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Allelic Variants of the Gene *bamy1* Barley in Eastern European and Central Asian Areas

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Abstract—Collections of varieties of spring barley cultivars from Eastern European and Central Asian areas were analyzed by exon-specific PCR (EPIC) for β-amylase genes. The endosperm β-amylase gene (*bamy*1) was differentiated by the presence of 126 bp MITE insertion into intron 3, which is associated with low activity β-amylase. The findings suggest that a low level of genetic variation for *bamy*1 gene within climatic zones is associated with individual breeding program for each climatic zone.

Keywords: β-amylase, intron, genetic diversity

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INTRODUCTION

Barley is one of the widely spread and highly productive crops. Barley seeds are used as the raw material for brewing and fine ground barley production and food for animals. Barley is easily adaptable to contrast climate conditions and different soils. Thus, it is cultivated in almost all regions. Vavilov et al. [1] identified three most important zones of barley cultivation in the Eurasian region: (1) Northern zone (food barley), (2) Southern zone (fodder barley) and (3) Western zone (Belorussia, Ukrainian forest steppe, northwestern part of Russia and Baltic countries) (brewing barley). However, this differentiation is very relative, because it does not exclude universal use of barley in regions. Barley breeds of the Western zone are the best corresponding to the requirements for brewing, because seeds containing a high level of polysaccharides but low level of protein are formed in this area. These kind of seeds are the most appropriate for brewing [1]. Grain with good malt properties is the main prerequisite for production of good malt [2]. Diastatic activity, i.e., amylolytic activity, which is considered to be one of the key parameters that determine malt properties, is also important for brewing industry [3]. β-amylase (1,4-α-D-glucanmaltohydrolase) is the key amylolytic enzyme. β-amylase cleaves α-1,4-glycoside bonds of starch during the seed sprouting leading to the formation of high molecular weight dextrins and disaccharide maltose, which diffuse easily and may be utilized by the sprouting corcule [4]. Maltose is also the main component of malt [5]. The endospermal β-amylase is encoded by the *bamy1* gene, which is located in the long shoulder of the chromosome 4H [6].

It seems important to study variability of the gene that encodes endospermal β-amylase of barley. Method of EPIC-PCR, i.e., exon-primed amplification of introns, is presently successfully used in genetic selection studies [7]. Several studies showed that the presence of 126 bp MITE-insertion (Stowaway transposon) in the third intron of the *bamy1* gene of barley endospermal β-amylase negatively affects the activity and thermostability of the enzyme [8–14]. Miniature inverted-repeat transposable element (MITE) belongs to the class of short dependent transposons often located near genes or within introns [15–21]. The activity of transposons located near genes may affect gene expression right up to its total inactivation or lead to duplication in case of unequal crossingover [22]. The effect of 126 bp MITE-insertion in the intron 3 on the synthesis of low activity β-amylase was confirmed in the studies of the activity of this enzyme barley grain [8, 13]. Therefore, this transposition may be used for creation of a PCR-marker for detection of low activity forms of β-amylase [8–14].

The goal of our study was to perform PCR-analysis of the allelic condition of the *bamy1* genes of barley breeds obtained from the collection of spring barley from Eastern European and Central Asian regions.

MATERIALS AND METHODS

The object of the study was the collection of 249 breeds of spring barley, which were regionalized at different time on the territories of East European and

† Deceased.

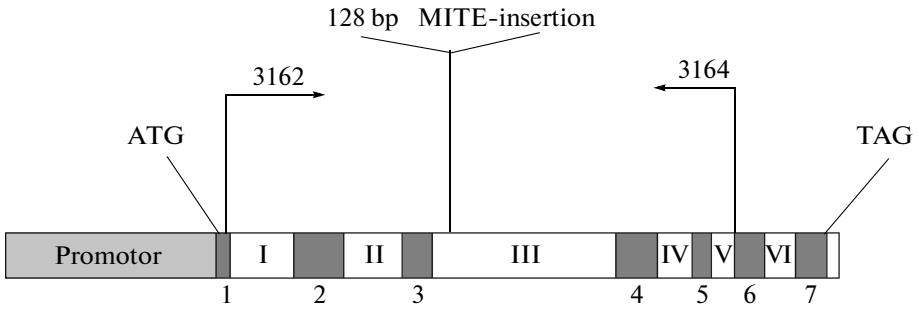

Fig. 1. Localization of primers in the *bamy1* locus: (1–7) exons, (I–VI) introns, (ATG) translation initiation site, and (TAG) stop-codon. Primers used in the study and their orientation are shown in the upper part of the figure.

Central Asian zones. The collection was provided by V.P. Netsvetaev and A.A. Pomortsev (collected at Vavilov Research Institute of Plant Industry). Catalogue numbers of the studied samples and their genotypes by the mentioned locus are shown in Table 1 (http://cytgen.com/articles/4920011s.pdf).

DNA was isolated from 5-day-old bleached-out seedlings with cetyltrimethylammonium bromide buffer (CTAB) that consisted of 2 M NaCl, 20 mM Na_3EDTA, 100 mM Tris-HCl, pH 8.0 at 25°C and 2% CTAB. DNA of five individual seedlings was combined in blocks. The concentration of DNA was measured with DNA-fluorimeter (Hoefer, United States).

The EPIC-primers specific to β-amylase genes were designed on the basis of multiple alignment of β-amylase gene sequences using the Multain program [23]. To perform the study, we used 45 DNA sequences of β-amylase genes obtained from NCBI database (screening was performed with BLAST [24]). Universal EPIC-primers for β-amylase genes were designed with the FastPCR program [25]. Primers were synthesized by Eurofins MWG Operon (Germany) and were of high purity salt free purification (HPSF) quality.

The following EPIC-primers were used for the study: 3162 (5'-TCCAAGTCTACGTCATGCTCC-3', primer localization 1389 ⟶ 1409 in exon 1 of the *bamy1* gene) and 3164 (5'-CAGCCGGAGGTAG-GTGAATCC-3', primer localization 4646 ⟵ 4666 in exon 6 of the *bamy1* gene).

PCR reaction mixture (20 μL) contained 50 mM KCl, 20 mM Tris-HCl, pH 8.4 (20°C), 2 mM $MgCl_2$, 0.01% Tween 20, 0.2 mM dNTP, 300 nM of each primer, 20 ng DNA, and 1 unit of *Taq*-polymerase.

The amplification conditions were as follows: first denaturation for 2 min, 95°C; denaturation for 20 s, 95°C; primer annealing for 60 s, 65°C; elongation for 2 min, 72°C; final elongation for 5 min, 72°C; 32 cycles in total. The amplification was carried out with Tertsik amplifiers (DNA-Technology, Russia).

The amplification products were analyzed by electrophoresis in 1% agarose gel stained with ethidium bromide and photographed with a digital camera equipped with an orange light filter.

RESULTS AND DISCUSSION

The analysis of 249 spring barley breeds covered nine cultivation regions. East European zone included European, Baltic, Belorussian, Middle Russian, Southern Russian, and Pre-Ural regions. Central Asian zone included Western Siberian, Far Eastern Amur-Ussuri, and Pre-Altai regions.

The present study of β-amylase genes was performed using a pair of specific primers aimed at the region between exon 1 and exon 6 of the *bamy1* gene and the same size region of the *bamy2* gene (Fig. 1). It was expected that analysis of such a long motive of barley β-amylase gene may reveal greater genetic diversity of *Hordeum vulgare* in the large collection analyzed. The primers studied were complementary to the highly conservative exons of genes of endospermal and general β-amylase. These primers were used in order to assess variability of the *bamy1* gene introns.

Amplification of DNA of barley breeds of the collection studied with the primers 3162 and 3164 revealed two alleles in the *bamy1* locus with PCR-fragments lengths 3278 and 3152 bp. By the *bamy2* locus, barley breeds were homogenous and contained 2260 bp PCR-fragments. The calculated length of DNA amplification products was obtained using the FastPCR program [25]. PCR-products of the *bamy1* gene differed from one another by the presence of 126 bp MITE-insertion in the third intron (Fig. 2).

PCR-analysis with EPIC-primers showed that barley breeds of the studied collection carried two types of the *bamy1* alleles: with (3278 bp allele) and without (3152 bp allele) 126 bp MITE-insertion. No other alleles have been found in the large region of the *bamy1* locus analyzed with specific primers (Table 1). It was confirmed that the difference between barley genotypes in the *bamy1* locus is due to 126 bp transposition of MITE-element.

Frequency distribution of the *barley1* in the studied samples was calculated in accordance with the Hardy–Weinberg equilibrium (if a breed was homogenous, the obtained allele frequency was taken as 1, if not, it was taken as 0.5). The *bamy1* allele frequency in representatives of the collection studied was 32% for

Table 1. Allelic variants of the *bamy1* gene in the collection of spring barley breeds cultivated in East Europe and Central Asia

No.	Collection number	Gene alleles β-*amy1*: **0** – 3152 bp, **1** – 3278 bp, **2** – 3152 + 3278 bp	Country/County/Region	Zone
1	k-2544	2	Estonia	Baltic
2	k-16921	0		
3	k-2143	2		
4	k-2787	2		
5	k-2548	0		
6	k-2547	0		
7	k-10607	1		
8	k-16926	0		
9	k-16925	2	Latvia	
10	k-16868	0		
11	k-17925	1		
12	k-17920	1		
13	k-17915	1		
14	k-16816	0	Lithuania	
15	k-1272	0		
16	k-1082	0		
17	k-357	0		
18	k-9379	0		
19	k-9355	1		
20	k-9349	0		
21	k-9338	0	Arkhangelsk oblast	European
22	k-9332	0		
23	k-9316	0		
24	k-9530	0		
25	k-9536	0		
26	k-9537	0		
27	k-9687	2		
28	k-9456	0		
29	k-9462	1		
30	k-9466	0	Komi Republic	
31	k-9460	2		
32	k-9486	0		
33	k-9475	0		
34	k-9478	0		
35	k-16380	2		
36	k-9751	0		
37	k-6433	0	Leningrad oblast	
38	k-6431	0		
39	k-9815	0		
40	k-9821	0		
41	k-4407	2		

Table 1. (Contd.)

No.	Collection number	Gene alleles β-*amy1*: **0** – 3152 bp, **1** – 3278 bp, **2** – 3152 + 3278 bp	Country/County/Region	Zone
42	k-4188	0		
43	k-2018	0		
44	k-9776	0		
45	k-9770	0		
46	k-9767	2		
47	k-9766	0		
48	k-9898	0		
49	k-9900	0	Pskov oblast	Baltic
50	k-4414	1		
51	k-4413	0		
52	k-4410	0		
53	k-4411	0		
54	k-6434	0		
55	k-16031	1		
56	k-9762	2		
57	k-9850	0	Novgorod oblast	
58	k-9852	2		
59	k-9855	1		
60	k-9864	0		
61	k-9878	0		
62	k-9860	0		
63	k-9880	0		
64	k-9885	0		
65	k-9886	1		
66	k-6459	0		
67	k-4869	0	Mari-El Republic	Middle Russian
68	k-10074	1		
69	k-4389	0	Kaliningrad oblast	Belorussian
70	k-5417	0		
71	k-5424	2		
72	k-6276	0		
73	k-6272	0		
74	k-6270	0		
75	k-6268	0		
76	k-6261	2		
77	k-6287	0		
78	k-6280	1		
79	k-4160	2	Kaluga oblast	Middle Russian
80	k-1955	1		
81	k-2193	0		
82	k-2201	1		
83	k-2202	0		
84	k-2208	1		
85	k-2223	2		
86	k-2186	0		
87	k-2234	0		
88	k-2021	1	Kursk oblast	
89	k-5340	1	Rostov oblast	Southern Russian

Table 1. (Contd.)

No.	Collection number	Gene alleles β-*amy1*: **0** – 3152 bp, **1** – 3278 bp, **2** – 3152 + 3278 bp	Country/County/Region	Zone
90	k-5338	1		
91	k-5337	1		
92	k-9213	2	Udmurtia	Pre-Ural
93	k-4637	2		
94	k-4154	0		
95	k-5108	0		
96	k-5014	0	Kuybyshev oblast	
97	k-9840	0	Vologda oblast	Middle Russian
98	k-9843	0		
99	k-9848	0		
100	k-9621	0		
101	k-9837	0		
102	k-9833	0		
103	k-9830	2		
104	k-9827	0		
105	k-9825	0		
106	k-9824	2		
107	k-6446	2	Yaroslavl oblast	
108	k-6443	1		
109	k-6441	2		
110	k-4435	0		
111	k-4433	0		
112	k-4320	0		
113	k-2697	1		
114	k-3696	2		
115	k-3694	0		
116	k-4321	2		
117	k-9509	0	Kostroma oblast	
118	k-9511	1		
119	k-9513	0		
120	k-3859	0		
121	k-3857	0		
122	k-3856	0		
123	k-3853	1		
124	k-4383	2		
125	k-4738	0	Kirov oblast	
126	k-4729	0		
127	k-4740	2		
128	k-4741	0		
129	k-9581	2		
130	k-4742	0		
131	k-9551	1		
132	k-4289	0		
133	k-16419	2		
134	k-16395	0		
135	k-4187	1	Perm oblast	Pre-Ural
136	k-4185	0		
137	k-4522	0	Belorussia	Belorussian
138	k-5321	0		
139	k-5320	2		
140	k-5315	1		
141	k-7118	0		
142	k-6741	2		

Table 1. (Contd.)

No.	Collection number	Gene alleles β-*amy1*: **0** – 3152 bp, **1** – 3278 bp, **2** – 3152 + 3278 bp	Country/County/Region	Zone
143	k-4267	0		
144	k-6420	0		
145	k-6412	1		
146	k-6410	0		
147	k-9895	2	Velikie Luki (Pskov oblast)	
148	k-9908	2		
149	k-9910	0		
150	k-9907	2		
151	k-9911	0		
152	k-6436	0		
153	k-4412	1		
154	k-5032	1	Smolensk oblast	Middle Russian
155	k-5027	2		
156	k-4881	1	Moscow oblast	
157	k-4878	0		
158	k-4877	0		
159	k-4876	0		
160	k-6439	0	Ivanovo oblast	
161	k-6438	2		
162	k-4380	2		
163	k-4375	2	Vladimir oblast	
164	k-18367	0	Sverdlovsk oblast	Western Siberian
165	k-4807	2		
166	k-18367	2		
167	k-4503	1	Tyumen oblast	
168	k-2139	2		
169	k-4961	0	Omsk oblast	
170	k-4955	2		
171	k-4959	0		
172	k-4962	2		
173	k-4972	0		
174	k-4963	1		
175	k-4964	0		
176	k-4965	0		
177	k-4968	0		
178	k-8399	0		
179	k-5043	0	Tomsk oblast	
180	k-4211	1		
181	k-4210	1		
182	k-10084	1	Novosibirsk oblast	
183	k-10336	0	Altai krai	Pre-Altai
184	k-16509	2		
185	k-16512	0		
186	k-16515	0		
187	k-16518	2		
188	k-16521	1		
189	k-5858	0		
190	k-16499	2		
191	k-5860	2		
192	k-5861	0		
193	k-18071	0	Krasnoyarsk oblast	
194	k-4812	0		
195	k-4813	1		

Table 1. (Contd.)

No.	Collection number	Gene alleles β-*amy1*: **0** – 3152 bp, **1** – 3278 bp, **2** – 3152 + 3278 bp	Country/County/Region	Zone
196	k-4814	0		
197	k-16023	2		
198	k-5824	0		
199	k-5820	0		
200	k-5819	2		
201	k-2948	1		
202	k-2925	2		
203	k-4836	2	Irkutsk oblast	
204	k-2556	0		
205	k-2935	0		
206	k-4815	0	Chita (Transbaikalia)	
207	k-4818	0		
208	k-4819	0		
209	k-4821	0		
210	k-4824	0		
211	k-4831	0		
212	k-4833	1		
213	k-10696	0	Yakutia	
214	k-10727	0		
215	k-10735	0		
216	k-8674	0		
217	k-7980	2		
218	k-7978	2		
219	k-2443	0		
220	k-5260	2		
221	k-10711	1		
222	k-11288	1	Buryatia	
223	k-6744	0		
224	k-2969	0	Amur	Far Eastern Amur-Ussuri
225	k-2965	1		
226	k-4999	1	Primorsky krai	
227	k-18645	0		
228	k-4994	1		
229	k-15130	1		
230	k-15124	1		
231	k-15123	1		
232	k-7993	1		
233	k-15147	1		
234	k-15144	1		
235	k-6376	2		
236	k-11077	0	Khabarovsk oblast	
237	k-11076	2		
238	k-11078	0		
239	k-11082	1		
240	k-18160	2	Southern Sakhalin	
241	k-3738	1	Kazakhstan	
242	k-4314	2	Karelia	
243	k-4313	0		
244	k-2174	0		
245	k-9745	0		
246	k-9743	0		
247	k-9739	2		
248	k-9734	0		
249	k-9727	0		

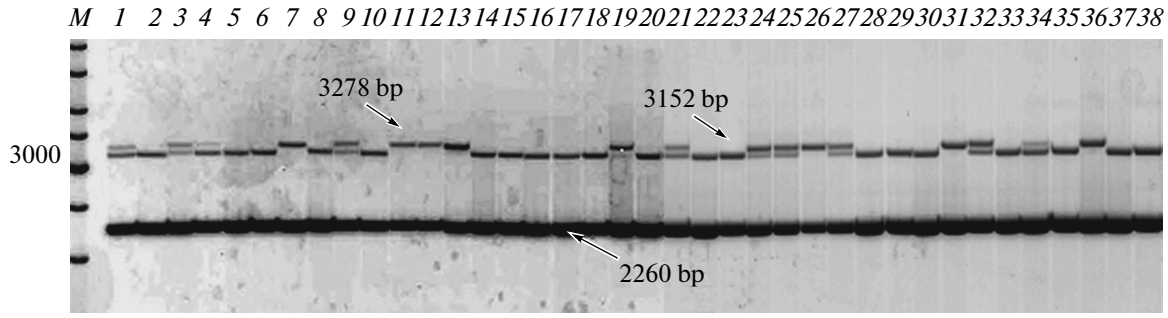

Fig. 2. Electrophoregram of DNA amplification products with the primers 3162 and 3164 of spring barley breeds cultivated in Baltic region. (M) molecular weight standard (DNA-ladder #SM1173).

the allele with molecular weight 3278 bp and 68% for the allele with molecular weight 3152 bp (Table 2).

It was shown that frequency of the *bamy1* alleles may vary significantly in the breeds of the collection studied. Although sizes of samples from different regions were not equal, it may be noted that 3152 bp *bamy1* allele, which does not carry 126 bp MITE-element in the intron 3 and is associated with high β-amylase activity, dominated in the northwestern regions of the studied territory (Table 2).

These results are consistent with the data on the soil and climate conditions of the North European region that appear to be optimal for cultivation of brewing breeds of barley [1]. Activity decreased in the direction of southeastern regions of barley cultivation frequency of the *bamy1* allele associated with β-amylase, while frequency of the allele associated with low activity of β-amylase increased conversely (Table 1).

Our study demonstrated uneven distribution of frequencies of the *bamy1* gene alleles in Eurasia. General ratio of the *bamy1* alleles, which do not carry 126 bp MITE-element in the intron 3 (high activity of β-amylase) and the alleles carrying 126 bp MITE-element (low activity of β-amylase) was approximately 2 : 1 respectively (Fig. 3a). In southern and eastern regions, this ratio was 1.5 : 1 (Fig. 3b), while it was 4 : 1 in North European and Baltic regions (zones of brewing barley cultivation) (Fig. 3c).

Study of the collection showed that geographic distribution of *bamy1* alleles depends on the heat supply of the regions in which the breeds studied regionalized. It was shown that frequency of the allele associated with high β-amylase activity (without 126 bp

Table 2. Distribution of the *bamy1* locus alleles in natural and agricultural regions

Region	Sample size	Allelic frequencies of the *bamy1* locus, %	
		3278 bp	3152 bp
East European zone			
European	28	12	88
Baltic	38	32	68
Belorussian	27	28	72
Middle Russian	60	33	67
Pre-Ural	7	29	71
Southern Russian	3	100	0
Central Asian zone			
Western Siberian	19	40	60
Far Eastern			
Amur-Ussuri	26	52	48
Pre-Altai	41	28	72
Total	249	32	68

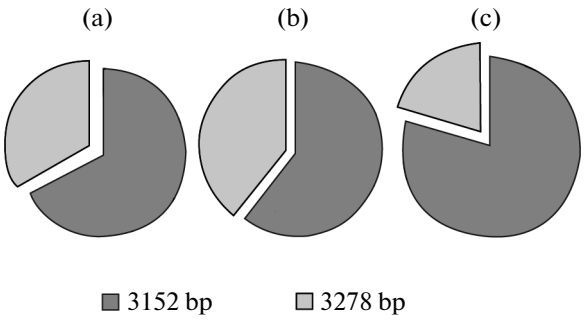

Fig. 3. Ratio of the *bamy1* gene alleles in the collection of spring barley breeds of Eurasian region: (a) breeds of all geographical zones, (b) breeds of Southern and Eastern regions, (c) breeds of North European and Baltic regions.

MITE-element) dominated in the regions with low or medium heat supply. Hence, use of EPIC-primers specific to the genes *bamy1* and *bamy2* allowed us to fulfill the comparative study of the properties of breeds cultivated in different climatic zones by the locus of endospermal β-amylase.

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