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Genetic diversity of bread wheat and durum wheat varieties at the *Ppd1*- and *ZCCT1* loci and identification genotypes by DNA markers

Abstract: The study of growth type and rate of development alleles gene of valuable cereal species, in particular wheat, determination of single alleles and their combinations in specific genetic material, identification of genotypes – carriers of different functional allelic variants is significant for the creation of varieties adapted to cultivation in specific climatic condition. In this regard, it is relevant to conduct molecular genetic analysis using DNA markers, which can identify allelic variations of *Vrn*- and *Ppd*-genes and their carriers in wheat, which is vital for the selection of genotypes and practical breeding of high-yielding varieties in certain climatic regions. The study object is the molecular genetic polymorphism of wheat varieties at the loci of growth type and rate of development. The study aimed to research the allelic diversity at the *Ppd-1* and *ZCCT-1* loci of bread wheat (*T. aestivum*) and durum wheat (*T. durum*) varieties of different origins and identification of genotypes carrying the corresponding alleles based on DNA markers. The objectives of the work included marker analysis of wheat varieties (*T. aestivum, T. durum*) at these loci and identification of the corresponding alleles and genotypes. Methods: DNA extraction, allele-specific PCR, agarose and polyacrylamide gel electrophoresis. Statistical analysis was performed using Microsoft Excel software. The genetic variability of 46 spring wheat varieties (25 – *T. aestivum*, 21 – *T. durum*) of various origins was analyzed at the *Ppd-1* and *ZCCT-1* loci. Possible combinations of alleles for each of the studied loci in bread and durum wheat varieties were identified, and the most common variants were determined. Among the studies of bread wheat varieties, eleven *Ppd-1* genotypes were identified. Two mutant recessive alleles were low-frequency in bread wheat varieties, whereas one was widely distributed in durum wheat. The *ZCCT-1* locus in the studied varieties is represented mostly by two genes. Absence of *ZCCT-B1* (null allele) is standard. In bread wheat varieties, only one gene (double null allele) and all three ZCCT-1 genes were detected once. The frequency of the null allele for *ZCCT-B1* is comparable to that of varieties carrying two other genes (*ZCCT-D1, ZCCT-A1*) of the *ZCCT-1* locus. The combination of *ZCCT-1* with the alleles *Ppd-A1b* or *Ppd-A1_del2ex7* is also comparable in the frequency of distribution in varieties. The distribution frequency in the combination

ZCCT-1 varieties with the alleles *Ppd-A1b* or *Ppd-A1_del2ex7* is also comparable. The analysis results can be used in genetic and plant breeding research to select genotypes and predict agronomically important traits in wheat.

Keywords: bread wheat (T. aestivum. L), durum wheat (T. durum. Desf.), Ppd1, ZCCT1, DNA polymorphism, DNA markers.

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Генетичне різноманіття сортів пшениці м'якої та твердої за локусами *Ppd1-* та *ZCCT1* та ідентифікація генотипів за ДНК-маркерами

Анотація. Вивчення алелів генів типу та темпів розвитку цінних видів злаків, в тому числі пшениці, визначення окремих алелів та їх комбінацій у певного генетичного матеріалу, виявлення генотипів – носіїв різних функціональних алельних варіантів має суттєве значення для створення сортів, адаптованих до вирощування в конкретних кліматичних умовах. У цьому зв'язку актуальним є проведення молекулярно-генетичного аналізу з використанням ДНК-маркерів, які здатні ідентифікувати алельні варіації *Vrn-* та *Ppd*-генів та їх носіїв у цінних злаків, зокрема пшениці для добору генотипів та ефективної селекції високоврожайних сортів у певних кліматичних регіонах. Об'єкт даного дослідження - молекулярно-генетичний поліморфізм сортів пшениці за локусами типу та темпів розвитку. Метою роботи було вивчення алельного різноманіття сортів пшениці м'якої (*T. aestivum*) та твердої (*T. durum*) різного походження за локусами *Ppd-1*, *ZCCT-1*, ідентифікація відповідних алелів та генотипів-носіїв на основі ДНК-маркерів. До завдань роботи входило проведення маркерного аналізу сортів пшениці (*T. aestivum, T. durum*) за даними локусами, виявлення відповідних алелів та ідентифікація генотипів. Методи: виділення ДНК, алель-специфічна ПЛР, електрофорез у агарозному та поліакриламідному гелях, аналіз отриманих даних з використанням Microsoft Excel. Результати. Проаналізовано генетичну варіабельність 46 сортів ярої пшениці (25 – *T. aestivum*, 21 – *T. durum*) різного походження за локусами *Ppd-1* та *ZCCT-1*, встановлено можливі комбінації алелів для кожного з досліджуваних локусів у сортів пшениці м'якої та твердої, визначено найбільш поширені варіанти. Серед досліджуваних сортів м'якої пшениці виявлено одинадцять *Ppd-1*-генотипів. У ярих сортів м'якої пшениці показана низька зустрічальність двох мутантних рецесивних алелів і поширеність одного з них у пшениці твердої. Локус *ZCCT-1* у досліджених сортів представлений здебільшого двома генами. Поширеним є відсутність *ZCCT-B1* (нульовий алель). У поодиноких випадках у сортів *T. aestivum* виявлено наявність лише одного гена (подвійний нуль-алель) та всіх трьох генів *ZCCT-1*. Частота зустрічальності нуль-алелю по *ZCCT-B1* порівняна з такою сортів-носіїв двох інших генів (*ZCCT-D1*, *ZCCT-A1*) локусу *ZCCT-1*. Також порівнянною є частота поширення у сортів комбінації *ZCCT-1* з алелями *Ppd-A1b* або *Ppd-A1_del2ex7*. Результати аналізу можуть бути використані в

генетико-селекційних дослідженнях для добору генотипів та прогнозування відповідних агрономічно-цінних ознак у пшениці.

Ключові слова: пшениця м'яка (T. aestivum. L), пшениця тверда (T. durum. Desf.), Ppd1, ZCCT1, поліморфізм ДНК, ДНК-маркери.

Abbreviations:

PRR is Pseudo-Response-Regulator,

FT is Flowering Time,

Vrn is Vernalization,

Ppd is Photoperiod,

$ZCCT$ is Zinc Finger CCT,

DNA is Deoxyribonucleic Acid,

$CTAB$ is Cetyltrimethylammonium Bromide,

PCR is Polymerase Chain Reaction.

Introduction

Increasing the adaptive potential of created genotypes of valuable cereal species, particularly wheat, due to optimal development rates in a specific growing region continues to attract significant attention. It is one of the essential directions of modern plant breeding programs (*Hasan et al., 2021*). In wheat, as in other cereal crops, the *Ppd* and *Vrn* gene systems are the main ones in controlling the adaptive responses of the plant organism to the environment. By controlling the duration and rate of the initial stages of organogenesis, the alleles of the *Vrn* and *Ppd* genes have a direct effect on many important traits, in particular, the level of formation of yield components, the resistance of genotypes to adverse conditions caused by the action of biotic and abiotic factors.

Ppd-1 genes of wheat are localized in the short arm of chromosomes of the second homeologous group. They belong to the family of *PRRs* (*Beales et al., 2007*; *Cockram et al., 2007*), whose protein products directly influence the activity of other genes, in particular *FT*, which are responsible for the induction of flowering (*Turck et al., 2008*; *Fernandez-Calleja et al., 2021*). There is a direct relationship, particularly in wheat, between the expression of dominant *Ppd-1* alleles, the transcriptional activity of the *FT* (*Vrn 3*) locus, and heading time. (*Nishida et al., 2013*).

Ppd-1 genes are important regulators of flowering in cereals. Thus, varieties that weakly respond to photoperiod are more adapted to growing conditions in areas with short winter, early spring, and high summer temperatures (*Law et al., 1997*; *Worland et al., 1998*). Photoperiod-insensitive alleles became widespread in bread wheat varieties after the "green revolution." Mutations identified in dominant alleles of *Ppd-1* are due to deletions or insertions in the promoter region, such as an increase in the number of copies of this gene (CNV mutants) (*Diaz et al., 2012*).

The influence of dominant alleles of *Ppd-1* from homeologous A, B, and D genomes of wheat on the reduction of sensitivity to photoperiod and heading time is not equivalent. Thus, the dominant allele *Ppd-D1a* is less sensitive to photoperiod and is associated with the earliest

flowering in short days. Lower expression levels were shown for dominant alleles of the *Ppd-A1* and *Ppd-B1* genes (*Bentley et al. 2013*).

PPD-1 proteins induce the *Vrn* 3 flowering locus, the repressor of which is the *Vrn2* locus, as one of the important elements of the adaptive regulatory mechanism of vernalization (*Kippes et al., 2016*).

It is known that the *VRN2* locus in wheat contains genes that encode transcription factors of the ZCCT type with the so-called "zinc finger" structure and the CCT motif, which is similar to other proteins in *A. thaliana* (*Kinmonth-Schultz et al., 2019*).

ZCCT genes were first mapped in the diploid wheat genome in the region of the long arm of chromosome 5A (translocation from 4A). In hexa- and tetraploid wheat, in addition to chromosome 5A, they are also localized in the long arms of chromosomes of the fourth homologous group of B- and D-genomes. Each polyploid wheat genome contains three copies of the *ZCCT* gene. One of these copies (*ZCCT-3*) is reduced and not functional. Only the *ZCCT-1* and *ZCCT-2* genes retain functional activity. In addition, duplication of the *ZCCT-B2* gene is frequently observed (*ZCCT-B2a* and *ZCCT-B2b* copies) (*Distelfeld et al., 2009*; *Zhu et al., 2011*).

The primary attention in wheat is paid to the study of *ZCCT-1*, which, according to many studies, is the most influential on the rate of development and the reduction of the response to vernalization. A dose-dependent effect on development rates characterizes the number of functional *ZCCT1* genes and, according to existing knowledge about epistatic interaction with other *VRN* genes, can potentially affect agronomic traits (*Yan et al., 2015*).

Decreased sensitivity to vernalization due to mutations in the *ZCCT* gene sequence encoding the CCT domain or the presence of null alleles for these genes (*Distelfeld et al., 2009*; *Zhu et al., 2011*). Recent studies have found that the *VRN-B2* gene has more influence on the need for vernalization in wheat than its homologous gene *VRN-D2* (*Kippes et al., 2016*).

The study aimed to research the allelic diversity at the *Ppd-1* and *ZCCT-1* loci of bread wheat (*T. aestivum*) and durum wheat (*T. durum*) varieties of different origins and identification of genotypes carrying the corresponding alleles based on DNA markers.

Since the rate of passage of the stage from germination to heading is controlled by the genes of these two genetic systems, obtaining data on the variability of both *Ppd* and *Vrn* loci, in particular *ZCCT-1*, is of scientific and practical interest. The use of molecular markers significantly increases the efficiency of identification of a large amount of genetic material, providing valuable information for understanding the adaptive value of single alleles or their combinations in specific wheat cultivation conditions.

Materials and methods

Genetic material for research

Analysis of DNA polymorphism in the *Ppd-1* and *ZCCT-1* loci was carried out using 46 spring wheat varieties of different origins, in particular, 25 varieties of *T. aestivum* (Anshlag, Vetka, Heroinya, Katyusha, Skorospelka 95; Skorospelka 99; Svitanok, Srebryanka, Kharkovskaya 30; Etud/UKR; Apu/FIN; Herakles, Capta /FRA; Chanate, Loros/USA; Ciano 67; Opata 85; Turaco/MEX; Norin 29; Norin 17; Konosu 25/JPN; Balaganka, Duvanka, Sarrubra, Saratovskaya 29; Sibiryachka 4; Shortandinka, Strela, Poltavka/RUS) and 21 varieties of *T. durum* (Chornokoloska, Gordeiforme 3; Kharkovskaya 1; Kharkovskaya 3; Kharkovskaya 15; Kharkovskaya 21; Kharkovskaya 33; Kharkovskaya 37; Kharkovskaya 39; Kharkovskaya 51;

Kuchumovka 46; Luganskaya 7; Mestnaya, Narodnaya /UKR; Beloturka/RUS; Trems, Presto de taviro/PRT; Merliuri, Tbilisuri 9/GEO; Shirvan 5/AZE; Gumillo/ITA).

Molecular genetic analysis

Total DNA from wheat grains and seedlings was isolated using the CTAB method. Gene marker analysis was carried out according to recommendations in the literature: *Ppd-A1* (*Wilhelm et al., 2009*), *Ppd-A1_del303*, *Ppd-A1_del2ex7* (*Takenaka et.al., 2012*), *Ppd-B1a* (*Diaz et.al, 2012*), *Ppd-D1, Ppd-B1c* (*Beales et.al, 2007*), *Ppd-D1a*, *Ppd-D1b*; *Ppd-D1c* (Show et.al, 2013), *Ppd-D1d* (*Guo et.al., 2010*), *ZCCT-1* (*Zhu et al., 2011*).

Samples of isolated DNA from the studied varieties were amplified by allele-specific PCR. PCR amplification products were tested using electrophoresis in agarose and polyacrylamide gels. The sequences of PCR primers, the composition of the reaction mixture, the conditions for PCR amplification, electrophoresis, and visualization of PCR products are also given in the above literature.

The DNA marking data analysis was performed using Microsoft Excel.

The results of the study

DNA markers analyzed the variability of 25 spring bread wheat (*T. aestivum*) varieties and 21 spring durum wheat (*T. durum*) varieties at the *Ppd-1* and *ZCCT-1* loci.

The study of the Ppd-1 locus

DNA marking of *Ppd-1* alleles was carried out, in particular *Ppd-D1a*, *Ppd-D1b, Ppd-D1c*, *Ppd-D1d*, *Ppd-B1c, Ppd-B1a, Ppd-A1b, Ppd-A1_del303, Ppd-A1_del2ex7*.

Marker fragments are 288 bp -*Ppd-D1a*, 414 bp - *Ppd-D1b,* 672 bp - *Ppd-D1c*, 174 bp - *Ppd-D1d*, 223 bp -*Ppd-B1a*, 452 bp – *Ppd-A1b*, 425 bp - *Ppd-B1c*, 220 bp - *Ppd-A1_del303,* 170 bp - *A1_del2ex7*.

Ppd-1 (T. aestivum)

Nine alleles were found at the locus *Ppd-1* due to the DNA marking analysis of the studied bread wheat varieties, based on the combination of which 11 *Ppd-1* genotypes were identified (*Table 1*). The most common allele in the studied sample of spring varieties is *Ppd-D1c*. This allele was detected in 10 (40%) of the studied varieties (*T. aestivum*) of different origins, including four of Ukrainian plant breeding.

The *Ppd-D1c* allele carriers were varieties Anshlag, Heroinya, Skorospelka 95, Kharkovskaya 30, Herakles, Balaganka, Duvanka, Saratovskaya 29, Poltavka, Sarrubra.

Nine out of 10 varieties with the *Ppd-D1c* allele also had the most common genotype in this sample, *Ppd-D1cPpd-B1Ppd-A1b*, the frequency of which was 36±9.6%. This genotype was identified in representatives of different origins, including four Ukrainian varieties: Anshlag, Heroinya, Skorospelka 95, and Kharkovskaya 30.

The genotype *Ppd-D1aPpd-B1bPpd-A1b* was present in the sample with a frequency of 20±8.0. This genotype was identified in varieties of different origins, including two Ukrainian varieties, Katyusha and Skorospelka 99, and in varieties Capta, Chanate, and Norin 17 from France, the USA, and Japan.

The *Ppd-B1* gene multicopy alleles are presented in various combinations with alleles of two other photoperiod genes as part of nine other genotypes, occurring in one or two varieties from the sample with a frequency of 4±3.9 and 8±5.4, respectively.

Varieties with dominant *Ppd-A1* have not been identified, but carriers of mutant recessive alleles *Ppd-A1_del303* and *Ppd-A1_del2ex7* are present.

Single cases of mutant alleles were identified in the *Ppd-1* genotypes of the Mexican varieties Ciano 67 (*Ppd-D1aPpd-B1bPpd-A1_del303*), Opata 85 (*Ppd-D1aPpd-B1bPpd-A1_del2ex7*), and the Eastern European variety Sarrubra (*Ppd-D1cPpd-B1bPpd-A1_del2ex7*).

Ppd-1 (T. durum)

Two Ppd-1 genes control the photoperiodic response in durum wheat. The main differences in the trait manifestation are associated with the *Ppd-A1* gene.

It is known that *T. durum* has two dominant alleles, *Ppd-A1a.2* and *Ppd-A1a.3*, the first of which has a higher expression level. In the set of spring varieties of durum wheat, the *Ppd-A1a.2* and *Ppd-A1a.3* alleles were detected in Luganskaya 7 and Merliuri, respectively (*Bentley et.al., 2011*).

In tetraploid wheat species, the *Ppd-A1* gene is highly polymorphic. According to the data of some authors (*Takenaka et.al., 2012*), it is considered to be more than 60 haplotypes, including haplotypes with a 303 bp deletion in exons 5,6 and a 2 bp deletion in exon 7. The *Ppd-A1_del303* allele was not detected in durum wheat varieties, unlike in bread wheat, which is consistent with literary sources. Royo et al. (*2018*) reported the presence of this allele only in ancient varieties of durum wheat and its absence in modern commercial ones. On the contrary, the *Ppd-A1_del2ex7* allele is widely distributed in durum wheat. The mutant allele in the sample studied was detected in 10 (47.6%) from 21 varieties of various origins, including seven Ukrainian (Kuchumovka, Chornokoloska 46; Kharkovskaya 21; Kharkovskaya 39; Kharkovskaya 33; Kharkovskaya 37; Narodnaya) and three others (Beloturka, Tbilisuri 9; Trems).

The *Ppd-A1b* allele was detected in nine (43%) of the *T. durum* studied varieties, including 6 Ukrainian (Gordeiforme 3; Kharkovskaya 1; Kharkovskaya 15; Kharkovskaya 3; Kharkovskaya 51; Mestnaya).

Thus, marker analysis made it possible to identify varieties of bread and durum wheat carriers of specific alleles of the *Ppd-1* genes and determine their *Ppd-1* genotypes. The indicated varieties can be used in plant breeding programs as donors of these or other alleles of photoperiod sensitivity genes or their various combinations.

The study of the ZCCT-1 locus

ZCCT-1 (T. aestivum)

Based on the results of the analysis, it was established that the ZCCT-1 locus in the studied spring bread wheat (T. aestivum) varieties is mainly represented by the *ZCCT-D1* and *ZCCT-A1* genes.

It was shown that most varieties have a null allele for ZCCT-B1 at this locus, similar to the theoretical data presented in the literature (*Zhu et al., 2010*).

The double null allele *ZCCT-A1 ZCCT-B1* was identified in the Skorospelka 99 variety. All three genes were present in two Russian-bred varieties, Balaganka and Duvanka.

ZCCT-1 (T. durum)

In durum wheat varieties (*T. durum*), the *ZCCT-1* locus is also represented by two genes. A combination of two genes, *ZCCT-A1* and *ZCCT-B1*, was detected in 11 studied varieties. Some of these varieties (Kharkovskaya 33; Kharkovskaya 37; Narodnaya, Kharkovskaya 3; Kharkovskaya 51; Mestnaya) are from Ukraine, and five varieties (Beloturka, Shirvan 5; Gumillo, Merliuri, Tbilisuri 9) are of other origin.

The null allele *ZCCT-B1* was detected in 10 (47.6%) varieties, including eight Ukrainian (Kuchumovka, Chornokoloska 46; Kharkovskaya 21; Kharkovskaya 39; Gordeiforme 3;

Kharkovskaya 1; Kharkovskaya 15; Luganskaya 7) and two (Trems, Presto de Tavira) of another origin.

In combination with the *Ppd-A1_del2ex7* allele, the null allele for *ZCCT-B1* was detected in 5 varieties (Kharkovskaya 33; Kharkovskaya 37; Narodnaya, Beloturka, Tbilisuri 9), in combination with *Ppd-A1b* it was found in 4 varieties (Gordeiforme 3; Kharkovskaya 1; Kharkovskaya 15; Presto de Tavira), which amounted to 23.8% and 19.0% in this sample, respectively.

The combination of *ZCCT-A1ZCCT-B1/Ppd-A1_del2ex7* and *ZCCT-A1 ZCCT-B1/Ppd-A1b* was found in ten varieties and equal numbers. (*Table 2*).

Therefore, varieties with different *Ppd-1* and *ZCCT-1* genotypes can create the genetic material necessary to study the effect of single specific alleles and their combinations on development rate and other traits.

Discussion

A wide range of studies of photoperiodic response in spring varieties of bread wheat indicate the predominance of carriers with recessive *Ppd-1*. In particular, carriers of recessive alleles are varieties from Scandinavian countries (Sweden, Finland), where spring comes late and daylight hours in summer are 18-19 hours.

Since multiple alleles were found for the *Ppd-1* genes, and each gene represents a series of sensitive and insensitive alleles, marking of recessive mutant alleles of the *Ppd-D1* and *Ppd-A1* genes made it possible to identify high variability in combinations of alleles of photoperiod genes.

Marking of *Ppd-1* loci was performed in 25 varieties of spring bread wheat (*T. aestivum*) of different origins, which allowed the identification of eleven *Ppd-1* genotypes with different combinations of *Ppd-1* alleles. In this sample, the most common genotype was *Ppd-D1cPpd-B1bPpd-A1b*. At the same time, in the sample of varieties, another genotype was detected with the most common allele among the studied varieties *Ppd-D1c* and with a rare allele *A1_ del2ex7* (*Ppd-D1cPpd-B1bPpd-A1_ del2ex7*), which has a corresponding mutation in *Ppd-A1*.

The decrease in photoperiodic response due to the influence of dominant alleles increases in temperate climate zones and, especially at low latitudes. In the total sample of varieties, the *Ppd-D1a* allele and the *Ppd-D1aPpd-B1bPpd-A1b* genotype were found with high frequency, detected in 20% of the varieties. The dominant *Ppd-D1a* allele is also present in a variety of four other *Ppd*-genotypes, including, in some, combinations with the dominant *Ppd-B1a* and *Ppd-B1c*.

In low-latitude varieties, in Mexico and Japan, where daylight hours are shortened and amount to 13-14 hours, varieties with a low reaction to photoperiod predominate. Among Japanese varieties, spring and winter varieties often have digenic dominant *Ppd* control. Only Norin 29 had the *Ppd-D1bPpd-B1cPpd-A1b* genotype in the Japanese varieties studied. A monogenic control involving the *Ppd-B1a* allele was not detected, most likely due to the small sample size. The Mexican variety Turaco with the genotype *Ppd-D1bPpd-B1aPpd-A1b* was one of the parents in creating the Ukrainian variety Etud, which inherited two dominant *Ppd* alleles. The high variability of *Ppd-1* genotypes is also due to allelic differences in the *Ppd-A1* gene, although the *Ppd-A1b* allele was mainly detected in the varieties presented in the sample. The *Ppd-A1_del303* allele was found only in the Mexican variety Ciano 67 in combination with *Ppd-D1a*. It can be noted that, unlike winter wheat, which often has mutant recessive alleles of the Ppd-

D1 gene, including in combination with Ppd-A1_del303, in spring wheat, the allele of the *Ppd-A1* gene with a deletion in exons 5,6 is rare.

In the sample of durum wheat varieties, this mutant allele was not detected. However, according to literature data, *Ppd-A1_del303* was detected in ancient European varieties but not in modern commercial ones (*Royo et al., 2018*).

The data on marking the *Ppd-A1_del2ex7* allele in bread wheat and durum wheat are particularly interesting. According to the marking results, the allele with a deletion in exon 7 is widely represented in the *T. durum* but is rare in common wheat. The *Ppd-A1_del2ex7* allele was detected in the Sarrubra (RUS) and Opata 85 (MEX) varieties. The Sarrubra variety was obtained from a combination of crossing the spring variety Poltavka (*T. aestivum, PpdD1cPpdB1bPpdA1b*) and Beloturka (*T. durum, Ppd-A1_del2ex7*). The Sarrubra variety inherited a mutant recessive allele of the *Ppd-A1* gene from a durum wheat variety, which resulted in the formation of the *PpdD1cPpdB1bPpdA1_ del2ex7* genotype. With such an allelic combination, it can be assumed that this variety has a later heading.

Mutations in the *ZCCT* gene encoding the *CCT* domain or the presence of null alleles for these genes cause decreased sensitivity to vernalization (*Distelfeld et al., 2009*; *Zhu et al., 2011*).

No polyploid wheat representative has been identified that simultaneously contains null alleles or non-functional variants of all *ZCCT* genes (*Distelfeld et al., 2009*; *Zhu et al., 2011*). Some known mutations lead to changes in amino acid sequences in the CCT domain of ZCCT1 proteins, which are associated with changes in heading time (*Distelfeld et al., 2009*).

Each of the polyploid wheat genomes contains three *ZCCT* genes, of which the primary attention is paid to the study of *ZCCT-1*, which, according to many studies, has more on the rate of development and the reduction of the response to vernalization (*Yan et al., 2015*). According to the results of this study at the *ZCCT-1* locus, it was found that among the analyzed of spring bread wheat genotypes of different development types and origins, there is a significant spread of the variant with a null allele for the *ZCCT-B1* gene, which correlates with literature data. It should be noted that the absence of *ZCCT-B1* is widely found, as is already known, in Ukrainian varieties and other European genotypes. At the same time, there is evidence in the literature of a relatively low frequency of the null allele for *ZCCT-B1* in bread wheat varieties of American and Chinese origin. However, double mutants were found in them (*Zhu et al., 2011*).

It is known that varieties lacking *ZCCT-B1* are more common among spring wheat genotypes than winter wheat genotypes and durum wheat genotypes of different growth types. As shown in this study, the *ZCCT-1* locus in spring durum wheat varieties was represented by two genes, and 47.6% of them had a null allele at *ZCCT-B1*. The presence of null alleles results in the absence of the translation product of the corresponding *ZCCT1* gene, which eliminates any competitive interaction with other proteins containing the *CCT* domain. It can be assumed that the presence of null alleles of *ZCCT1* genes can affect the indicators of traits directly or indirectly regulated by genes encoding *CCT*-domain-containing proteins that enter into competitive interaction with *ZCCT* proteins. Identifying and assessing the prevalence of null-allele mutants for the *ZCCT1* genes in hexaploid and tetraploid wheat is particularly interesting in this connection.

Since the rate of passage of the stage from germination to heading is controlled by genes of two genetic systems, *Vrn* and *Ppd*, information on the variability of both *Ppd*-genotypes and *Vrn*-genotypes, in particular the genes of the *ZCCT-1* locus, is necessary and valuable.

Using molecular markers significantly increases the efficiency of identifying large amounts of genetic material, providing initial information for understanding the adaptive value of single alleles or their combinations in specific wheat cultivation conditions (*Fait & Balashova, 2022*).

Conclusion

Variability of *Ppd-1* genotype varieties of two commercial wheat species (*T. aestivum*, *T. durum*) is shown.

The allele *Ppd-D1c* and the genotype *Ppd-D1cPpd-B1bPpd-A1b* were the most common in spring-bread wheat varieties.

The *Ppd-D1a* allele, which became widespread as a result of the green revolution, is presented in five combinations with alleles of two other photoperiod genes, of which the *Ppd-D1aPpd-B1bPpd-A1a* genotype is predominant.

A rare presence of mutant recessive alleles *Ppd-A1_del303* and *Ppd-A1_del2ex7* encoding non-functional proteins was detected in spring bread wheat varieties and a broad prevalence of the one latter in durum wheat varieties.

The *ZCCT-1* locus in *T. aestivum* varieties is mainly represented by the *ZCCT-D1* and *ZCCT-A1* genes (null alleles for *ZCCT-B1*). In the *T. durum* varieties, the proportion of carriers of the *ZCCT-A1* genotypes (null alleles for *ZCCT-B1*) and *ZCCT-B1ZCCT-A1* is comparable.

Varieties with different *Ppd-1* and *ZCCT-1* genotypes can be used in genetic and plant breeding research to study the effects of single alleles and allelic combinations, create new genetic material, and in wheat breeding practice.

Conflict of interest

The authors declare that there is no conflict of interest.

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Appendix

Table 1. *Ppd*-genotypes of spring bread wheat (*T. aestivum*) varieties

Ppd-genotype	Varieties	n	$P \pm S_p$
Ppd-D1cPpd-B1bPpd-A1b	Anshlag, Heroinya, Skorospelka 95, Kharkovskaya 30, Herakles, Balaganka, Duvanka, Saratovskaya 29, Poltavka	9	36±9,6
PpdD1d Ppd-B1bPpd-A1b	Apu, Sibiryachka 4	2	8±5,4
Ppd-D1aPpd-B1bPpd-A1b	Capta, Chanate, Katyusha, Norin 17, Skorospelka 99	5	20±8,0
Ppd-D1aPpd-B1bPpd-A1_del303	Ciano 67	1	4±3,9
PpdB1a Ppd-D1aPpd-B1cPpd-A1b	Konosu 25	1	4±3,9
Ppd-D1aPpd-B1aPpd-A1b	Etud, Turaco	2	8±5,4
PpdD1d Ppd-B1aPpd-A1b	Loros	1	4±3,9
PpdD1d Ppd-B1cPpd-A1b	Strela	1	4±3,9
Ppd-D1bPpd-B1cPpd-A1b	Norin 29	1	4±3,9
Ppd-D1aPpd-B1bPpd-A1_del2ex7	Opata 85	1	4±3,9
Ppd-D1cPpd-B1bPpd-A1_ del2ex7	Sarrubra	1	4±3,9
Total		25	100

Table 2. *Ppd-A1* and *ZCCT-1* genotypes of spring durum wheat (*T. durum*) varieties

Variety	*Ppd-A1*	*ZCCT-1*	n	$P \pm S_p$
Kuchumovka, Chornokoloska 46, Kharkovskaya 21, Kharkovskaya 39, Trems	*PpdA1_del2ex7*	*ZCCT-A1*	5	23,8±9,29
Gordeiforme 3, Kharkovskaya 1, Kharkovskaya 15, Presto de taviro	*PpdA1в*	*ZCCT-A1*	4	19,0±8,56
Beloturka, Kharkovskaya 33, Kharkovskaya 37, Narodnaya, Tbilisuri 9	*PpdA1_del2ex7*	*ZCCT-A1 ZCCT-B1*	5	23,8±9,29
Kharkovskaya 3, Kharkovskaya 51, Mestnaya, Shirvan 5, Gumillo	*PpdA1в*	*ZCCT-A1 ZCCT-B1*	5	23,8±9,29
Luganskaya 7	*PpdA1a.2*	*ZCCT-A1*	1	4,8±4,66
Merliuri	*PpdA1a.3*	*ZCCT-A1 ZCCT-B1*	1	4,8±4,66
Total		21		100