

## HAPLOTYPES OF *PPD-D1* GENE AND ALLELES OF *PPD-A1* AND *PPD-B1* IN UKRAINIAN BREAD WHEAT VARIETIES

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Modern Ukrainian bread wheat varieties are not enough characterized by the alleles of *Ppd* genes and there is very restricted information about haplotypes of these genes in Ukrainian wheat genetic pool. The dominant alleles (*a*) of *Ppd-A1* (2A), *Ppd-B1*(2B), *Ppd-D1* (2D) genes reduce sensitivity to photoperiod thus shorten the period until earing and genotypes with recessive alleles (*b*) have a strong reaction to the photoperiod. Haplotypes of *Ppd-D1* gene could also affect photoperiod sensitivity. According to PCR analysis with allele specific primers most investigated varieties were characterized by *Ppd-A1b* and *Ppd-B1b* alleles. 40 varieties (81.2 %) had the deletion upstream the coding region of *Ppd-D1* gene, that distinguish *Ppd-D1a* allele. Also, we analyzed haplotypes of *Ppd-D1* gene. There was shown presence of VII haplotype of *Ppd-D1* gene for 79.6 % of varieties. The varieties with I (2 %), II (6 %), III (10 %) haplotypes of the *Ppd-D1* gene were less frequent. One variety was heterogeneous by *Ppd-D1a/b* alleles. From the tested ones only varieties of winter bread wheat from The V.M. Remeslo Myronivka Institute of Wheat (MIP) were polymorphic by investigated genes that highlight the importance of *Ppd-A1b*, *Ppd-B1b* and *Ppd-D1a* alleles for environmental conditions in the Ukraine. The haplotype (III) was detected in genotypes of 5 varieties – three of them from MIP and in varieties Etos and Yevdokiia. Also this haplotype was found in the well known old variety created in 1938 year – Odes'ka 3 – by Guo et al. [14], thus transposable element (TE) insertion in the 1 intron of *Ppd-D1* gene was historically present in the background of Ukrainian wheats. Simultaneously, spring wheat varieties have higher level of genetic polymorphism at *Ppd-D1* loci than winter bread wheat varieties, maybe this locus and sensitivity to photoperiod is not critical for adaptability of spring wheat.

*Keywords:* photoperiod sensitivity, haplotypes, *Ppd*-genes, wheat varieties

The growth and development of plants are significantly affected by light period. Cereals, including bread wheat (*Triticum aestivum* L.), react to change in the duration of light day by accelerating or slowing down the development. The degree of such reaction in bread wheat depends mainly from three genes that are localized in the second chromosome of each wheat subgenome A, B and D: *Ppd-A1* (2A), *Ppd-B1*(2B), *Ppd-D1* (2D). The dominant alleles (*a*) of *Ppd* genes reduce sensitivity to duration of the day thus shorten the period until earing and genotypes with recessive alleles (*b*) have a strong reaction to the photoperiod [17].

According to Langer et al. [16], *Ppd-D1* gene is the major factor affecting flowering time in population of 410 winter wheat varieties (that was evaluated in multi-location field trials), explaining 58 % of the genotypic variance. Copy number variation at the *Ppd-B1* locus explains only 3.2 % of the genotypic variance [16].

Previously well characterized *Ppd-D1a* and sensitive *Ppd-D1b* alleles did not perfectly explain the broad adaptation of wheat to photoperiod variation. Guo et al. [14] detected several mutations in nucleotide sequences of *Ppd-D1* which designated six haplotypes (I – VI) of the *Ppd-D1* gene (Fig. 1). The highest expression level (pick time in the morning in short-day conditions) was observed for haplotype I with shortest days to heading [14]. All haplotypes also affected significantly other agronomic traits: days to heading, plant height and 1000-kernel weight tested in three environments in China [14].

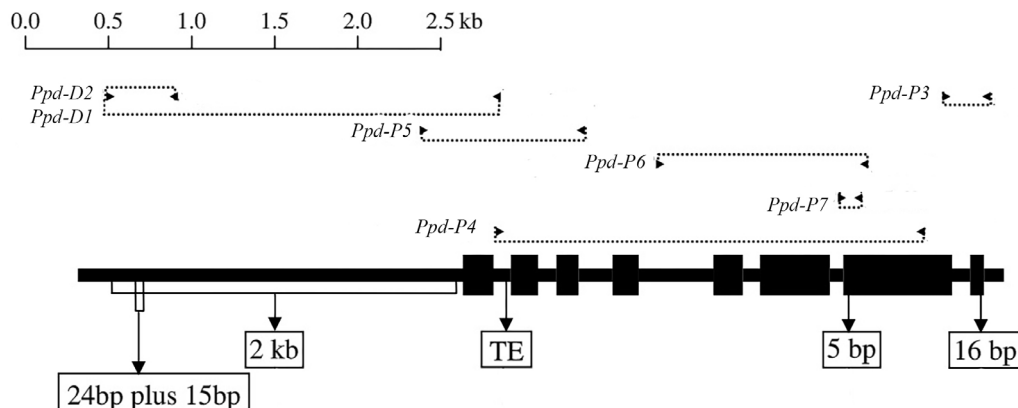


Fig. 1. Schematic structure of *Ppd-D1* gene according to Guo et al. [14] with localization of the primers that have been applied in this investigation. Tall rectangles represent coding regions, low rectangles represent the introns, 5'UTR and 3'UTR regions

Previously there was no information about haplotypes of *Ppd-D1* in Ukrainian varieties.

The aim of the work was to determine alleles of genes *Ppd-A1*, *Ppd-B1* and haplotypes of *Ppd-D1* for modern winter and spring wheat varieties using molecular markers.

#### Materials and methods

As the material there were used the mostly modern winter wheat varieties from: Bilatserkovska Experimental Breeding Station (BEBS; 41°64' N 31°08' E) – Bilatserkovska polukarlikova (1999), Olesya (2001), Pearlyna lisostepu (2001), Elegiya (2003), Yasochka (2006), Lybid (2006), Tsarivna (2008), Lisova pisnya (2008), Romantyka (2009), Vidrada (2010), Tschedra nyva (2011), Charodiyka bilotserkivska (2011), Vodogray bilotserkivsky (2014); The V.M. Remeslo Myronivka Institute of Wheat (MIP; 49°64' N; 31°08' E) – Beregynya myronivs'ka (2016), Horlytsya myronivs'ka (2016), Economka (2008), Zymoyarka (2007), Kryzhynka (2002), Legenda myronivs'ka (2012), Myronivs'ka zolotovercha (at the varietal testing), Myronivs'ka 65 (2000), Myronivs'ka storichna (2009), Myronivs'ka rann'ostyhla (2002), Oberig myronivs'ky (2014), Pamyati Remesla (2009), Svitanok myronivs'ky (2014), Juviliyar myronivs'ky (2009); Poltava State Agrarian Academy (PSAA; 49°71' N, 34°51' E) – Dykan'ka (2005), Levada (2005), Sahaydak (2010), Sydor Kovpak (2008\*), Tsarichanka (2013), Lyut'enka (2009\*), Sonata poltavaska (2018), Vilshana (2010), Govtva, Orzhytsa (2013), Karmelyuk (2015), Kolomak 3 (1997), Kolomak 5 (1997), Ukrainka poltavaska (2000); spring wheat varieties – Krasa Polissia (2003) (Nosivska Breeding and Research Station), Ethos (Saaten-Union GmbH), Struna myronivs'ka (2008) (MIP), Yevdokiia (2007) (The Plant Production Institute nd. a. V. Ya. Yuryev of National Academy of Agrarian Sciences of Ukraine), Etiud (2006) (MIP), Nedra (2007) (National Scientific Center "Institute of Agriculture of NAAS"), Natasa (2009) (Institute of Field and Vegetable Crops, Novi Sad, and "NS SEME-UKRAINA", Torchyns'ka (Peasant farming "Roden' 10").

DNA was isolated according to [5]. Allele-specific PCR with the primers recommended by Beales et al. [10], Nishida et al. [19] and Gao et al. [14] to *Ppd-A1*, *Ppd-B1*, *Ppd-D1* genes were used (Table 1).

The amplification products were fractionated by electrophoresis in 1 % agarose gel and visualized using the Gel Doc™ XR + System Bio-Rad video system (USA) and also in 7 % polyacrylamide gel strained with AgNO<sub>3</sub>, according to Promega [20]. The sizes of the amplified fragments were estimated using the standard molecular weight pUC19 / Msp I, Gene ruler 100 bp and ladder mix.

Table 1

PCR primers for detecting alleles of *Ppd-A1*, *Ppd-B1* and *Ppd-D1* in bread wheat, that have been applied in the investigation

| Locus         | Allele                           | Forward and reverse primers                               | Expected band size (bp) | Reference           |
|---------------|----------------------------------|---|-------------------------|---------------------|
| <i>Ppd-A1</i> | <i>Ppd-A1a</i>                   | 5'-cgtactccctcgtttcttt-3'<br>5'-aatttacggggaccaataacc-3'  | 338                     | Nishida et al. [19] |
| <i>Ppd-A1</i> | <i>Ppd-A1b</i>                   | 5'-cgtactccctcgtttcttt-3'<br>5'-gttggggtcgtttggtggtg-3'   | 299                     | Nishida et al. [19] |
| <i>Ppd-B1</i> | <i>Ppd-B1b</i>                   | 5'-acactagggtgctgcaaga-3'                                 | 1292 /                  | Nishida et al. [19] |
|               | <i>Ppd-B1a</i>                   | 5'-ccgagccagctgcaaattaac-3'                               | 1600                    |                     |
| <i>Ppd-D1</i> | <i>Ppd-D1a</i>                   | 5'-acgcctcccactactg-3'<br>5'-cactgggtgtagctgagatt-3'      | 288 or 2377             | Beales et al. [10]  |
| <i>Ppd-D2</i> | <i>Ppd-D1b</i>                   | 5'-acgcctcccactactg-3'<br>5'-gttgggtcaaacagagagc-3'       | 414 or 453              | Beales et al. [10]  |
| <i>Ppd-P3</i> | 16 bp insertion<br>Exon 8        | 5'-gatgaacatgaacggg-3'<br>5'-gtctaaatagtagtactagg-3'      | 320 or 336              | Beales et al. [10]  |
| <i>Ppd-P4</i> | TE deletion                      | 5'-aggctctactcactcaatctca-3'<br>5'-ctcccattgtggtgtgta-3'  | 2612                    | Guo et al. [14]     |
| <i>Ppd-P5</i> | 2 kb deletion or<br>TE insertion | 5'-ccattcgaggagacgattcat-3'<br>5'-ctgagaaagaacagagctca-3' | 1005                    | Guo et al. [14]     |
| <i>Ppd-P6</i> | 5 bp deletion<br>Exon 7          | 5'-gaatggctctcctggtc-3'<br>5'-gatggcgaaaccttatt-3'        | 1,032 or 1,027          | Guo et al. [14]     |
| <i>Ppd-P7</i> | 5 bp deletion<br>Exon 7          | 5'-gtgtccttgcgaatcct-3'<br>5'-ttggagcctgctcatct-3'        | 184 or 179              | Guo et al. [14]     |

### Results

According to our previous data [1] MIP wheat varieties have been differentiated into three groups, with different genotypes corresponding to the alleles of *Ppd-1* genes: I group – genotype *Ppd-A1a Ppd-B1b Ppd-D1a* – Horlytsya myronivs'ka (in later investigations we detected also *Ppd-A1b* allele in this variety); II group – genotype *Ppd-A1b Ppd-B1b Ppd-D1a* – Kryzhynka, Legenda myronivs'ka, Myronivs'ka 65, Myronivs'ka rann'ostyhla, Oberig myronivs'ky, Pamyati Remesla, Svitanok myronivs'ky, Juviliyar myronivs'ky, Economka; III group – genotype *Ppd-A1b Ppd-B1b Ppd-D1b* – Beregynya myronivs'ka, Zymoyarka, Myronivs'ka zolotovercha, Myronivs'ka storichna.

Almost all of these varieties had significant pair differences according to the earing date on the natural photoperiod, with a minimum difference of 5.38 days for Myronivs'ka storichna and Beregynya myronivs'ka, the maximum difference was observed between Myronivs'ka storichna and Zymoyarka – 27.82 days [1]. The difference in flowering time between Myronivs'ka storichna and Zymoyarka could not be precisely explained by the alleles of the *Ppd-A1b Ppd-B1b Ppd-D1b*. Also we detected significant differences ( $P=0,05$ ;  $P=0,01$ ) between MIP varieties with *Ppd-A1b Ppd-B1b Ppd-D1a* genotype in natural photoperiod in vegetable vessels in each year (2014, 2015 and 2016). Thus, we analyzed haplotypes of *Ppd-D1* gene in MIP wheat varieties.

Winter wheat varieties from the breeding stations of Central (BEBS) and East (PSAA) region of Ukraine were characterized by alleles *Ppd-A1b/Ppd-B1b/Ppd-D1a*. The start of the ear emergence for BEBS varieties differ not significantly in average 5 days in three years field trials.

Spring wheat varieties were divided into two groups: *Ppd-A1b/Ppd-B1b/Ppd-D1a* and *Ppd-A1b/Ppd-B1b/Ppd-D1b*. Plants from varieties Krasa Polissia, Yevdokiia, Nedra and Torchyns'ka were characterized as strongly sensitive to photoperiod, whereas Etiud was weakly sensitive to photoperiod; Struna myronivs'ka, Natasa and Ethos have middle sensitivity to photoperiod according to State Register of Plant Varieties Suitable for Dissemination in Ukraine [3, 4].

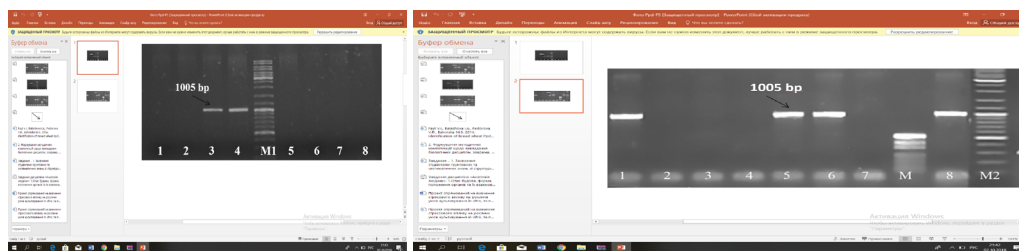
As a result of investigation of *Ppd-D1* sequence structure by using molecular markers recommended by Beales et al. [10] and Guo et al. [14] different haplotypes have been revealed among investigated varieties (Table 1). Two polymorphisms: a 2089-bp deletion upstream the coding region and the *mariner*-like transposable element (TE) insertion in intron 1 were found in Ukrainian bread wheat varieties (Table 2). Thus among 49 wheat varieties analyzed in this study, 40 varieties (81.6 %) had a 2089-bp deletion upstream of the coding region corresponded to the photoperiod-insensitive *Ppd-D1a* allele. One variety – Nedra – was heterogeneous according the presence of this deletion. The photoperiod-sensitive *Ppd-D1b* allele was detected in the 8 remaining varieties. In the genotypes of 5 varieties absence of the *mariner*-like TE in intron 1 of the *Ppd-D1* gene were detected (Fig. 2, A, B). Thereby, 4 combinations of the 2 mentioned polymorphisms (Table 2) were detected in the tested wheat varieties.

Table 2

*Ppd-D1* haplotypes identified in the investigated bread wheat varieties

| <i>Ppd-D1</i> haplotype | Variety   | 24 bp + 15 bp | 2 kb | TE | 5 bp | 16 bp |
|-------------------------|---|---------------|------|----|------|-------|
| I*                      | Etiud   | –             | –    | –  | +    | –     |
| I/II                    | Nedra   | –             | +/-  | –  | +    | –     |
| II*                     | Krasa Polissia, Torchyns'ka,<br>Zymoyarka                     | –             | +    | –  | +    | –     |
| III*                    | Myronivs'ka storichna, Myronivs'ka<br>zolotovercha, Beregynya | –             | +    | +  | +    | –     |
| VII**                   | myronivs'ka, Etos, Yevdokiia<br>Other 39 varieties            | –             | –    | +  | +    | –     |

**Notes.** \* – Haplotypes designated by Guo et al. [14]; \*\* – Haplotype designated by Chen et al. [12]. Insertions and deletions are indicated by + and -, respectively



A

B

Fig. 2. Electrophoresis of PCR amplification fragments of wheat varieties with marker Ppd-P5: A – 1 – Beregynya myronivs'ka; 2 – Myronivs'ka storichna; 3 – Zymoyarka (1); 4 – Zymoyarka (2); B – 1 – Krasa Polissia; 2 – Ethos; 3 – Struna myronivs'ka; 4 – Yevdokiia; 5 – Etiud; 6 – Nedra; 7 – Natasa; 8 – Torchyns'ka; M – *pUC 19 / Msp I*; M1, M2 – molecular weight marker ladder mix

In variety Zymoyarka (MIP) TE insertion from the first intron was absent (Fig. 2A), all other varieties from MIP had this insertion. The presence of TE insertion in the first intron reduces the expression level of *Ppd-D1b* [12] and, as we assume, due to this, MIP varieties with *Ppd-D1b*

earied later for 11–28 days than Zymoyarka on the natural photoperiod in artificial conditions [1]. Although in this variety the biggest difference between time of earing was detected on the natural and short photoperiod in artificial conditions as compared to with winter wheat varieties from MIP. According to Vlasenko et al. [2] variety Zymoyarka is not a descendent of varieties Ukrainka or Myronivs'ka 808 as a majority of other investigated MIP varieties, but could have in the pedigree cv. Krymka or germplasm of some other Ukrainian varieties. It is known that Krymka is one from the old and very heterogenic varieties. Deletion in exon 7 (size 5 bp) was absent in tested varieties from MIP.

Investigated varieties from BEBS and PSAA have not polymorphism in *Ppd-D1* locus, all these varieties have 2089-bp deletion upstream of the coding region, TE insertion in 1 intron; 5 bp deletion in exon 7 and 16 bp insertion in exon 8 were absent in their genotypes and thus these varieties were corresponded to the VII haplotype.

### Discussion

According to Langer et al. [16] allele *Ppd-D1a* is rare in the UK, Denmark, Germany, Poland, the Czech Republic and in Austria, and it is predominant in Eastern Europe and Russia. In our previous investigations among the 27 varieties of spring wheat from the collection of National Centre of Genetic Resources of Ukraine, 22.2 % of tested varieties were characterized by the presence of the *Ppd-D1a* allele, indicating weak sensitivity of varieties to the photoperiod, 11 % of varieties were heterogeneous, and 66 % had the *Ppd-D1b* allele. All 34 investigated varieties from Institute of Cytology and Genetics of Siberian Branch of Russian Academy of Sciences were the carriers of allele *Ppd-D1b* [7]. We revealed the widespread distribution of the *Ppd-D1b* allele among the spring varieties of various climatic zones of Ukraine and the Russian Federation. It should be noted that among the modern winter wheat varieties from Ukraine, the varieties created in the south region mostly have the allele *Ppd-D1a* [1, 8, 11]. In this work we revealed that 79.6 % of varieties corresponds to the VII haplotype and only one variety – to the I haplotype. In the investigations of Guo et al. [14], the varieties with I haplotype have the highest frequency among world wheat germplasm. The same situation was detected by Chen et al. [12] in the bread wheat from the Yellow and Huai Valley of China. The difference between I and VII haplotypes can be explained by absence of TE insertion in the first intron in the I haplotype. Absence of TE insertion can be identified by presence of fragment 1005 bp which is generated in PCR with marker Ppd-P5 and we have not tested amplification fragment 1005 bp with marker Ppd-P5 for 39 wheat varieties. The III haplotype was found in the famous old variety Odes'ka 3 by Guo et al. [14], thus TE insertion in the 1 intron of *Ppd-D1* gene was historically present in the gene pool of Ukrainian wheats. In this investigation of Ukrainian wheat varieties the 5-bp deletion in exon 7, 16-bp insertion in exon 8 and a 24-bp plus a 15-bp insertions in the 2-kp upstream region were not found. Earlier Guo et al. [14] have found the genotypes that have 24-bp plus 15-bp insertions in the 2-kb upstream region among synthetic wheats and *Aegilops tauschii* Coss. accessions. In previous work Chebotar et al. [8] also detected 24-bp plus a 15-bp insertions and new variant of polymorphism – only 15-bp insertion (without 24-bp insertion) in the collection of *Aegilops tauschii* accessions.

Most Ukrainian varieties investigated in this work have *Ppd-D1a* (81.2 %) photoperiod insensitive allele, according to the previously known nomenclature, or characterized by the VII haplotype (79.6 %). Observed significant differences in different years between varieties with the same genotype that leveled off after combining of three years data, that could be due to the multidirectional effects of environmental factors in different years or the effects of other genetic systems that affect time of plants development, or modification variability, or epigenetic marks

(such as levels of methylation).

Investigated in our work spring varieties Krasa Polissia, Yevdokiia, Etiud, Struna myronivs'ka, Torchyns'ka were divided into the haplotypes of *Ppd-Alb* gene by Muterko et al. [18], they are: I – Krasa Polissia, Yevdokiia; III – Etiud and IV – Struna myronivs'ka, Torchyns'ka and haplogroups AII – Krasa Polissia, Yevdokiia, Etiud and AI – Struna myronivs'ka, Torchyns'ka.

According to Fayt et al. [6] among 129 winter wheat varieties from different regions of Ukraine and Russia that have been created mostly at the end of the 20-th century the frequency of allele *Ppd-D1a* was 77.5 %. Among varieties and landraces of wheat from Turkey [9], the frequency of *Ppd-D1a* is 60 % in spring wheats, and in winter wheat varieties the frequency is 54 %. Among the 20 varieties created from 1960 to 2003 in Bulgaria, a large majority of which are winter, the frequency of this allele is 93.3 % [15]. Yang et al. [21] in the study of 926 landrace and wheat varieties from China, showed the presence of *Ppd-D1a* in 66 % of genotypes, while its frequency in landrace – 38.6 %, and in varieties – 90.6 %. The authors note that *Ppd-D1a* is present in all “improved varieties” developed and zoned after 1970, with the exception of spring wheat, created in high latitudes of Northwest China and winter varieties in Gansu and Xinjiang. According to Chen et al. [12] 89,6 % of bread wheat from the Yellow and Huai Valley of China have *Ppd-D1a*. Most tested wheat varieties that are grown in Southern and Central Europe are insensitive to the photoperiod, while British varieties are the most susceptible [13]. Unfortunately, further large-scale genotyping of wheat germplasm from different geographic regions (except China) has not yet been performed in terms of photoperiod response alleles. In Ukraine there were few investigations of *Ppd-D1* haplotypes earlier. The results of this study are useful as description of Ukrainian wheat germplasm and could be in demand for breeding of wheat varieties for better maturity and adaptability.

### Conclusions

We have determined allelic characteristic of *Ppd-A1*, *Ppd-B1* and *Ppd-D1* loci for modern wheat varieties from Central and East regions of Ukraine. Most varieties have *Ppd-Alb* allele. With the primers recommended by Nishida et al. [19], the PCR fragment 1292 bp that corresponds to *Ppd-B1b* allele were detected in all varieties. We have also detected haplotypes of *Ppd-D1* gene. Among all investigated varieties the haplotype VII was the most common (it was present in 79.6 % of varieties). Simultaneously, spring wheat varieties have higher level of genetic polymorphism at *Ppd-D1* loci than winter bread wheat varieties. In 8 spring wheat varieties to the each of haplotypes II, III and VII were corresponded 25 % of varieties (2 varieties), and by 12.5 % (1 variety) accounted to haplotype I and heterogeneous variety Nedra. Among winter wheat varieties 90.3 % (37 varieties) corresponds to the haplotype VII, 2.4 % (1 variety) – II haplotype and 7.39 % (3 varieties) – III haplotype. Maybe *Ppd-D1* locus and sensitivity to photoperiod is not critical for adaptability of spring wheat.

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## ГАПЛОТИПИ *PPD-D1* ГЕНА Й АЛЕЛІ *PPD-A1* І *PPD-B1* В УКРАЇНСЬКИХ СОРТАХ М'ЯКОЇ ПШЕНИЦІ

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Сучасні українські сорти м'якої пшениці недостатньо охарактеризовані за алелями генів *Ppd*, також недостатньо інформації щодо гаплотипів за цими генами у вітчизняному генетичному пулі пшениці. Домінантні алелі (*a*) генів *Ppd-A1* (2A), *Ppd-B1* (2B), *Ppd-D1* (2D) знижують чутливість рослин пшениці до фотоперіоду, тим самим скорочуючи період до колосіння, а рецесивні алелі (*b*) характерні для генотипів зі сильною реакцією на фотоперіод. Гаплотипи гена *Ppd-D1* також можуть впливати на фотоперіодичну чутливість. За результатами ПЛІР-аналізу з алель-специфічними праймерами більшість досліджених сортів характеризувалися алелями *Ppd-A1b* та *Ppd-B1b*. 40 сортів (81,2 %) мали делецію перед кодуєчим регіоном *Ppd-D1* гена, яка відрізняє *Ppd-D1a* алель. Також ми проаналізували гаплотипи гена *Ppd-D1*. Було показано наявність VII гаплотипу гена *Ppd-D1* у 79,6 % сортів. Сорти з гаплотипами I (2 %), II (6 %), III (10%) гена *Ppd-D1* траплялися з меншою частотою. Один сорт був гетерогенним за алелями *Ppd-D1a/b*. Із досліджених лише сорти озимої м'якої пшениці Миронівського інституту пшениці імені В.М. Ремесло (МІП) були поліморфними за генами *Ppd-1*, що підкреслює важливість алелів *Ppd-A1b*, *Ppd-B1b* та *Ppd-D1a* для вирощування рослин в еколого-географічних умовах України. Гаплотип (III) був виявлений у генотипах 5 сортів – у трьох з МІП і в ярих сортах Етос та Євдокія, також цей гаплотип детектовано у стародавньому та відомому сорті Одеська 3 (створеному у 1938 р.) в роботі Guo et al. [14], таким чином, TE в 1 інtronі гена *Ppd-D1* був історично наявним у сортах пшениці в Україні. Цікаво, що сорти ярої пшениці мають більш високий рівень генетичного поліморфізму в локусі *Ppd-D1*, ніж сорти озимої м'якої пшениці. Можливо, цей локус і чутливість до фотоперіоду не є критичними для адаптивності ярої пшениці.

**Ключові слова:** чутливість до фотоперіоду, гаплотипи, *Ppd*-гени, сорти пшениці