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IDENTIFICATION OF *Psy1* GENES ALLELES RESPONSIBLE FOR CAROTENOID ACCUMULATION IN WHEAT GRAINS

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The aim of the research was to select and optimize marker systems for identification of *Psy1* genes alleles, which are responsible for different levels of carotenoid pigments accumulation in wheat grains, as well as to screen varieties for the selection of valuable genotypes. 162 wheat samples were analyzed by the polymerase chain reaction method. Among them, varieties and lines with different allelic states of *Psy-A1* and *Psy-B1* genes were identified. The *Psy-D1* gene did not show any polymorphism. As a result, samples with valuable alleles of *Psy1* genes, which potentially contain increased carotenoids content in grains, were selected.

Key words: *Psy1* genes, carotenoids, molecular markers, *Triticum aestivum*.

Technological quality indicators define the use of wheat for the production of a certain type of product. Yellow pigment content is an important factor both for *Triticum durum* Dest. and *T. aestivum* L., which is determined mainly by accumulation carotenoid in grains. This feature affects the final quality and nutritional value of pasta and bakery products. Intense yellow color of products is attractive in yellow noodle that consumed mainly in Japan and Southeast Asia. Therefore, a high level of pigments in wheat is desirable for varieties used in that region [1]. White flour is preferable in soft wheat variety breeding, while a yellow pigment is considered undesirable characteristic for baking wheat [2]. White flour is also attractive for the production of steam bread and Chinese wheat noodle [3]. Endosperm color of wheat determines flour color and controls the main content of carotenoids in grain.

There are more than 750 compounds in carotenoid pigments family present in plants, bacteria and fungi [4, 5]. All carotenoids are formed from phytoene and most of them— C40 polyenes. They play a key role in photosynthesis, as required for proper construction of photosystems and light-absorbing complexes and performing photoprotection, reducing oxidative damage [6]. Provitaminic activity of β-carotene, α-carotene, β-cryptoxanthin and other pigments with at least one same oxygen-free ion in one ring determine nutritive value of carotenoids [4]. Carotenoids synthesis occurs by metabolic pathway, which involves at least 10 different enzymes [7, 8]. Yellow pigment content is mainly determined by plant genotype but also depends on the environmental conditions [9].

Duplicated genes *Psy1*, *Psy2* and *Psy3* were detected, in cereals on chromosomes 7, 5 and 5, respectively, which are responsible for the carotenoids synthesis. In subsequent studies it was shown that *Psy1* only is related to the yellow pigment content. Silencing *Psy1* gene expression using RNA interference to 54–76% led to a significant reduction of carotenoids in grains at 26–35% [10, 11]. Phytoene synthase (PSY) is the key enzyme in carotenoid biosynthesis that shows high correlation with yellow pigment content in wheat grain [12]. The phytoene synthase enzyme catalyzes condensation of two geranylgeranyl pyrophosphate molecules to form the phyton [13].

Homeological wheat genes *Psy-A1*, *Psy-B1*, *Psy-D1* are located on chromosomes 7A, 7B and 7D, respectively. *Psy1* genes contain six exons and five introns in wheat and its relatives, and

also in maize, rice and other cereals [14–16]. It was determined that carotenoids are the only 30–50% of endosperm yellow pigment in cereals and the remaining substances that provide color has not been identified yet. The main carotenoids in wheat endosperm are lutein and zeoxanthin, while α-carotene, β-carotene and β-cryptoxanthin present in much lower quantities [17].

The aim of the study was to develop biotechnological approaches based on molecular marker systems for the detection of *Psy-1* genes alleles, which are responsible for the biosynthesis of carotenoid pigments in cereals and screening a collection of domestic and foreign wheat accessions for proper genotype selection.

Materials and Methods

The subjects of the research were 156 samples of wheat Ukrainian and foreign selection, 1 sample of *Aegilops cylindrica*, 1 sample of *Aegilops tauschii*, 1 wheat amfiploid and 3 samples of emmer from different originators.

Total plant DNA was isolated with the CTAB method [18] with some modifications. The reaction mixes for PCR included: specific primers, 0.25 mM (Table 1), reaction buffer B (Solis BioDyne), 2 mM $MgCl_2$ solution (Solis BioDyne), 0.2 mM of each deoxyribonucleotide-3-phosphate (Thermo Fisher Scientific), 0,5 units of polymerase FIREPol® DNA Polymerase (Solis BioDyne), 30 ng of total DNA and deionized water Milli-Q (Merck Millipore) to a final volume of 20 µl.

Separation of amplification products was performed by horizontal electrophoresis in agarose gel with either sodium borate or lithium borate buffer containing 0.5 mg/ml ethidium bromide and vertical electrophoresis in polyacrylamide gels. Visualization of results was performed in UV-light with the photosystem Canon EOS 600D, image processing with MS PowerPoint and GIMP.

Results and Discussion

A number of varieties and lines with different allelic composition of *Psy-A1* and *Psy-B1* genes have been discovered in our study. At the same time the *Psy-D1* gene didn't show any polymorphism.

Three varieties (Glenlea, Antonivka and Bobwhite) with *Psy-A1b* allele responsible for the low grain carotenoid content, according to the published data [19, 21], were detected among 162 studied samples in our collection. Two varieties Zymoiarka and Nedra did not reveal any presence of the expected alleles. Allele *Psy-A1c* was not identified among the samples. The rest of the samples carried *Psy-A1a* allele, which defines high carotenoid content (Fig. 1).

The differentiation between varieties carrying *Psy-B1a* and *Psy-B1b* alleles was not practically possible with the use of agarose electrophoresis gels. However, according to the literature these alleles do not result in

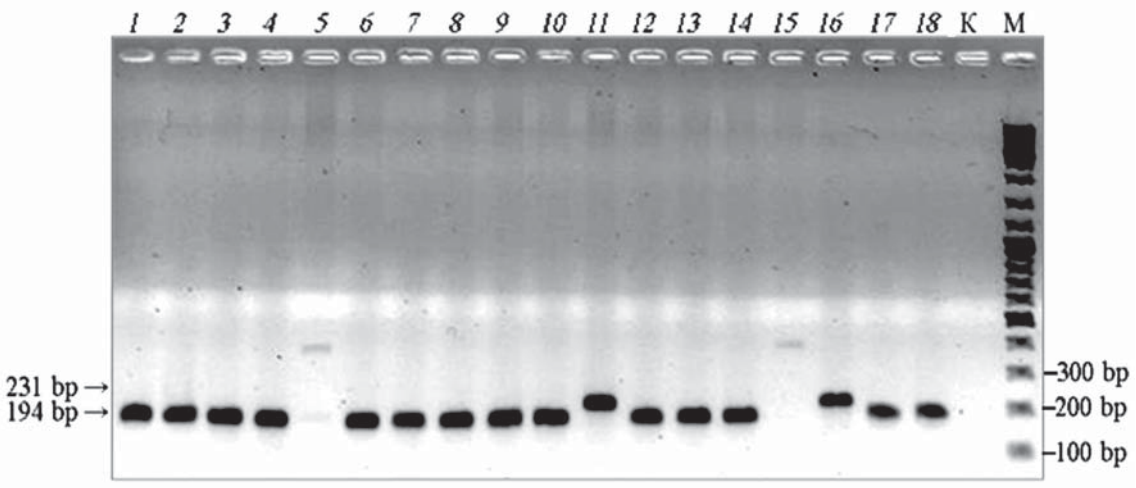

Fig. 1. **Agarose gel showing the results of *Psy-A1* gene amplification to identify *Psy-A1a* and *Psy-A1b* alleles:**
Lane *1* — Drevlianka; *2* — Khutorianka; *3* — Dvorianka; *4* — Trizo; *5* — Nedra; *6* — Nyva Kyivshchyny; *7* — Tybalt; *8* — Torchynska; *9* — Pereiaslavka; *10* — Granny; *11* — Antonivka; *12* — Podolianka; *13* — Favorytka; *14* — Zolotokolosa; *15* — Zymoiarka; *16* — Glenlea; *17* — Volodarka; *18* — Novokyivska;
hereafter K— negative control without DNA;
M — molecular weight marker GeneRuler™ DNA Ladder Mix

Table 1. **List of primers used in research and the length of expected amplification products**

Primer name	Sequence 5′ to 3′, reference	Allele –amplicon length, bp	Gene
YP7AF YP7AR	GGACCTTGCTGATGACCGAG TGACGGTCTGAAGTGAGAATGA [12]	*Psy-A1a*— 194 *Psy-A1b*— 231	*Psy-A1*
YP7A2F YP7A2R	GCCAGCCCTTCAAGGACATG CAGATGTCGCCACACTGCCA [19]	*Psy-A1a*— 1686 *Psy-A1b*— 1686 *Psy-A1c*— 1001	*Psy-A1*
YP7B-1F YP7B-1R	GCCACAACTTGAATGTGAAAC ACTTCTTCCATTTGAACCCC [19]	*Psy-B1a*— 151 *Psy-B1b*— 156	*Psy-B1*
YP7B-2F YP7B-2R	GCCACCCACTGATTACCACTA CCAAGGTGAGGGTCTTCAAC [19]	*Psy-B1c*— 428	*Psy-B1*
YP7B-3F YP7B-3R	GAGTAAGCCACCCACTGATT TCGCTGAGGAATGTACTGAC [19]	*Psy-B1d*— 884	*Psy-B1*
YP7B-4F YP7B-4R	AGGTACCAGCCAGCCCATA CTCGTCAAAGTTCGTGTACC [19]	*Psy-B1e*— 716	*Psy-B1*
YP7D-2F YP7D-2R	ACTCCCACAAACCTACAACG ACGCTCATCAACCCCACG [14]	*Psy-D1a*— 967 *Psy-D1g*— 1046	*Psy-D1*
RTF RTR	CAACGCTAGCTGCACCACTAACT ACTCCTCCTTGATAGCAGCCTT [20]	934	*TaTM20*

increasing carotenoid content in cereals and the levels of pigments in case of presence *Psy-B1a* or *Psy-B1b* do not differ significantly. Among the analyzed wheat sample 62 accessions that carry *Psy-B1d* allele, 14 accessions with *Psy-B1c* allele and also 1 hybrid with *Psy-B1e* allele were found. Some accessions with *Psy-B1c* allele had unexpected additional amplicon of about 900 bp. It indicates the additional polymorphisms of *Psy-B1* gene. Allele *Psy-B1c* is responsible for high carotenoid content in grains, according to the literature, so this data is valuable for further research and biofortification of wheat.

To optimize the analysis the multiplex PCR detecting alleles *Psy-B1d*, *Psy-B1s* and *Psy-B1e* together with the reference *TaTM20* gene was developed. Typical electrophoregrams are shown in Fig. 2 and 3.

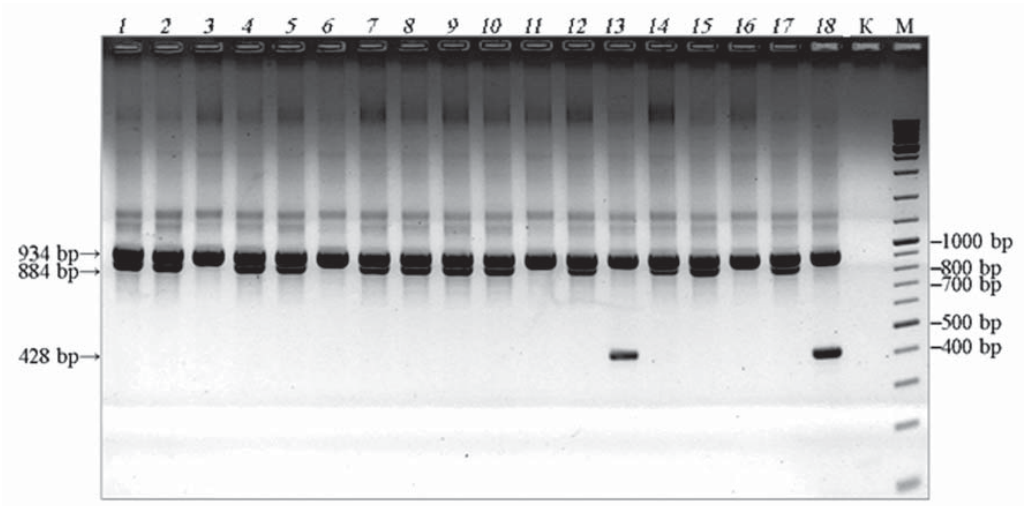

Fig. 2. **Agarose gel showing the results of multiplex PCR of *Psy-B1* gene to identify *Psy-B1d* and *Psy-B1c* alleles with *TaTM20* reference gene:**
Lane *1* — Diuk; *2* — Dvorianka; *3* — Khutorianka; *4* — Nikoniia; *5* — Khyst; *6* — Nyva Kyivshchyny; *7* — Ukrainka; *8* — Zorepad; *9* — Pereiaslavka; *10* — Antonivka; *11* — Granny; *12* — Podiaka; *13* — Nedra; *14* — Yatran 60; *15* — Yednist; *16* — Federer; *17* — positive control carrying *Psy-B1d* allele; *18* — positive control containing *Psy-B1c* allele

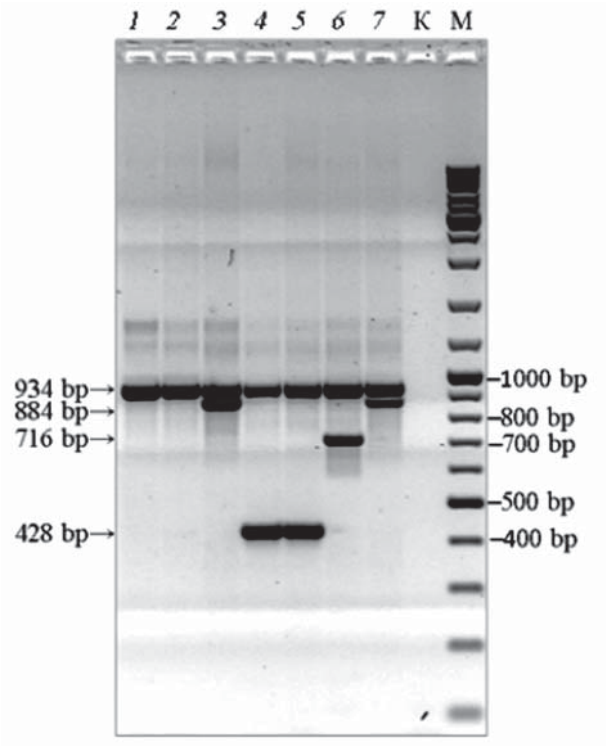

Fig. 3. **Agarose gel showing the results of multiplex PCR of gene to identify *Psy-B1d*, *Psy-B1c* and *Psy-B1e* alleles with *TaTM20* reference gene:**
Lane *1* — Khutorianka; *2* — Aranka; *3* — Blahodarka; *4* — Dobrochynna; 5 — control which carries *Psy-B1c* allele; *6* — control containing *Psy-B1e* allele; *7*— control bearing *Psy-B1d* allele

There is the wild *Psy-D1a* allele only found among the studied accessions. Typical electrophoregram is shown in Fig. 4.

The list of the detected samples with identified alleles of *Psy1* genes shown at Table 2.

Allele frequency of studied genes for the Ukrainian varieties are the following: 97.8% for *Psy-A1a*, 0.8% for *Psy-A1b*, and 1.4% for the samples that did not reveal any of the expected alleles; 47% of the total for the both alleles *Psy-B1a* and *Psy-B1b* together, 43.3% for *Psy-B1d*, and, finally, 9.7% for *Psy-B1c*.

According to the information set out in the paper by He et al. (2009) the highest content of carotenoids is observed in the presence of allele *Psy-A1a* and *Psy-B1c*. Therefore, the highest concentration of carotenoids is expected in the following accessions: wheat varieties Dobrochynna, Kosovytsia, Odeska 265, Biliava, Pysanka, Panna, Lider, Odeska 51, Selianka, Povaha, Lanovyi, Suputnytsia and the Emmer accession originating from Germany. Also, the presence of 1B·1R translocation increases carotenoid content in grain. Among the samples with *Psy-A1a* and *Psy-B1c* alleles, and therefore potentially high carotenoids content, there are no accessions with 1B·1R translocation. The least carotenoid content is expected in accessions with *Psy-A1b* and *Psy-B1b* alleles and without 1B·1R translocation.

To confirm phenotypic expression of the studied genes as well as the relationship between their allelic state and the manifestation of a sign the carotenoid content in wheat was measured by the AACC International Method 14-60.01 (2012) in grains. It was found that the carotenoid content in studied wheat samples is so low that it cannot be identified by standard method, designed specifically for the measurement of carotenoid pigments in cereals and flour. At the same time, we conducted the control experiment which allowed us to reliably distinguish among carotenoid pigments content in corn samples, which confirmed the adequacy and reproducibility of chosen measurement method. The carotenoid pigments content in wheat, which is below the sensitivity threshold of common methods, clearly confirms the importance of the study subjects. The development of wheat with a high

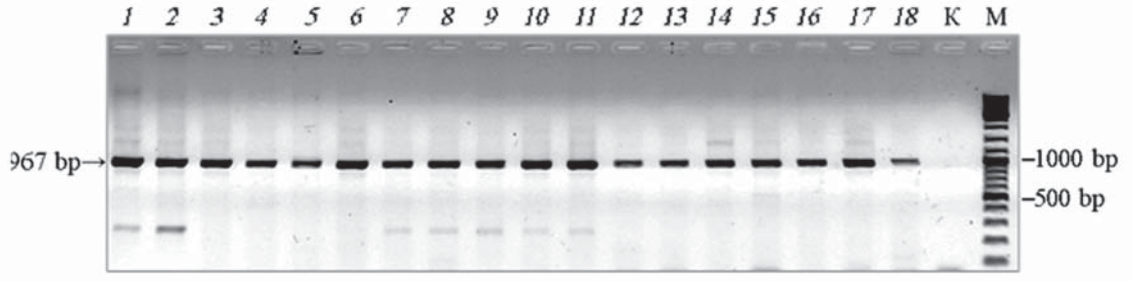

Fig. 4. **Agarose gel showing the results of *Psy-D1* gene amplification:**
1 — Drevlianka; *2* — Khutorianka; *3* — Dvorianka; *4* — Trizo; *5* — Nyva Kyivshchyny; *6* — Tybalt; *7* — Torchynska; *8* — Pereiaslavka; *9* — Granny; *10* — Podolianka; *11* — Zymoiarka; *12* — Zolotokolosa; *13* — Favorytka; *14* — Volodarka; *15* — Novokyivska; *16* — Sonechko; *17* — Glenlea; *18* — Smuhlianka

Table 2. **Allelic profiling of genes responsible for accumulation of carotenoids in wheat grains**

№	Genotype	*Psy-A1*	*Psy-B1*	*Psy-D1*
1	*Aegilops cylindrica* 220/34	*a*	*a/b*	*a*
2	*Aegilops tauschii* 232/87	*a*	*d*	*a*
3	Akter	*a*	*a/b*	*a*
4	Albatros	*a*	*d*	*a*
5	Amphiploid 4 242/59	*a*	*e*	*a*
6	Antonivka	*a*	*d*	*a*
7	Antonivka 2	*b*	*d*	*a*
8	Aranka	*a*	*a/b*	*a*
9	Bankuti 1201	*a*	*a/b*	*a*
10	Bezosta 1	*a*	*a/b*	*a*
11	Biliava (Kyiv)	*a*	*c*	*a*
12	Biliava (Odesa)	*a*	*d*	*a*
13	Bilotserkivska napivkarlykova	*a*	*a/b*	*a*
14	Blahodarka	*a*	*d*	*a*
15	Bobwhite (Kyiv)	*a*	*a/b*	*a*
16	Bobwhite (Odesa)	*b*	*a/b*	*a*
17	Bohdana	*a*	*a/b*	*a*
18	Boriia	*a*	*a/b*	*a*
19	Borviy	*a*	*d*	*a*
20	Bunchuk	*a*	*a/b*	*a*
21	Chinese spring	*a*	*a/b*	*a*
22	Chinese spring 2	*a*	*a/b*	*a*
23	Chorniava	*a*	*a/b*	*a*
24	Chornobrova	*a*	*a/b*	*a*
25	Dalnytska	*a*	*d*	*a*
26	Dobrochyn	*a*	*d*	*a*
27	Dobrochynna	*a*	*c*	*a*
28	Dobroslava	*a*	*d*	*a*
29	Donetska 46	*a*	*a/b*	*a*
30	Donetska 48	*a*	*a/b*	*a*
31	Donska napivkarlykova	*a*	*a/b*	*a*
32	Doskonala	*a*	*a/b*	*a*
33	Drevlianka	*a*	*a/b*	*a*
34	Dvorianka	*a*	*d*	*a*
35	Diuk	*a*	*d*	*a*
36	Emmer (commer)	*a*	*a/b*	*a*
37	Emmer (Germany)	*a*	*c*	*a*
38	Emmer (Hungary)	*a*	*a/b*	*a*
39	Epokha	*a*	*d*	*a*
40	Favorytka	*a*	*a/b*	*a*
41	Federer	*a*	*a/b*	*a*

Continuation Table 2

№	Genotype	*Psy-A1*	*Psy-B1*	*Psy-D1*
42	Glenlea	*b*	*d*	*a*
43	Granny	*a*	*a/b*	*a*
44	Harmoniia	*a*	*a/b*	*a*
45	Hileia	*a*	*a/b*	*a*
46	Hoduvalnytsia	*a*	*a/b*	*a*
47	Hospodynia	*a*	*d*	*a*
48	Hrezdivlytsia	*a*	*a/b*	*a*
49	Hurt	*a*	*d*	*a*
50	Istyna	*a*	*d*	*a*
51	Khersonska b/o	*a*	*d*	*a*
52	Khutorianka	*a*	*a/b*	*a*
53	Khyst	*a*	*d*	*a*
54	Kiriia	*a*	*d*	*a*
55	Kniahynia Olha (Kyiv)	*a*	*d*	*a*
56	Kniahynia Olha (Odesa)	*a*	*d*	*a*
57	Kolleha	*a*	*d*	*a*
58	Kolumbiia	*a*	*a/b*	*a*
59	Koreli	*a*	*a/b*	*a*
60	Kosovytsia	*a*	*c*	*a*
61	Krasen	*a*	*d*	*a*
62	Kryzhynka	*a*	*a/b*	*a*
63	Kuialnyk (Kyiv)	*a*	*a/b*	*a*
64	Kuialnyk (Odesa)	*a*	*d*	*a*
65	Kyivska ostysta	*a*	*a/b*	*a*
66	Lad	*a*	*d*	*a*
67	Lanovyy	*a*	*c**	*a*
68	Lastivka	*a*	*d*	*a*
69	Lasunia	*a*	*a/b*	*a*
70	Lebidka	*a*	*d*	*a*
71	Lider	*a*	*c*	*a*
72	Liona	*a*	*d*	*a*
73	Lira	*a*	*d*	*a*
74	Lybidka	*a*	*d*	*a*
75	Lytanivka	*a*	*d*	*a*
76	Marquis	*a*	*a/b*	*a*
77	Misiia	*a*	*d*	*a*
78	Myronivska 30	*a*	*a/b*	*a*
79	Myronivska 61	*a*	*a/b*	*a*
80	Myronivska 65	*a*	*a/b*	*a*
81	Myronivska 808	*a*	*a/b*	*a*

Continuation Table 2

№	Genotype	*Psy-A1*	*Psy-B1*	*Psy-D1*
82	Natalka	*a*	*a/b*	*a*
83	Natalka (Kyiv)	*a*	*d*	*a*
84	Nebokray	*a*	*d*	*a*
85	Nedra	empty	*c*	*a*
86	Nikoniia	*a*	*d*	*a*
87	Nirit	*a*	*a/b*	*a*
88	Norin 16	*a*	*a/b*	*a*
89	Norin 29	*a*	*a/b*	*a*
90	Norin 35	*a*	*a/b*	*a*
91	Novokyivska	*a*	*a/b*	*a*
92	Novosibirskaya 67	*a*	*a/b*	*a*
93	Novosmuhlianka	*a*	*a/b*	*a*
94	Nyva	*a*	*d*	*a*
95	Nyva Kyivshchyny	*a*	*a/b*	*a*
96	Nyva Odeska	*a*	*a/b*	*a*
97	Odeska 51 (Kyiv)	*a*	*c*	*a*
98	Odeska 51 (Odesa)	*a*	*a/b*	*a*
99	Odeska 265	*a*	*c*	*a*
100	Odeska 267 (Kyiv)	*a*	*a/b*	*a*
101	Odeska 267 (Odesa)	*a*	*a/b*	*a*
102	Oslo	*a*	*a/b*	*a*
103	Otaman	*a*	*d*	*a*
104	Panna	*a*	*c*	*a*
105	Pereiaslavka	*a*	*d*	*a*
106	Podiaka	*a*	*d*	*a*
107	Podolianka	*a*	*a/b*	*a*
108	Polianka	*a*	*a/b*	*a*
109	Poliska 90	*a*	*a/b*	*a*
110	Polovyk	*a*	*d*	*a*
111	Poshana	*a*	*a/b*	*a*
112	Povaha	*a*	*c**	*a*
113	Pylypivka	*a*	*a/b*	*a*
114	Pysanka	*a*	*c**	*a*
115	Pyvna	*a*	*a/b*	*a*
116	Selianka	*a*	*c**	*a*
117	Shchedrivka Kyivska	*a*	*a/b*	*a*
118	Skarbnytsia (Kyiv)	*a*	*a/b*	*a*
119	Skarbnytsia (Odesa)	*a*	*d*	*a*
120	Sluzhnytsia	*a*	*d*	*a*
121	Smuhlianka	*a*	*a/b*	*a*
122	Solokha	*a*	*a/b*	*a*

End Table 2

№	Genotype	*Psy-A1*	*Psy-B1*	*Psy-D1*
123	Solomiia	*a*	*d*	*a*
124	Sonata	*a*	*a/b*	*a*
125	Sonechko	*a*	*d*	*a*
126	Sotnytsia	*a*	*a/b*	*a*
127	Spasivka	*a*	*a/b*	*a*
128	Statna	*a*	*a/b*	*a*
129	Suputnytsia	*a*	*c*	*a*
130	Torchynska	*a*	*a/b*	*a*
131	Trizo	*a*	*a/b*	*a*
132	Trypilska	*a*	*a/b*	*a*
133	Turunchuk	*a*	*d*	*a*
134	Tybalt	*a*	*a/b*	*a*
135	Ukrainka	*a*	*d*	*a*
136	Ukrainska 0246	*a*	*a/b*	*a*
137	Uzhynok (Kyiv)	*a*	*a/b*	*a*
138	Uzhynok (Odesa)	*a*	*a/b*	*a*
139	Vatazhok	*a*	*a/b*	*a*
140	Vesnianka	*a*	*a/b*	*a*
141	Viktoriia	*a*	*d*	*a*
142	Volodarka	*a*	*a/b*	*a*
143	Vykhovanka	*a*	*d*	*a*
144	Yatran 60	*a*	*d*	*a*
145	Yednist	*a*	*d*	*a*
146	Zadumka	*a*	*a/b*	*a*
147	Zahrava	*a*	*d*	*a*
148	Zamozhnist	*a*	*d*	*a*
149	Zaporuka	*a*	*d*	*a*
150	Zdobutok	*a*	*a/b*	*a*
151	Zemliachka (Kyiv)	*a*	*a/b*	*a*
152	Zemliachka (Odesa)	*a*	*a/b*	*a*
153	Zhaivir	*a*	*d*	*a*
154	Zhuravka	*a*	*d*	*a*
155	Zmina	*a*	*d*	*a*
156	Znakhidka	*a*	*d*	*a*
157	Zoloto Ukrainy	*a*	*d*	*a*
158	Zolotokolosa	*a*	*a/b*	*a*
159	Zorepad	*a*	*d*	*a*
160	Zvytiaha	*a*	*a/b*	*a*
161	Zymoiarka	empty	*a/b*	*a*
162	Zysk	*a*	*d*	*a*

*— samples have additional unexpected amplicon.

content of carotenoids in grains is a promising area of plant breeding for getting crops of special nutritional purposes.

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ІДЕНТИФІКАЦІЯ АЛЕЛІВ ГЕНІВ *Psy1*, ЩО ВІДПОВІДАЮТЬ ЗА НАКОПИЧЕННЯ КАРОТИНОЇДІВ У ЗЕРНІ ПШЕНИЦІ

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Метою дослідження було підібрати та оптимізувати маркерні системи для ідентифікації алелів генів *Psy1*, що відповідають за різний рівень накопичення каротиноїдних пігментів у зерні пшениці, та здійснити скринінг вибірки сортів для добору необхідних генотипів. Методом полімеразної ланцюгової реакції проаналізовано 162 зразки пшениці. Серед вибірки було виявлено сорти і лінії з різним алельним складом генів *Psy-A1* та *Psy-B1*. Ген *Psy-D1* не виявив поліморфізму. В результаті відібрано зразки з необхідними алелями генів *Psy1*, що потенційно мають підвищений вміст каротиноїдів у зернівках.

Ключові слова: гени *Psy1*, каротиноїди, молекулярні маркери, *Triticum aestivum*.

ИДЕНТИФИКАЦИЯ АЛЛЕЛЕЙ ГЕНОВ *Psy1*, КОТОРЫЕ ОТВЕЧАЮТ ЗА НАКОПЛЕНИЕ КАРОТИНОИДОВ В ЗЕРНЕ ПШЕНИЦЫ

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Целью исследования были подбор и оптимизация маркерных систем для идентификации аллелей генов *Psy1*, отвечающих за разный уровень накопления каротиноидных пигментов в зерне пшеницы, а также скрининг выборки сортов для отбора нужных генотипов. Методом полимеразной цепной реакции проанализированы 162 образца пшеницы. Среди выборки были выявлены сорта и линии с различным аллельным составом генов *Psy-A1* и *Psy-B1*. Ген *Psy-D1* не проявил полиморфизма. В результате отобраны образцы с нужными аллелями генов *Psy1*, которые потенциально имеют повышенное содержание каротиноидов в зерновках.

Ключевые слова: гены *Psy1*, каротиноиды, молекулярные маркеры, *Triticum aestivum*.