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ALLELE FREQUENCIES OF *PPD-D1A*, *PPD-B1A*, AND *PPD-B1C* OF PHOTOPERIODIC SENSITIVITY GENES IN SPRING BREAD WHEAT VARIETIES (*TRITICUM AESTIVUM* L.) OF VARIOUS ORIGIN

I. A. Balashova, V. I. Fait

National Centre of Seed and Cultivar Investigation «*The Plant Breeding and Genetics Institute*» *NAAS*
3, Ovidiopolska Rd, Odesa, Ukraine, 65036

E-mail: ibalashova@ukr.net, faygen@ukr.net

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Aim. To identify and evaluate allele frequencies of *Ppd-D1a*, *Ppd-B1a*, *Ppd-B1c* and *Ppd-1* of the genotypes of spring bread wheat varieties from various climatic zones. **Methods.** DNA isolation, allele-specific PCR, electrophoresis in agarose and polyacrylamide gel, statistical analysis. **Results.** 137 varieties of spring bread wheat of various origin were detected to identify *Ppd-1* genotypes of *Ppd-D1a*, *Ppd-B1a* and *Ppd-B1c* allele carriers. The results for the total sampling of the varieties under investigation and the sampling of Asian varieties yielded six different *Ppd-1* genotypes in each. As for samplings of other regions, there were from two (Mexico) to four (Europe, the USA, Canada, Ukraine) *Ppd-1* genotypes. In the total sampling of varieties, there was a high incidence (20.5 %) of genotypes, dominant only in allele *Ppd-D1a*, varying from 0 (Russia) to 85.0 % (Mexico). The incidence of the genotypes with monogenically dominant *Ppd-B1a* (7.3 %) or *Ppd-B1c* (5.1 %) in the total sampling, was considerably lower. These genotypes were most common for the sampling of the varieties from the USA and Canada (25.0 and 16.7 % respectively). Digenically dominant *Ppd-D1a Ppd-B1a* genotypes were found in the total sampling with relatively low incidence (7.3 %), and were notable for the varieties from Asia (33.4 %), Mexico (15.0 %), Ukraine (13.1 %), and Europe (3.1 %). The digenically dominant genotype *Ppd-D1a Ppd-B1c* was found only in the Japanese variety Konosu-25. Gene *Ppd-A1* was present in all the spring varieties under investigation in its recessive state. **Conclusions**. Out of three dominant alleles in the studied sampling, the highest incidence was noted for allele *Ppd-D1a* (28.5 %). All the varieties from Mexico, present in the set, carry this allele. At the same time, it was not found in any variety from Russia. Allele *Ppd-B1a* was detected in the varieties from all the regions with the incidence of 7.7 (Russia) – 44.4 % (Asia). Allele *Ppd-B1c* was sporadically present in the varieties from Russia, Ukraine, the USA, Japan, and Brazil, and its incidence in the total sampling was insignificant (5.8 %). The varieties, identified by the allelic status of *Ppd-1* genes, may be used as donors for selection and determination of the influence of alleles for each gene by the development rate and related economically valuable traits of bread wheat.

Key words: *Triticum aestivum*, spring type of development, photoperiod, *Ppd-1* genes, genotype, allele-specific PCR.
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INTRODUCTION

The photoperiod-sensitivity of bread wheat plants (*Triticum aestivum* L.) is mainly controlled by genes of the orthologue series *Ppd-1: Ppd-D1*, *Ppd-B1,* and *Ppd-A1* (McIntosh RA et al, 2003), localized in short arms of chromosomes II of the homologous group 2A, 2B, and 2D, respectively (Scarth R, Law CN, 1984) and encoding proteins, which induce the manifestation of the blossoming locus *TaFT* (*VRN3*) (Yan L et al, 2006; Chen F et al, 2013). At present, each photoperiod gene is viewed as a series of alleles, occurring due to various mutations of their older predecessor forms (Shaw LM et al, 2012; Bentley AR et al, 2013). Wild alleles *Ppd-1* contain a site in their promoter, which is involved in suppressing the expression at nighttime. Therefore, "sensitive" or recessive alleles of photoperiod genes are expressed after dawn and prior to the darkness phase. Poor photoperiod-sensitivity is caused

by the influence of one or more mutant "insensitive" or dominant alleles. The occurrence of the latter is a result of the impaired structure of the promoter due to extensive deletions (genes *Ppd-A1a* and *Ppd-D1a*), or insertion (gene *Ppd-B1a.1*). The presence of these mutations leads to 24-hour-long gene expression under higher activity at nighttime and at dawn. Therefore, the availability of a dominant allele ensures faster accumulation of the inductor protein, which is reflected in the acceleration of the rate for the period from seedlings to heading/blossoming.

The differences in the rate of development for bread wheat are mainly related to the impact of dominant gene *Ppd-D1a*, which spread widely among the varieties in temperate zones (Langer SM et al, 2014), predominantly affecting winter type of development. Allelic differences for gene *Ppd-D1* are among the most relevant factors, defining the variability of winter wheat varieties in terms of adaptivity and performance in specific cultivation regions (Worland AJ et al, 1998; Síp V et al, 2010; Grogan SM et al, 2016), including Ukraine (Bakuma AO et al, 2018). On the contrary, allele *Ppd-A1a.1* and *Ppd-B1a.1* (Nishida H et al, 2013; Seki M et al, 2011) are rare. Allele *Ppd-B1a.1* with MITE type insertion in the promoter was detected only in a dozen of related Japanese varieties and not found in the varieties from other regions. However, in *Ppd-B1*, contrary to two other genes *Ppd-1*, the dominant form is also presented by multi-copy alleles, which occurred due to CNV-mutations. It is known that there are two, three, and four-copy alleles, indicated as *Ppd-B1d*, *Ppd-B1a*, *Ppd-B1c*, respectively (Cane K et al, 2013). The multi-copy nature is a common phenomenon, including several known copies of the recessive *Vrn-A1* (Diaz A et al, 2012; Würschum T et al, 2015) in the genes of CBF family (Würschum T et al, 2017), etc. CNV-mutants of *Ppd-B1* do not have any impairments in the promoter structure. At the same time, the increase in transcription activity at nighttime was determined in multi-copy alleles, contrary to the recessive one without any copies (Diaz A et al, 2012; Shaw LM et al, 2012). In addition, it has been recently shown that a difference in the methylation levels for the promoter zone of *Ppd-B1a* reflects on its transcription activity and the carriers of the same allele differ in their heading rate (Sun H et al, 2014). Contrary to *Ppd-D1a,* the frequencies and distribution of dominant *Ppd-B1* are practically not studied. The analysis of intramolecular structure of *Ppd-1* genes ensured the elaboration of PCR-tests, the application of which allows for the identification of varieties by allelic status of photoperiod genes. The first test of this kind was multiplex PCR, suggested by Bealis (Bealis J et al, 2007) for the detection of dominant and recessive alleles of *Ppd-D1* gene by the presence or absence of deletion in the promoter, respectively. The authors also demonstrated that in addition to the functional gene, Chinese Spring variety, a carrier of dominant *Ppd-B1*, has a pseudogene with partially deleted exon 8, to detect which a respective PCR-test was developed. It was later found that in the Chinese variety *Ppd-B1* has four copies, including the shortened one – allele *Ppd-B1c* (Cane K et al, 2013). The complexity of labelling CNV-mutants is conditioned by the fact that copies are practically identical, just like inter-copy zones, including the insertions of transposons. However, a three-copy gene of varieties Sonora 64 and Timstein was found to have a polymorphous area, which served as a foundation for the elaboration of a PCR-test, identifying allele *Ppd-B1a*. The varieties of hexaploid wheat *T. aestivum* were found to have allele *Ppd-A1a.1*, containing a deletion in the promoter region (Nishida H et al, 2013). However, this allele is rarely detected, and a recessive allele of the gene prevails in bread wheat varieties. Several PCR-tests were developed to detect allelic status of *Ppd-A1* and to identify the absence/presence of deletions in the promoter (Wilhelm EP et al, 2009).

The aim of this study was to identify and assess the incidence frequencies for alleles *Ppd-D1a*, *Ppd-B1a*, *Ppd-B1c*, and *Ppd-1* of genotypes of spring bread wheat varieties from different climatic zones. The identification of carriers of genes *Ppd-D1a*, and especially multi-copy genes *Ppd-B1* is actual, as the information about *Ppd-1* genotypes, including Ukrainian spring varieties, is rather scarce. Such information about *Ppd-1* genotypes of the varieties is relevant when the latter are used as donors in breeding programs, aimed at the develop of early-maturing varieties, and as the initial material to determine the effects of the alleles of each gene in terms of development rate and related agronomic traits of bread wheat.

MATERIALS AND METHODS

137 varieties of spring bread wheat *Triticum aestivum* L. of various origin were used as the initial material; the complete list is presented in Table 1. Among the investigated varieties, 32 varieties were from European countries (Germany – 14, Sweden – 4, France, Switzerland – 3, Great Britain, the Netherlands, Finland – 2, Austria, Czech Republic – 1), 23 – from Ukraine, 26 – Russia, 4 – Kazakhstan. The sampling also contained 12 varieties from the USA and Canada, 20 vari-

eties from Mexico, 9 varieties from Asia (Japan, India), 6 – from Africa, 3 – South America and 2 – Australia.

To detect the alleles of *Ppd-1* family, we used PCR-tests, the primers for which are specified in Table 1. The marking of gene *Ppd-D1* was done according to the recommendations of Beales (Beales J et al, 2007). This test envisages the identification of recessive alleles of the gene by the absence of deletions in the promoter – fragment 414 bp and dominant allele *Ppd-D1a* – marking product of 288 bp. The detection of allele *Ppd-B1c* (four copies of the gene) was also performed by the test, developed by Beales (Beales J et al, 2007), where the marker was a fragment of 425 bp. A variant of allele-specific PCR, suggested by Chen (Chen F et al, 2013), was used to detect a three-copy gene *Ppd-B1a*. A marker of this gene was an amplification fragment of 223 bp. As a reference control, Chinese Spring variety was used in the former case and Timstein – in the latter. To detect the intact status of the promoter of gene *Ppd-A1*, a PCR-test was used where a fragment of 452 bp indicated recessive status of this gene (Williams EP et al, 2009). The absence of the product allows for an alleged presence of a deletion in the promoter and a presence of a dominant allele.

DNA extraction was conducted using CTAB-buffer from dry grain or five-day-old seedlings. The reaction buffer for PCR contained: 50 mM KCl; 20 mM tris-HCl, pH 8.4; 2.0 mM $MgCl_2$; 0.01 % Tween-20; 0.15 mM of each dNTP; 5 pM of each primer; 20 ng of DNA and 1 unit of Taq-polymerase. The volume of the reaction mixture was 20 µl. Amplification: denaturation – 94 °C – 2 min (initial), then 30 s; 60 °C – 30 s annealing; elongation – 72 °C – 50 s; 35 cycles, final elongation – 72 °C – 3 min.

Terzik amplifier (DNA-technology, Russia) was used for the amplification. The amplification products were fractioned in 10 % polyacrylamide gel and their visualization in PAAG was done by staining with 0.012 M $AgNO_3$. The molecular mass of amplification products was determined regarding markers pUC19/MspI and Ledder 1000.

The statistical processing of the obtained results was done by common methods (Rokitskiy PF, 1973).

RESULTS

We identified the genotypes of 137 varieties of spring bread wheat of various geographical origin for genes *Ppd-A1*, *Ppd-B1*, *Ppd-D1* (Table 2). The locus *Ppd-A1* polymorphism was not found in the sampling of varieties under investigation. A marker fragment of 452 bp was found in all the investigated varieties during DNA amplification, which indicated the absence of deletions in the promoter and the presence of a recessive allele *Ppd-A1b* in the genotypes.

At the same time, the carriers of dominant and recessive alleles of genes *Ppd-D1* and *Ppd-B1* were detected in the varieties of the investigated sampling (Fig. 1, 2). As a result, six different *Ppd-1* genotypes were identified in the varieties: recessive in three genes of *Ppd-1*, monogenically dominant in *Ppd-D1a*, or *Ppd-B1a*, or *Ppd-B1c*, and digenically dominant in *Ppd-D1a, Ppd-B1c* or *Ppd-D1a, Ppd-B1a*. The samplings of the varieties from most regions demonstrated from two (Mexico) to three (Russia), or four (Europe, the USA, Canada, Ukraine) *Ppd-1* genotypes. And only the sampling of Asian varieties had 6 genotypes (Table 3).

Among 137 spring wheat varieties, 28 samples or 20.5 ± 3.45 % have only a dominant allele *Ppd-D1a* in

Table 1. The sequences of primers and expected sizes of a PCR product for the labelling of genes *Ppd-D1a, Ppd-Db, Ppd-B1a, Ppd-B1c*, *Ppd-A1b*

Gene	Primer	Primer sequence	Fragment size, bp
Ppd-D1a/Ppd-D1b			
	Ppd-D1_F	acgcctcccactacactg	288 bp/414 bp
	Ppd-D1_R1	cactggtggtagctgagatt	[Beales J et al 2007]
	Ppd-D1_R2	tgttggtcaaacagagagc	
Ppd-B1c	PpdB1_2copyL	taactgctcgtcacaagtgc	425 bp
	PpdB1_2copyR	ccggaacctgaggatcatc	[Beales J et al 2007]
Ppd-B1a	PpdB1son_ L	ccaggcgagtgatttacaca	223 bp
	PpdB1son_ R	gggcacgttaacacacctt	[Chen F et al 2013]
PpdA1b	durum_Ag5del_F2	tgtcacccatgcactctgtt	452 bp
	durum_Ag5del_R2	ctggctccaagaggaaacac	[Wilhelm EP et al 2009]

their genotype. The frequencies of genotypes, monogenically dominant in *Ppd-B1a* (10 varieties or 7.3 ± ± 2.09 %) or *Ppd-B1c* (7 varieties or 5.1 ± 1.88 %) were considerably lower by 13.2 ± 4.03 % (t = 3.27 at $t_{0.05}$ = 1.96) and 15.4 ± 3.93 % (t = 3.91 at $t_{0.05}$ = 1.96), respectively. The frequency of digenically dominant genotype *Ppd-D1a Ppd-B1a* equals that of the genotype, monogenically dominant in *Ppd-B1a*. The frequency of digenically dominant genotype *Ppd-D1a Ppd-B1c* is practically close to zero (1 grade or 0.7 ± 0.71 %).

The varieties from different countries or regions did not differ ($d < S_d' t_{0.05}$) among themselves in frequencies of genotypes, monogenically dominant in *Ppd-B1a* or *Ppd-B1c* and digenically dominant in *Ppd-D1a Ppd-B1a* or *Ppd-D1a Ppd-B1c*. In general, the frequencies of the four mentioned genotypes were rather low, varying in different samplings from 0 to 33.4 %.

At the same time, the frequency of monogenically dominant *Ppd-D1a* genotypes in Mexican varieties is 85.0 ± 7.98, which is reliably higher by 62.8 ± 15.98 % than that for the sampling of Asian varieties (t = 3.93 at $t_{0.05}$ = 2.05) and four other regions, which, in their turn, did not differ much from the Asian varieties.

In general, the share of monogenically or digenically dominant varieties, the carriers of alleles *Ppd-D1a*, *Ppd-B1a* or *Ppd-B1c* in spring varieties from Europe and Russia, is rather low, amounting to 15.5 ± 6.40 and 15.4 % ± 7.07, respectively. This value is insignificantly ($d < S_d' t_{0.05}$) increasing up to 30.4 ± 9.59 % for Ukrainian varieties and up to 48.4 ± 14.23 % for the varieties from the USA and Canada. But for the Indian variety Kalyansona, all the investigated varieties of Asia and Mexico carry dominant alleles of genes *Ppd-D1* and/or *Ppd-B1*. The presence of dominant alleles of one or two genes of orthologue series *Ppd-1* in the genotype of the variety may demonstrate low photoperiodic sensitivity.

The remaining varieties (81 samples or 59.1 ± ± 4.20 %) within this investigation are characterized as carriers of recessive alleles *Ppd-1*.

Higher frequency in the total sampling of the varieties was noted for allele *Ppd-D1a* – 28.5±3.86 % or 39 varieties (Table 4). The share of the carriers of allele

Table 2. *Ppd-1* genotypes of various origin

Genotype	Variety (the originating country)
Ppd-D1a	Capta (France), Orello (Switzerland), Barton, Red River 68 (USA), Alondra, Bob White, Chanate, Catbird, Cettia, Ciano 67, Kauz, Jahana, Opata 85, Saric-70, Seri, Sitta, Sitella, Cocoraque-75, Olat, Tia-3, Nesser (Mexico), Norin 17, Norin 61 (Japan), Beacon (Kenya), Frontana (Brazil), Katyusha, Rannia 93, Skorospilka 99 (Ukraine)
Ppd-B1a	Albis (Switzerland), Aranka (Czech Republic), Big club, Loros (USA), Losprout (Canada), C591 (India), Salmayo (Kenya), Desconocida (Ethiopia), Udarnitsa, Kometa (Russia)
Ppd-B1c	Transec, Chul (USA), Norin 29 (Japan), Bage (Brazil), Zhnitsa, Strela (Russia), Struna myronivska (Ukraine)
Ppd-D1a Ppd-B1a	Atys (France), Alondra, Mexique-45, Turaco (Mexico), Shiroganekomugi, Zenkojkomugi (Japan), Sonalika (India), Azhurnaya, Elehia myronivska, Etiud (Ukraine)
Ppd-D1a Ppd-B1c	Konosu-25 (Japan)
Recessive in alleles of genes *Ppd-1*	Apu, Touko (Finland), Kadett, Weilbus algat, Weilbus sappo, Svalofs amy (Sweden), Bali, Famos, Herakles, Koga, Kolibri, Mujket, Triso, Star, Solo, Sirius, Tupic, Shirocco, Cesar, Arcos (Germany), Cardinal (France), Hinal (Switzerland), Atilla (Austria), Arabel, Sicco (Netherlands), Aintree, Broom (Great Britain), Borach, Axminster, Hope, Lee, Murquillo (USA), Lin calel (Argentina), Kalyansona (India), Zambezi (Zambia), Kenia farmer (Kenya), Red Egypet (Egypt), Pronto federaton, Amby (Australia), Anshlah, Vitka, Yevdokia, Heroinia, Kolektyvna 3, Krasa Polissia, Myronivska 5, Myronivska yara, Skorospilka 95, Skorospilka 98, Sribnianka, Stavyska, Suita, Torchynska, Kharkivska 26, Kharkivska 30 (Ukraine), Bashkirskaya 8, Botanicheskaya 3, Balaganka, Buriatskaya 34, Duvanka, Lutescence 55/11, Lutescence 53/12, Kommunar 29, Milturum 162, Narymskaya 3, Omskaya 9, Poltavka, Sarrubra, Saratovskaya 29, Saratovskaya 46, Saratovskaya 210, Sibakovskaya 3, Sibiriachka 4, Sibiriachka 8, Rusak, Tsezium 111, Tulun 14 (Russia), Akmolinka, Koktunkulskaya 322, Pirotriks 28, Shortardinka (Kazakhstan)

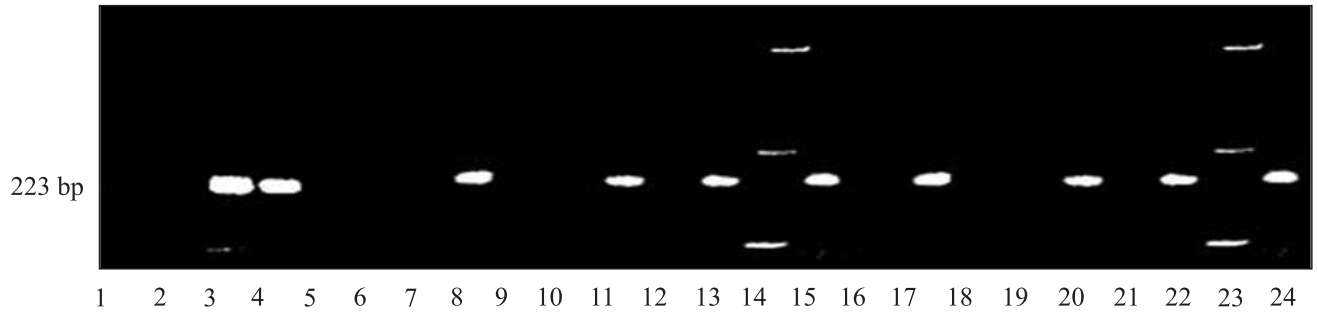

Fig. 1. The labelling of allele *Ppd-B1a* of spring bread wheat of various origin: 1, 2, 5, 6, 7, 9, 10, 12, 16, 18, 19, 21 – varieties Herakles, Koga, Capta, Cocoraque-75, Transec, Chul, Norin 29, Saratovskaya 29, Strela, Struna myronivska, Katiusha, Vitka – absence of allele *Ppd-B1a*; 3, 4, 8, 11, 13, 15, 17, 20, 22, 24 – varieties: Albis, Aranka, Atys, Loros, Losprout, Timstein (control sample), Udarnitsa, Kometa, Etiud, Azhurna – carriers of allele *Ppd-B1a;* 14, 23 – marker pUC19/MspI

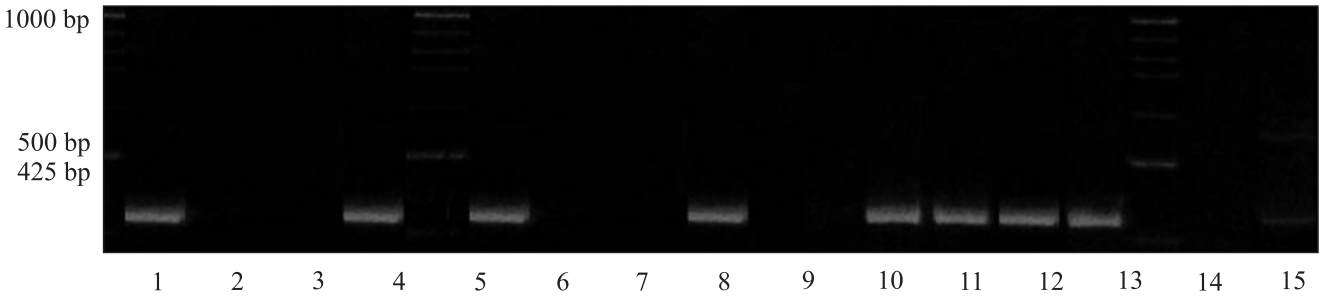

Fig. 2. The labelling of allele *Ppd-B1c* in spring bread wheat of various origin: 1, 4, 6, 9, 11, 12, 13, 14 – Chinese Spring (control sample), Transec, Bage, Norin 29, Konosu-25, Struna myronivska, Zhnitsa, Kometa – carriers of allele *Ppd-B1c*; 2, 3, 7, 8, 9, 10 – varieties Aranka, Big club, Atys, Turaco, Udarnitsa – absence of allele *Ppd-B1c*; 5, 15 – marker Ledder 1000

Ppd-B1a was almost twice smaller ($t = 2.84$ at $t_{0.05} = 1.96$), and allele *Ppd-B1c* – almost five times smaller ($t = 5.22$ at $t_{0.05} = 1.96$). The frequencies of alleles *Ppd-B1a* and *Ppd-B1c* did not demonstrate considerable differences in the samplings of varieties from different regions. For instance, allele *Ppd-B1a* was detected in the varieties from all regions, but its incidence in most regions did not exceed 15 %. The exception was found only for the varieties from the USA, Canada and Asia, where a tendency towards an increase in the frequency of this allele was noted up to 25.0 ± 12.50 % and 44.4 ± 16.56 %, respectively. The frequencies of allele *Ppd-B1c* did not demonstrate significant differences in the samplings of varieties from different regions, which may be explained by their low number. This allele was found in some varieties from Russia, Ukraine, USA, Japan and Brazil with the frequency of 4.3 ± 4.23 – 22.2 ± 13.85 %. Among the varieties from Europe and Mexico, the carriers of allele *Ppd-B1c* were not detected.

At the same time, significant differences in the frequencies of gene *Ppd-D1a* were noted between some regions. No variety, carrier of this dominant allele, was found in the sampling of Russian varieties. On the contrary, among the specified Mexican spring varieties, 100 % of them had allele *Ppd-D1a* in their genotype, which was reliably 73.9–100 % higher as compared with the value for other regions ($t = 8.06–29.94$ at $t_{0.05} = 1.96$), except for the sampling of Asian varieties, the differences with which were found to be insignificant ($d = 33.3 \pm 15.71$ %). In its turn, the frequency of this allele in Asian varieties was considerably exceeding the similar index ($t = 2.62–4.15$ at $t_{0.05} = 1.96–2.09$) in the samplings of the varieties from the USA, Canada, Europe and Russia. The latter three regions did not differ in this index. Significant differences in the frequencies of gene *Ppd-D1a* were also noted during the comparison of varieties from Ukraine and Russia ($t = 2.69$ at $t_{0.05} = 1.96$).

DISCUSSION

Many genetic studies found that winter and spring wheat varieties, grown in countries of higher latitude, usually have a higher incidence of photoperiod-sensitive alleles. On the contrary, the genotypes, grown in lower latitudes, usually carry photoperiod-insensitive alleles.

The results, presented in this article, demonstrate not a high incidence of dominant alleles of genes *Ppd-*

Table 3. The frequencies of *Ppd-1* genotypes in the samplings of spring bread wheat of various origin

Region, country	n	*Ppd-D1a*		*Ppd-B1a*		*Ppd-B1c*		*Ppd-D1a Ppd-B1a*		*Ppd-D1a Ppd-B1c*		*Recessive*	
		n	$p \pm S_p$	n	$p \pm S_p$	n	$p \pm S_p$	n	$p \pm S_p$	n	$p \pm S_p$	n	$p \pm S_p$
Europe	32	2	6.2 ± 4.26	2	6.2 ± 4.26	0	0.0 ± 2.86	1	3.1 ± 3.06	0	0.0 ± 2.86	27	84.5 ± 6.40
Russia	26	0	0.0 ± 3.44	2	7.7 ± 5.2	2	7.7 ± 5.2	0	0.0 ± 3.44	0	0.0 ± 3.44	22	84.6 ± 7.07
Ukraine	23	3	13.1 ± 7.03	0	0.0 ± 3.84	1	4.2 ± 4.18	3	13.1 ± 7.03	0	0.0 ± 3.84	16	69.6 ± 9.59
Mexico	20	17	85.0 ± 7.98	0	0.0 ± 4.34	0	0.0 ± 4.34	3	15.0 ± 7.98	0	0.0 ± 4.34	0	0.0 ± 4.34
USA and Canada	12	2	16.7 ± 10.77	3	25.0 ± 12.50	2	16.7 ± 10.77	0	0.0 ± 7.43	0	0.0 ± 7.43	5	41.6 ± 14.23
Asia (Japan, India)	9	2	22.2 ± 13.85	1	11.1 ± 10.47	1	11.1 ± 10.47	3	33.4 ± 15.72	1	11.1 ± 10.47	1	11.1 ± 10.47
Other	15	2	13.3 ± 8.77	2	13.3 ± 8.77	1	6.7 ± 6.46	0	0.0 ± 5.54	0	0.0 ± 5.54	10	66.7 ± 12.17
Total	137	28	20.5 ± 3.45	10	7.3 ± 2.09	7	5.1 ± 1.88	10	7.3 ± 2.09	1	0.7 ± 0.71	81	59.1 ± 4.20

Note. n – number of varieties in the sampling, p – genotype frequency, S_p – standard error

Table 4. The frequencies of alleles *Ppd-D1a*, *Ppd-B1a* and *Ppd-B1c* in the samplings of spring bread wheat of various origin

Region, country	n	*Ppd-D1a*		*Ppd-B1a*		*Ppd-B1c*		*Recessive*	
		n	$p \pm S_p$	n	$p \pm S_p$	n	$p \pm S_p$	n	$p \pm S_p$
Europe	32	3	9.4 ± 5.16	3	9.4 ± 5.16	0	0.0 ± 2.86	27	84.5 ± 6.40
Russia	26	0	0.0 ± 3.44	2	7.7 ± 5.2	2	7.7 ± 5.2	22	84.6 ± 7.07
Ukraine	23	6	26.1 ± 9.16	3	13.0 ± 7.01	1	4.3 ± 4.23	16	69.6 ± 9.59
Mexico	20	20	100.0 ± 0.00	3	15.0 ± 7.98	0	0.0 ± 4.34	0	0.0 ± 4.34
USA and Canada	12	2	16.7 ± 10.77	3	25.0 ± 12.50	2	16.7 ± 10.77	5	41.6 ± 14.23
Asia (Japan, India)	9	6	66.7 ± 15.71	4	44.4 ± 16.56	2	22.2 ± 13.85	1	11.1 ± 10.47
Other	15	2	13.3 ± 8.77	2	13.3 ± 8.77	1	6.7 ± 6.46	10	66.7 ± 12.17
Total	137	39	28.5 ± 3.86	20	14.6 ± 3.02	8	5.8 ± 2.00	81	59.1 ± 4.20

Note. n – number of varieties in the sampling, p – gene frequency, S_p – standard error

B1 and *Ppd-D1* in the sampling of European varieties (northern and central Europe mainly). A total of 5 varieties (or 15.6 %) carry these dominant alleles. In particular, monogenically dominant *Ppd-D1a* – the control of the trait was noted in the varieties Capta (France) and Orello (Switzerland), and *Ppd-B1a* – in varieties Albis (Switzerland) and Aranka (Czech Republic). The variety Atys (France) was found to have a digenically dominant genotype *Ppd-D1a Ppd-B1a*. The remaining 27 varieties (or 84.4 %) from Europe have only recessive alleles of all three genes *Ppd-1* in their genotype. A low frequency of allele *Ppd-D1a* in the sampling of spring varieties from Europe was noted previously (Shcherban A et al, 2015). Among 245 varieties, the authors found allele *Ppd-D1b* in 91.5 % of them. Allele *Ppd-D1a*, controlling insensitivity to the shorter day, was mainly found in wheat varieties from South Europe, while in other regions of Europe it is rare (only 3 varieties). At the same time, as noted in scientific literature (Síp V et al, 2010), 36.8 % varieties from Czech Republic and 90 % varieties from Slovakia have allele *Ppd-D1a.* Unfortunately, we have not managed to find any data about the frequency of dominant alleles of gene *Ppd-B1* in spring varieties from European countries.

The marking of varieties from the USA and Canada demonstrated that dominant genes *Ppd-1* are present in the genotype of seven out of 12 investigated samples, and allele *Ppd-D1a* was detected only in varieties Barton and Red River 68. The donor of gene *Ppd-D1a* for the variety Barton was a Brazilian spring variety, Frontana (*Ppd-D1a*), created using an Italian winter variety, Mentana, whose parents, in its turn, are a Japanese variety, Akakomugi, and a Dutch variety, Wilhelmina. The variety Red River 68, in whose development a spring variety, Sonora 64, had been used (*Ppd-D1a Ppd-B1a*), was often used in breeding as a donor of *Ppd-D1a*, but it did not inherit the second dominant allele *Ppd-B1a* from the Mexican parent. Allele *Ppd-B1a* was marked in varieties Big club, Loros (USA), Losprout (Canada). For instance, a Canadian variety, Lorsprout, also has a Mexican variety, Sonore 64, listed among its parental varieties. Varieties Chul and Transec were detected to have allele *Ppd-B1c*, inherited by the former of these from the ancient Uzbek variety (1913), and by the latter – from the Chinese variety, Chinese Spring. It is evident that the presence of dominant genes *Ppd-1* in the varieties from North America is not a rare event, which is noted in scientific literature (Whittal A. et al 2018), but it concerns only allele *Ppd-D1a*. It should also be noted that no varieties with digenically dominant control of the trait were found among the investigated spring samples from the USA and Canada.

In the sampling of Mexican varieties, allele *Ppd-D1a* was detected in all the varieties under investigation. It is clear that the distribution of *Ppd-D1a* among Mexican spring varieties results from the selection under naturally shorter light day, aimed at development a genotype with a poor response to photoperiod. In the pedigree data of varieties Seri-82, Sitta, Sitella, Bobwhite, the donors of gene *Ppd-D1a* are winter varieties Bezostaya 1, Kavkaz, Avrora. However, most varieties of the sampling inherited allele *Ppd-D1a* from Mexican or Brazilian spring varieties, whose pedigree data also contain the varieties, created using a Japanese variety Akakomugi. Three Mexican varieties (Alondra, Mexique-45, Turaco) were also found to have allele *Ppd-B1a* in addition to gene *Ppd-D1a* in the genotype which is probably caused by the involvement of variety Timstein, a carrier of a three-copy gene *Ppd-B1a*, in the breeding. It should be noted that no carriers of allele *Ppd-B1c* were found among the investigated Mexican varieties.

Out of the occasional identified spring samples from South America (a total of three varieties), the most widespread use in the breeding was noted for the variety Frontana, which inherited allele *Ppd-D1a* from an Italian winter variety, Mentana. A Brazilian variety, Bage, was detected to have allele *Ppd-B1c*, probably transferred from an Argentinian variety, Chino, created using the variety Chinese Spring. An ancient Argentinian variety, Lin calel (1927), carries only recessive alleles of *Ppd-1*. Even though the sampling of South American spring varieties is limited, it is possible to assume that ancient varieties had strong response, the decrease in which was achieved during the breeding. Over 70 % of the investigated Argentinian varieties carry dominant alleles of *Ppd-1* (Vanzetti LS et al, 2013).

The sampling under investigation has only two Australian varieties, which carry recessive alleles of genes *Ppd-1*. Cane et al (Cane K et al, 2013) noted a wide variability of *Ppd*-genotypes in Australian wheat varieties. For instance, the authors demonstrated the presence of monogenically dominant *Ppd-D1a,* or *Ppd-B1a,* or *Ppd-B1c* and digenically dominant varieties, carriers of multi-copy genes *Ppd-B1* in the combination with gene *Ppd-D1a*, and the varieties with strong photoperiod-sensitivity, where only recessive alleles are present.

High variability of *Ppd*-genotypes was demonstrated for Asian varieties, the sampling of which was small. Three Indian varieties were identified, out of which the ones, widely used in the breeding, were Kalyansona – recessive *Ppd-1* genotype, Sonalika – digenically dominant in genes *Ppd-D1a Ppd-B1a*. Line C591 has allele *Ppd-B1a*. Among Japanese varieties, Norin 29 was detected to have gene *Ppd-B1c*, varieties Norin 61 and Norin 17 – *Ppd-D1a*; Shiroganekomugi, Zenkojkomugi were shown to have a combination of alleles *Ppd-D1a* and *Ppd-B1a*, Konosu-25 – *Ppd-D1a* and *Ppd-B1c*. The sampling of Japanese spring varieties was not found to have samples with the presence of only recessive allele *Ppd-1* and the varieties with monogenically dominant *Ppd-B1a*-control, which is likely to result from a limited sampling. At the same time, (Seki M et al, 2013) demonstrated that most (90 %) Japanese spring wheat varieties did not carry photoperiod-sensitive alleles of genes *Ppd-1*. In Korea, the varieties-carriers of genes *Ppd-B1b* and *Ppd-D1a* (Eun et al 2015) are mostly wide-spread. All 59 Pakistani spring varieties, investigated by M. Iqbal (Iqbal M et al, 2011), with a single exception, carry gene *Ppd-D1a*.

African varieties are presented in a limited number, including three varieties from Kenya. The variety Beacon was identified to have allele *Ppd-D1a*, inherited from the variety Frintana, and the variety Salmayo – allele *Ppd-B1a*, most probably inherited from its Mexican parent. A recessive status of photoperiod genes was demonstrated for the variety Kenia farmer, widely used in the breeding of Australian and American varieties.

The results of marking spring varieties from the CIS countries and Ukraine are of most interest. The sampling of Russian spring varieties mainly included ancient local varieties and those, developed in the late previous century in the Volga region, Western Siberia, and the Urals. No variety from this sampling had allele *Ppd-D1a*. Likhenko et al. (Likhenko IE et al, 2014) analyzed 48 modern early-maturity and mid-early spring bread wheat varieties from Siberia and found only one variety Tulun 15, which carried allele *Ppd-D1a*. All the remaining ones had allele *Ppd-D1b* in the genotype. At the same time, the marker analysis demonstrated that Russian varieties Udarnitsa and Kometa had allele *Ppd-B1a* in their genotype, and varieties Zhnitsa and Strela – allele *Ppd-B1c*. Four varieties from northern Kazakhstan, investigated by us, carry recessive alleles of genes *Ppd-D1* and *Ppd-B1*, and by the phenotype, they are characterized as strongly photoperiod-sensitive genotypes (Fayt VI, Fedorova VR, 2014).

Contrary to the varieties from Russia and Kazakhstan, Ukrainian spring varieties have both ancient and modern varieties, created in this century. In the investigated sampling of Ukrainian varieties, allele *Ppd-D1a* was detected in 6 varieties, three of which – Azhurna, Elehia myronivska, Etiud – were found to have allele *Ppd-B1a* in addition to allele *Ppd-D1a* in the genotype. The variety Struna myronivska carries only allele *Ppd-B1c*, and this allele was inherited by the latter from one of parental varieties – a winter variety, Ekspromt. The variety Etiud was developed by crossing the variety TAM-200 (USA) and a Mexican spring variety, Turaco (*Ppd-D1a Ppd-B1a*-genotype). Parental genotypes of the variety Elehia myronivska are a spring variety, Maris Dove (Great Britain), and a winter variety, Myronivska 40. As British varieties are traditionally the ones with the highest photoperiod-sensitivity, the donor of two dominant genes may be a winter variety, Myronivska 40, created using a Mexican spring variety, Siete-Cerros. In general, spring varieties of different climatic zones of Ukraine were shown to have a wide distribution of allele *Ppd-D1b*. According to actual data, almost all modern winter wheat varieties of Ukrainian breeding, including the ones, created in the south of Ukraine, carry allele *Ppd-D1a* (Fayt VI et al, 2014). The results of the marker analysis and the pedigree data demonstrate that until the last decade of the previous century the varieties with high photoperiod-sensitivity prevailed among spring varieties both in Ukraine in most European countries. The decrease in the response from some modern spring varieties may be a targeted process, but there might be a possibility of a chance in these events, which is conditioned by the application of parental genotypes of the carriers of dominant genes *Ppd-1*. The urgency of creating genotypes with poor photoperiod-sensitivity requires the study of the impact on development rate of both specific alleles and *Ppd*-genotype in general, which may be facilitated by the information, obtained while marking the varieties of Ukrainian and foreign breeding.

In this study, *Ppd-1* genotypes were characterized only from the standpoint of the presence of dominant *Ppd-D1a*, *Ppd-B1a* and *Ppd-B1c*, which have a more considerable influence on the increase in the photoperiod-sensitivity. Allele *Ppd-A1a.1* is absent from the investigated spring varieties, and its distribution in the global assortment is limited. The donor of this allele is considered to be an ancient variety, Purplestraw (1822), and Purcam, used in the breeding in the USA, and later in the breeding of Japanese varieties (Seki M. et al 2013). In addition to alleles *Ppd-B1a* (three cop-

ies) and *Ppd-B1c* (four copies), gene *Ppd-B1* is known to have one more dominant allele – *Ppd-B1d* (two copies), which was not marked in this study. The scientific data demonstrate that a two-copy gene is sometimes present in bread wheat varieties but is more likely to be in combination with *Ppd-D1a*. For instance, in spring Australian varieties *Ppd-B1d* is present only in combination with another dominant gene (Chen F et al, 2013). Due to the low level of polymorphism, reliable PCR-tests for detection of a two-copy allele have not been developed. In some studies, it was suggested that it is relevant to mark dominant *Ppd-B1* using microsatellite analysis for loci *Xgwm257* and *Xgwm148*, the difference in allelic status between which are associated with recessive and dominant status of the gene (Chen F et al, 2013; Mohler V et al, 2004). The application of associative markers is possible, but the effectiveness of the analysis is not absolute, especially considering the data of scientific literature and the results of marking, obtained in this study. Presuming that *Ppd-B1d* is present in the combination with *Ppd-D1a*, the possibility of the presence of carriers of a two-copy cnv-mutant, for instance, in the investigated Ukrainian spring varieties, is incredibly low. There could be single cases of the presence of *Ppd-B1d* in European varieties under investigation.

Certainly, the variability of *Ppd-1* genotypes is not limited by the presence of marked dominant alleles. The varieties of the specified sampling may have allelic differences in terms of genes *Ppd-A1* and *Ppd-D1* with known mutant recessive alleles. The differentiation between genotypes, carriers of different recessive alleles of *Ppd-1* will ensure obtaining more detailed information about *Ppd*-genotypes of the varieties, presented in this study.

CONCLUSIONS

The carriers of alleles *Ppd-D1a*, *Ppd-B1a*, and *Ppd-B1c* were identified in 137 spring bread wheat varieties of various origin using molecular markers. The highest variability of *Ppd-1* genotypes was observed in Asian varieties. The varieties from other regions had from two (Mexico) to four (Europe, the USA, Canada, Ukraine) *Ppd-1* genotypes. Most varieties of the investigated sampling are carriers of only recessive *Ppd-1* (81 variety). A different ratio of genotypes, carriers of dominant *Ppd-D1* and *Ppd-B1* (39 and 28 varieties, respectively) is mostly conditioned by the priority use of *Ppd-D1a* carriers in the breeding. According to the pedigree data, the donor of *Ppd-D1a* for most varieties was a winter Japanese variety, Akakomugi. Most frequently, the predecessor varieties-donors for alleles *Ppd-B1a* and *Ppd-B1c* were varieties Sonora 64, Timstein, and Chinese Spring. The incidence of carriers of the alleles of a three-copy *Ppd-B1a* is higher than for four-copy *Ppd-B1c* (20 and 8 varieties, respectively). Modern Ukrainian spring monogenically dominant *Ppd-D1a* or *Ppd-B1c* varieties and the varieties with digenically dominant *Ppd-D1a Ppd-B1a* control were identified. The digenically dominant genotype *Ppd-D1a Ppd-B1c* was found only in a Japanese variety, Konosu-25. The varieties, identified by *Ppd-1* genes, may be used to study the influence of specific alleles and their combinations on the development tempo and other economic traits, and the application of marker analysis ensures the selection of breeding material with optimal combination of alleles of photoperiod genes.

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Поширення алелів *Ppd-D1a*, *Ppd-B1a* і *Ppd-B1c* генів фотоперіодичної чутливості у ярих сортів м'якої пшениці (*Triticum aestivum* L.) різного походження

І. А. Балашова, В. І. Файт

Національний центр насіннєзнавства та сортовивчення «Селекційно-генетичний інститут» НААН, Овідіопольска дорога 3, Одеса, Україна, 65036

E-mail: ibalashova@ukr.net, faygen@ukr.net

Мета. Метою цієї роботи є ідентифікація і оцінка частот зустрічальності алелів *Ppd-D1a*, *Ppd-B1a*, *Ppd-B1c* та *Ppd-1* генотипів ярих сортів м'якої пшениці різного походження. **Методи**. Виділення ДНК, алель-специфічна ПЛР, електрофорез в агарозному та поліакриламідному гелях, статистичний аналіз. **Результати.** Проведено маркування 137 сортів м'якої пшениці ярого типу розвитку різного походження для ідентифікації Ppd-1 генотипів носіїв алелів *Ppd-D1a*, *Ppd-B1a*, *Ppd-B1c*. У загальній вибірці вивчених сортів і вибірці сортів із Азії виявлено по шість різних *Ppd-1* генотипів. У вибірках інших регіонів – від двох (Мексика) до чотирьох (країни Європи, США і Канада, Україна) *Ppd-1* генотипів. У загальному наборі сортів з більшою частотою (20,5 %) виявлені домінантні тільки за алелем *Ppd-D1a* генотипи, з варіюванням від 0 (Росія) до 85,0 % (Мексика). Частоти моногенно домінантних за алелями *Ppd-B1a* (7,3 %) або *Ppd-B1c* (5,1 %) в загальному наборі були істотно нижче. Ці генотипи набули найбільшого поширення в вибірці

сортів США і Канади (25,0 і 16,7 %, відповідно). Дігенно домінантні *Ppd-D1aPpd-B1a* генотипи зустрічаються в загальному наборі з відносно низькою частотою (7,3 %) і відзначені у сортів країн Азії (33,4 %), Мексики (15,0 %), України (13,1 %) і країн Європи (3,1 %). Дігенно домінантний генотип *Ppd-D1a Ppd-B1c* виявлено тільки у японського сорту Konosu-25. Ген *Ppd-A1* у всіх досліджених ярих присутній у рецесивному стані. **Висновки**. З трьох домінантних алелів у вивченому наборі найбільшого поширення набув алель *Ppd-D1a* (28,5 %). Всі представлені в наборі сорти Мексики є носіями даного алеля. У той же час у жодного сорту Росії такого не виявлено. Алель *Ppd-B1a* присутній у сортів всіх регіонів з частотою 7,7 (Росія) – 44,4 % (країни Азії). Алель *Ppd-B1c* присутній у поодиноких сортів Росії, України, США, Японії і Бразилії, і його частота в загальній вибірці незначна (5,8 %). Сорти, ідентифіковані за алельним станом генів *Ppd-1,* можуть бути використані в якості донорів у селекції і для визначення ефектів тих чи інших алелів кожного з генів за темпами розвитку та пов'язаними з ними господарсько цінними ознаками м'якої пшениці.

Ключові слова: пшениця м'яка яра, фотоперіод, *Ppd-1* гени, алель-специфічна ПЛР.

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