	Don 95	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd, 5917
65	Dominanta	B1B1	A1b	Federal Rostov Agricultural Center, Rostov, 223
66	Don 105	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd
67	Donstar	B1B1	A1b	Federal Rostov Agricultural Center, Rostov, 223
68	Donskoi mayak	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd, 5917
69	Donskoi syurprize	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd
70	Deviz	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd
71	Dzhangal'	B1B1	A1b	Federal Center of Agriculture Research of the Southeast Region, Saratov
72	Donshchina	B1B1	**A1a**	Donskoy, Agricultural Research Center, Zernograd
73	Ershovskaya 11	B1B1	**A1a**	Federal Center of Agriculture Research of the SoutheastRegion, Saratov
74	Zhemchuzhina Povolzh'ya	B1B1	A1b	Federal Center of Agriculture Research of the Southeast Region, Saratov
75	Zernogradka 9	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd
76	Zernogradka 11	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd
77	Zimnitsa	B1B1	A1c	National Grain Center, Krasnodar, 25
78	Zolushka	B1B1	A1b	Federal Rostov Agricultural Center, Rostov, 223
79	Izyuminka	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd
80	Kalach	B1B1	A1b	Federal Center of Agriculture Research of the Southeast Region, Saratov
81	Kaprizulya	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd
82	Konkurent	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd

Table 3. (Contd.)

No.	Cultivar	Phenotypes according to genes		Originator
		B1/b1	*b-Amy-A1*	
83	Krasa Dona	B1B1	A1b + **A1a**	Donskoy, Agricultural Research Center, Zernograd
84	Laureat	B1B1	A1b	National Grain Center, Krasnodar, 25
85	Levoberezhnaya 1	B1B1	A1c	Federal Center of Agriculture Research of the Southeast Region, Saratov
86	Lilit	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd
87	L'govskaya 4	B1B1	A1b	Experimental Breeding Station, L'gov, 95
88	Marafon	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd
89	Melodia	B1B1	**A1a**	Agricultural Scientific Center, Omsk
90	Morozko	B1B1	A1b	National Grain Center, Krasnodar, 25
91	Nakhodka	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd
92	Nemchinovskaya 24	B1B1	A1b	Nemchinovka, Federal Research Center, Moscow, 168
93	Niva Stavropol'ya	B1B1	A1b	Federal Research Agricultural Center, Stavropol, 53
94	Omskaya 5	B1B1	**A1a**	Agricultural Scientific Center, Omsk
95	Omskaya 6	B1B1	A1b	Agricultural Scientific Center, Omsk
96	Paritet	B1B1	A1b	Federal Research Agricultural Center, Stavropol, 53
97	Tabor	B1B1	A1b	National Grain Center, Krasnodar, 25
98	Tanais	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd
99	Timiryazevskaya 150	B1B1	A1b	National Grain Center, Krasnodar, 25
100	Chernozemka 115	B1B1	**A1a**	Federal Agricultural Research Center, Voronezh
101	Etyud	B1B1	A1b	Donskoy, Agricultural Research Center, Zernograd
102	Yuka	B1B1	A1b	National Grain Center, Krasnodar, 25

Recombinant phenotypes by *B1*, *b-Amy-A1* alleles are in bold.

controls the synthesis of beta-amylase dominates among winter wheat cultivars. In its frequency, this allele significantly exceeds that of the *b-Amy-A1a* alternative allele (t = 4.87***). Within the Rostov oblast, the frequencies of these alleles are close to each other and the differences in frequencies are within the experimental error (Table 4). Northward (region IV, Central Chernozem region + Lower Volga region), the *b-Amy-A1a* allele dominates and the frequency of *b-Amy-A1b* is decreased. In this case, the differences in the frequencies of these alleles are significant: t = 4.32**. A similar situation is observed further northward. Thus, at the latitude of the Moscow oblast (region V, northern Central Federal District + Middle Volga region), the frequency of the *b-Amy-A1a* allele was 72.5%, and that of the alternative allele was only 25.0%. Differences in frequencies are significant, t = 3.27* (Table 4).

Next, we assessed allele frequency variations among the selected regions. Herein, the most contrasting differences were observed when geographically more distant regions were compared. For example, a comparison of the *b-Amy-A1b* allele frequency in region V (northern Central Federal District + Middle Volga region) with the frequency of this allele in region I (Northern Caucasus) revealed a significant decrease in its frequency from south to north, i.e., by 50.8% (t = 2.60*; P > 0.95). A comparison of this allele frequency in region IV (Central Chernozem region + Lower Volga region) and region I (North Caucasus) revealed similar regularities. As well, its frequency from south to north decreased by 47.2% (t = 3.26**; P > 0.99). At the same time, the differences between geographically close regions were insignificant. Thus, variations in frequencies of the *b-Amy-A1a* allele between regions V (Northern Central Federal District + Middle Volga region) and IV (Central Chernozem region + Lower Volga region) were only 1.1% (t = 0.06; P < 0.95).

In general, the *b-Amy-A1b* allele frequency tends significantly to a decrease and the frequency of the *b-Amy-A1a* alternative allele increases from south to north of European Russia.

It had been shown that the collection of self-pollination cultivars can be characterized as a homozygous $F_{\rightarrow\infty}$ population. In this case, such population may be used to evaluate linkage of the genes located in the same linkage group [21]. Hence, since wheat cultivars were grouped according to traits awn/awnless spike (genes *b1* and *B1*), as well as according to the *b-Amy-A1a*

Table 4. Distribution of the *b-Amy-A1* locus alleles in winter common wheat cultivars released in the Russian Federation that were combined into groups according to the altitude origin

Regions	Occurrence of alleles of the *b-Amy-A1* locus in the European parts of the Russian Federation from south to north				Error in occurrence frequency	Significance (*t*)
	quantity of cultivars with alleles		allele frequency			
	A1a	*A1b*	*A1a*	*A1b*		
I. Northern Caucasus (National Grain Center, Krasnodar; Federal Research Agricultural Center, Stavropol)	4	12.5	0.242	0.758	0.106	**4.87*****
II. South of the Rostov oblast (Donskoy Agricultural Research Center)	14	18.5	0.431	0.569	0.087	1.59
III. North of the Rostov oblast (Federal Rostov Agricultural Center, Rostov oblast)	4.5	6.5	0.409	0.591	0.148	1.23
IV. Central Chernozem Region + Lower Volga region (Federal Agricultural Center, Belgorod; Experimental Breeding Station, L'gov, Saratov)	15	6	0.714	0.286	0.099	**4.32****
V. Central Federal District + Middle Volga region (Nemchinovka, Moscow; Selektsiya, Research and Production Center, Ulyanovsk)	6	2	0.725	0.250	0.153	**3.27***
Total	43.5	45.5	0.489	0.511	0.053	0.415

Significant differences between frequencies are in bold.
*, **, *** Probabilities are 0.95, 0.99, and 0.999, respectively.

Table 5. Analysis of $F_{\rightarrow\infty}$ winter common wheat for estimation of linkage between loci *B1/b1* and *b-Amy-A1*

Alleles	Phenotypic classes in F_4			Sample size	χ^2_L	Recombination, %
$\frac{A}{a}\times\frac{B}{b}$		BB	bb			
$\frac{B1}{b1}\times\frac{b\text{-}Amy\text{-}A1a}{b\text{-}Amy\text{-}A1b}$	AA aa	6.5 39.5	42.5 7.0	95.5	49.13	5.56 ± 1.90

and *b-Amy-A1b* alleles located on chromosome 5A, we estimated occurrence of the corresponding phenotypes and determined the amount of linkage between the *B1* and *b-Amy-A1* loci.

The ratio of the phenotypes shown in Table 3 was as follows: b1b1 + A1a : b1b1 + A1b : B1B1 + A1a : B1B1 + A1b = 39.5 : 7.0 : 6.5 : 42.5. Segregation in $F_{\rightarrow\infty}$ according to the expected segregation at monogenic inheritance should be 1 : 1. Compliance of segregation by awn/awnless to this ratio of phenotypes was expressed by the value $\chi^2_{b1} = 0.063$, which is significant at $P > 0.25$. Alleles of the *b-Amy-A1* locus reveal close values $\chi^2_{b\text{-}Amy\text{-}A1} = 0.124$, $P > 0.25$. In digenic inheritance (segregation 1 : 1 : 1 : 1), the value $\chi^2_o = 49.32$; $P < 0.01$. This indicates that these genetic factors deviate from independent inheritance. Hence, Table 5 shows the value of χ^2 associated with linkage of the studied loci $\left(\chi^2_L\right)$.

The recombination value calculated on the basis of the studied collection of winter common wheat was 5.56 ± 1.90%.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest.

This article contains no studies involving animals or human participants performed by any of the authors.

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