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### SOLUBLE PROTEIN CONTENT IN SEEDLINGS AND CALLI OF ISOGENIC *PPD* GENES OF BREAD WHEAT LINES

**Aim.** To study the influence of the *PPD* gene system – photoperiodic insensitivity of common wheat on growth and synthetic activity and soluble protein content in seedlings and calli of different origin of isogenic wheat lines under *in vivo* and *in vitro* conditions. **Methods.** Seedlings and calli of near isogenic *PPD* lines of *Triticum aestivum* L. created in the genome of Myronivska 808 variety were used as plant material. Seedlings were analyzed for growth response and accumulation of easily soluble protein in axial organs. Primary calli were obtained using mature embryos, primary aseptically leaves, and apical root sections as explants. Morphophysiological characterization of calli was performed, proliferation frequency and soluble protein content were analyzed. **Results.** It was found that isolines *Ppd 1* and *Ppd 3*, which exhibit photoperiodic neutrality, are characterized by maximum rates of linear growth, biomass accumulation and protein content in the early stages of ontogenetic development *in vivo*. Under *in vitro* culture conditions, isolines *Ppd 2* and *Variety* were characterized by the highest rates of callus proliferation and the lowest soluble protein content, which leads to a reduced potential morphogenetic activity. **Conclusions.** The *PPD* gene system determines the growth response and synthetic activity of seedlings of isogenic lines under *in vivo* conditions and the processes of primary callus proliferation, synthesis and accumulation of soluble protein in them under *in vitro* conditions.

**Keywords:** *Triticum aestivum* L., *PPD* gene system, soluble protein, seedlings, organ specificity, *in vitro* callus culture.

Common wheat *Triticum aestivum* L. is one of the most important food crops in the world, which is grown in different ecological and geographical zones under various temperature and photoperiod conditions. These factors – temperature and photoperiod – largely determine the adaptabil-

ity, resistance to stressors, productivity and quality of the wheat crop. The photoperiod response of common wheat plants is controlled by the *PPD* (photoperiod) gene system located in the second homeologous group of chromosomes: chromosome 2D - *PPD-D1a*, *PPD-B1a* and *PPD-A1a* [1]. Reduced sensitivity to photoperiod (photoperiodic neutrality) is caused by dominant alleles of the *PPD* genes, and a strong response to photoperiod is characteristic of genotypes with only recessive *ppd* alleles of all three genes [1]. *PPD* genes, in addition to photoperiod sensitivity, determine a number of agronomic traits of winter common wheat, such as developmental rates, duration of the sprouting-earning period, individual productivity, frost resistance, nitrogen uptake and optimal nitrogen assimilation [1–3]. Currently, the molecular biological characteristics of the *PPD* gene system – allelic state, promoter sequences, protein products, gene networks – are being actively studied, as well as the relationship with the *VRN* gene system and floral morphogenesis genes, etc. [2, 4]. The use of *in vitro* culture methods is becoming one of the most common research tools in plant physiology. The basis of the methods of culture of isolated cells, tissues and organs of plants is a unique property of plant cells – totipotency. *In vitro* culture methods are widely used in the study of the main agricultural crop, common wheat *Triticum aestivum* L. [5, 7]. When introducing common wheat into *in vitro* culture, modern biotechnological research uses a variety of explants - mature and immature embryos, leaf explants, tillering nodes, roots, anthers [5–8]. Currently, most experiments are conducted to improve the stress resistance of wheat in order to increase yields [5]. However, to date, studies of the influence of individual genes or gene systems on the manifestation of cell totipotency *in vitro* on objects with difficult regeneration, which include common wheat, are few. Since the *PPD* gene system determines the rate of development of common wheat

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plants *in vivo*, we assumed that these genes might also have an effect on morphogenetic processes *in vitro*.

### Materials and methods

**Plant material.** Nearly isogenic monogenic dominant lines (NILs) for the genes controlling photoperiodic insensitivity of common wheat – *PPD* genes – were used as plant material, genes created in the genome of winter variety Myronivska 808 with genotypes *PPD-D1aB1bA1b* (*Ppd 1*), *PPD-D1bB1aA1b* (*Ppd 2*), *PPD-D1bB1bA1a* (*Ppd 3*) and *PPD-D1bB1bA1b* (*variety*). Under *in vivo* conditions, the experiments were carried out in vegetative experiments in the growth chamber of the Department of Physiology and Biochemistry of Plants and Microorganisms of V. N. Karazin Kharkiv National University. Plants were cultivated to the tillering stage (21 days old) in soil culture at 22 / 18°C, 15 lux illumination, 16-hour photoperiod. Indicators of the growth response were determined - the length and biomass of the seedling and axial organs, and the protein content was determined by the Lowry colorimetric method [9]. Under *in vitro* culture conditions, the experiments were carried out at the biotechnological laboratory of the Department of Morphogenesis of Higher Plants in Vitro, using generally accepted biotechnological methods [9]. NILs were introduced into the culture according to the developed protocol [9], using mature embryos and aseptic sprouts as explants. Callus cultures were grown on Murashige-Skoog (MS) medium + 2 mg/L 2.4 D in a thermostat at + 26°C for 4–6 weeks. The frequency of callusogenesis and soluble protein content in primary callus of different origin were determined. The results were statistically analyzed using one-factor analysis of variance ANOVA; the tables show the mean values and their standard deviations.

### Results and discussion

**Studies under *in vivo* conditions.** Growth response – growth and accumulation of biomass is an integral indicator of the phenotypic implementation of the genetic program of plant development. The *PPD* gene system is one of the most important genetic systems that determine the rate of development of common wheat. It is generally believed that the effects of photoperiod sensitivity genes are realized when wheat plants transition from the vegetative to the generative stage of development or under

the influence of a provocative photoperiod. In our experiments, we studied the growth response of 21-day-old seedlings of isogenic wheat lines, which corresponds to the beginning of the tillering phenophase or the third stage of common wheat plant organogenesis. The results of the experiments showed that the length of seedlings at this ontogenetic stage of development ranges from 37.35 to 45.60 cm (Table 1), with the maximum values characterized by the *Ppd 3* isoline and the minimum values by the *Ppd 2* isoline.

Under the conditions of the vegetation experiment, in all isolines, the aerial part of the seedling significantly exceeds the root system in terms of growth response, and the same trend persists among the studied isolines – the maximum length of both the aerial and underground parts of the seedling is characterized by isoline *Ppd 3*, and the minimum – by isoline *Ppd 2*. The accumulation of biomass reflects the growth and synthetic processes of the plant organism. According to the experimental results, it was found that the biomass of 21-day-old seedlings of isogenic lines is 553.77–621.45 mg (Table 2). The maximum values were observed in isolines *Ppd 1* and *Ppd 3*, which have genotypes *PPD-D1aB1bA1b* and *PPD-D1bB1bA1a*, respectively. The dominant genes *PPD-D1a* and *PPD-A1a* determine photoperiod insensitivity, i.e., determine the photoperiod neutrality of bread wheat plants. The maximum biomass of the aboveground part of 493.75 mg is shown for isoline *Ppd 1*, and the minimum 385.23 mg – for isoline *Ppd 2*. The accumulation of biomass in the root system has a different picture: the maximum development of the root system of 168.54 mg was set for isoline *Ppd 2*, and the minimum values of 108.12 mg – for isoline *Ppd 1*.

Thus, we have established that the photoperiod insensitivity genes *PPD* determine the growth response at the early stages of common wheat plant ontogeny. The dominant alleles *PPD-D1a* and *PPD-A1a*, which determine photoperiod neutrality, contribute to intensive growth processes both in terms of linear growth parameters and biomass accumulation, an indicator that reflects general biosynthetic processes. Seedlings in the genotype with the dominant allele of the *PPD-B1a* gene and the winter variety in which all *PPD* genes are represented by recessive alleles are characterized by minimal growth response.

Table 1. Growth response of seedlings of wheat variety Myronivska 808 isogenic for *PPD* genes (NILs) ( $M \pm \sigma$ ,  $n = 30$ ), cm

Isoline	Genotype	Length, cm		
		total	seedling	roots
<i>Ppd 1</i>	<i>PPD-D1aB1bA1b</i>	40.87 ± 2.02	29.15 ± 1.23	11.72 ± 0.52
<i>Ppd 2</i>	<i>PPD-D1bB1aA1b</i>	37.35 ± 1.63	27.06 ± 1.14	10.29 ± 0.43
<i>Ppd 3</i>	<i>PPD-D1bB1bA1a</i>	45.60 ± 1.93	31.65 ± 1.42	13.95 ± 0.57
<i>Variety</i>	<i>PPD-D1bB1bA1b</i>	40.85 ± 1.97	28.82 ± 1.27	12.05 ± 0.61
	<i>LSD</i> <sub>0,5</sub>	2.06	1.98	0.53

Table 2. Biomass accumulation of seedlings of isogenic *PPD* gene lines (NILs) of wheat variety Myronivska 808 ( $M \pm \sigma$ ,  $n = 30$ ), mg

Isoline	Genotype	Biomass, mg		
		total	seedling	roots
<i>Ppd 1</i>	<i>PPD-D1aB1bA1b</i>	601.87 ± 25.6	493.75 ± 18.1	108.12 ± 4.6
<i>Ppd 2</i>	<i>PPD-D1bB1aA1b</i>	553.77 ± 19.3	385.23 ± 15.6	168.54 ± 5.4
<i>Ppd 3</i>	<i>PPD-D1bB1bA1a</i>	621.45 ± 27.3	474.84 ± 17.2	146.61 ± 5.9
<i>Variety</i>	<i>PPD-D1bB1bA1b</i>	571.53 ± 22.4	458.35 ± 16.8	113.18 ± 4.1
	<i>LSD</i> <sub>0,5</sub>	22.31	18.54	8.26

The readily soluble proteins of common wheat are represented by albumin, globulins and cytoplasmic proteins, including various enzymes, make up approximately 10–12 % of dry biomass and can serve as markers of the synthetic activity of plant tissue. In our studies, we analyzed the content of easily soluble protein in the organs of common wheat plants used as explants for obtaining callus tissue, namely in mature seed embryos that have hatched and in the leaves and roots of NILs seedlings (Table 3). The results of the analyzes showed that the content of easily soluble protein significantly depends on the organ of the plant organism and is associated with metabolic activity. The maximum protein content was found in wheat germ - 110.2–146.4 mg/g, almost twice lower in seedling leaves – 62.3–64.1 mg/g, and the minimum in seedling roots – 16.3–19.6 mg/g.

Nowadays, wheat germ is widely used in nutrition as an indispensable part of a healthy diet [10]. Isolated germs contain up to 30 % of pro-

teins/peptides and are a complete source of vegetable protein. Isolated embryos of hatching seeds are represented by meristematic tissues and contain the maximum amount of soluble protein, but there is a certain distribution of content depending on the genotype of the isolines. Isolines *Ppd 1* and *Ppd 3*, which have dominant *PPD-D1a* and *PPD-A1a* alleles in the genotype that determine photoperiodic insensitivity, are characterized by the maximum soluble protein content, while isolate *Ppd 2* and the variety have the minimum content. In leaves, as the main metabolic organs of the plant organism, soluble proteins are mainly represented by enzymes, among which ribulose biphosphate carboxylase / oxygenase (RBFco) prevails and accounts for up to 50 % of the total protein content. In general, the protein content is almost two times lower than that in the embryos, but the distribution by genotype has the same trend (Table 3). According to the soluble protein content, the isolines are ranked as follows: *Ppd 3* > *Ppd 1* > *Variety* ≥ *Ppd 2*.

Table 3. Soluble protein content in seedlings of *PPD* gene isogenic lines (NILs) of wheat variety Myronivska 808 *in vivo*, mg/g fresh weight ( $M \pm \sigma$ ,  $n = 9$ )

Isoline	Genotype	Protein content, mg/g		
		germs	roots	roots
<i>Ppd 1</i>	<i>PPD-D1aB1bA1b</i>	138.3 ± 5.2	64.1 ± 2.8	19.3 ± 0.8
<i>Ppd 2</i>	<i>PPD-D1bB1aA1b</i>	110.2 ± 3.5	62.3 ± 2.8	16.3 ± 0.7
<i>Ppd 3</i>	<i>PPD-D1bB1bA1a</i>	146.4 ± 6.2	65.7 ± 2.8	19.6 ± 0.9
<i>Variety</i>	<i>PPD-D1bB1bA1b</i>	119.1 ± 4.1	62.6 ± 2.8	16.5 ± 0.5
	<i>LSD</i> <sub>0,5</sub>	25.6	7.82	0.75

According to our experiments, the lowest soluble protein content was found in the roots of common wheat seedlings. The root proteome differs from the leaf proteome not only in quantitative but also in qualitative terms, i.e., the functional groups of identified soluble proteins [11]. Compared to the wheat leaf proteome, proteins involved in metabolism and transport are overrepresented in the roots, while proteins involved in energy, disease resistance and defense, transcription, and signal transduction are underrepresented [11]. The distribution between the isolines in terms of quantitative content has the same pattern as in other plant organs of the isolines:  $Ppd\ 3 \geq Ppd\ 1 > Variety \geq Ppd\ 2$ . The study of soluble protein content in isolines by *PPD* genes under *in vivo* conditions showed organ specificity and revealed a certain influence of the system of genes controlling the photoperiodic response over soluble protein synthesis. Indicators of growth response and protein content among the studied isolines have similar trends, which also confirms the genetic control (direct or indirect) of the *PPD* gene system over the developmental rates of common wheat plants at this stage of ontogenetic development.

*Studies under in vitro conditions.* Since the *PPD* gene system determines the rate of development of common wheat plants *in vivo*, we assumed that these genes might also have an effect on morphogenetic processes *in vitro* culture, which is a common model system for studying soft wheat plants. Different types of explants were used to obtain the primary callus: mature embryos, leaf explants, and apical root sections of aseptic seedlings of isogenic wheat lines. The morphological characteristics of primary calli obtained from different types of explants were almost identical (Table 4).

The callus tissues were represented by dense, saponified, transparent or matte calli of yellowish or whitish color. Significantly, primary calli differed in terms of induction of callusogenesis: calli were formed most rapidly from explants of mature embryos, rather quickly from apical root areas, and the longest period of primary calli formation was observed when using leaf explants (Table 4).

This is due to the degree of meristematicity / differentiation of explant tissues: isolated embryos and root apical areas contain more meristematic cells, leaf explants are more differentiated and, accordingly, require more time for the processes of

dedifferentiation and proliferation of primary callus.

The results of the study of the frequency of callus proliferation of soft wheat lines isogenic for *PPD* genes showed that the type of explant significantly affects this indicator. The primary callus is formed most efficiently when mature embryos are used as explants - the proliferation rate is 62.92–98.51 %. When using leaf explants and apical parts of roots of aseptic seedlings, the indicators are almost the same and amount to 40.36–53.63 % (Table 5). The frequency of proliferation among the isolines, regardless of the type of explants (germs, leaves, roots), has a certain pattern: the maximum rates are characteristic of isolate *Ppd 2* and the *Variety*, the minimum – for isolates *Ppd 3* and *Ppd 1*. It should be noted that isolines characterized by more efficient growth response and synthetic activity (soluble protein synthesis) show less tendency to proliferation of primary callus and vice versa, those isolines characterized by slower rates of development and growth response demonstrate more efficient indicators of callusogenesis frequency regardless of the type of explant selected.

The results of the study of soluble protein content in primary calli of different origin showed that under *in vitro* conditions the protein content was significantly lower than in plant tissues under *in vivo* conditions (Table 6). Depending on the type of explant and the genotype of the isolate, the protein content was 3.17–6.82 mg/g of fresh weight. It should be noted that when cultivated *in vitro*, there was no organ-dependence of protein content on a particular type of explant, which is associated with the processes of plant cell dedifferentiation during the initiation and proliferation of callus tissues.

The content of soluble protein in primary calli formed from mature embryos, leaf explants, and apical areas of aseptic roots was almost the same. However, the genetic predetermination of soluble protein accumulation among the studied isolines *in vitro* retains the same trends as *in vivo*.

The maximum soluble protein content in primary calli of different origin is characterized by isolines *Ppd 1* and *Ppd 3*, which show photoperiodic neutrality, while isolate *Ppd 2* and *Variety* show the lowest protein content in calli. This indicates similar effects of the *PPD* gene system on the processes of soluble protein synthesis and accumulation *in vivo* and *in vitro*. According to the literature, the soluble protein content in calli can be a marker of the morphogenetic potential of callus cells.

Table 4. Morphological characteristics of primary calli (primary callus) of common wheat lines isogenic for *PPD* genes obtained from different types of explants




Type of explant	Morphological characteristics	Time of primary callus appearance, days	Color	Photo
Mature embryos	Dense, globular, with meristematic zones	3.72 ± 0.15	Yellowish, matte	
Leaf explants	Dense, but more watered down, heterogeneous	14.57 ± 0.72	Whitish, light yellow, shiny	
Apical areas of roots	Dense, watered down, no elements of differentiation	5.91 ± 0.31	Transparent, light yellow	

Table 5. The frequency of primary callus proliferation from different types of explants of *PPD* gene isogenic lines (NILs) of common wheat variety Myronivska 808 *in vitro*, % (M ± σ, n = 15)

Isoline	Genotype	Frequency of callusogenesis, %.		
		germs	leaves	roots
<i>Ppd 1</i>	<i>PPD-D1aB1bA1b</i>	74.02 ± 3.2	45.71 ± 2.2	44.84 ± 1.9
<i>Ppd 2</i>	<i>PPD-D1bB1aA1b</i>	98.51 ± 5.7	53.63 ± 2.8	48.93 ± 2.2
<i>Ppd 3</i>	<i>PPD-D1bB1bA1a</i>	62.92 ± 2.8	44.92 ± 2.0	40.36 ± 1.8
<i>Variety</i>	<i>PPD-D1bB1bA1b</i>	90.31 ± 4.2	50.27 ± 3.1	47.78 ± 1.9
<i>LSD</i> 0.5		18.11	6.27	5.48

Table 6. Soluble protein content in calli of *PPD* gene isogenic lines (NILs) of wheat variety Myronivska 808 *in vitro*, mg/g of fresh weight (M ± σ, n = 9)

Isoline	Genotype	Protein content in calli, mg/g		
		germs	leaves	roots
<i>Ppd 1</i>	<i>PPD-D1aB1bA1b</i>	5.72 ± 0.3	6.21 ± 0.4	6.62 ± 0.4
<i>Ppd 2</i>	<i>PPD-D1bB1aA1b</i>	3.17 ± 0.2	4.34 ± 0.2	5.41 ± 0.3
<i>Ppd 3</i>	<i>PPD-D1bB1bA1a</i>	5.84 ± 0.3	6.67 ± 0.4	6.82 ± 0.4
<i>Variety</i>	<i>PPD-D1bB1bA1b</i>	4.01 ± 0.2	4.42 ± 0.3	5.51 ± 0.3
<i>LSD</i> 0.5		0.05	0.04	0.06

Embryogenic calli and suspension cultures have a higher soluble protein content than non-embryogenic ones and differ in the spectra of detected intracellular polypeptides and those secreted into the culture medium [12]. Thus, according to our results, isolates *Ppd 1* and *Ppd 3*, which have lower callus proliferation rates, show the highest soluble protein content and are potentially more effective *in vitro* morphogenesis, and vice versa, isolate *Ppd 2* and the *Variety* show the highest callusogenesis frequency and the lowest soluble protein content, which may be due to the reduced morphogenic potential.

## Conclusions

The results of *in vivo* studies have shown that the *PPD* gene system genetically determines the growth response and synthetic activity in seedlings of isogenic lines at the early stages of ontogenetic development. The soluble protein content in NILs seedlings has a clear organ specificity only under *in vivo* conditions. The *PPD* gene system affects the processes of primary callus induction, proliferation, and soluble protein content in callus cells regardless of the type of explant. The genetic predetermination of soluble protein synthesis and accumulation indi-

cates similar effects of *PPD* genes under *in vivo* and *in vitro* culture conditions.

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## ВМІСТ РОЗЧИННОГО БІЛКА В ПРОРОСТКАХ ТА КАЛЮСАХ ІЗОГЕННИХ ЗА ГЕНАМИ *PPD* ЛІНІЙ М'ЯКОЇ ПШЕНИЦІ

**Мета.** Вивчення впливу системи генів *PPD* – фотоперіодичної нечутливості пшениці м'якої на ростову та синтетичну активність і вміст розчинного білка в проростках і калюсах різного походження ізогенних ліній пшениці за умов *in vivo* та *in vitro*. **Методи.** Як рослинний матеріал використовували проростки та калюси майже ізогенних за системою генів *PPD* ліній *Triticum aestivum* L., створені в генофоні сорту Миронівська 808. Проростки аналізували за ростою реакцією та накопиченням легкорозчинного білка в осьових органах. Первинні калюси отримували, використовуючи як експланти зрілі зародки, первинні асептичні листки та апікальні ділянки коренів. Проводили морфологічну характеристику калюсів, аналізували частоту проліферації та вміст розчинного білка. **Результати.** Встановлено, що ізолінії *Ppd 1* та *Ppd 3*, які проявляють фотоперіодичну нейтральність, характеризуються максимальними показниками лінійного росту, накопичення біомаси та вмісту білка на ранніх етапах онтогенетичного розвитку за умов *in vivo*. За умов культури *in vitro* ізолінії *Ppd 2* та *Sopt* характеризувались максимальними показниками частоти проліферації калюсів і мінімальним вмістом розчинного білка, що обумовлює знижену потенційну морфогенетичну активність. **Висновки.** Система генів *PPD* детермінує ростою реакцію та синтетичну активність проростків ізогенних ліній за умов *in vivo* й процеси проліферації первинних калюсів, синтезу та накопичення в них розчинного білка за умов *in vitro*.

**Ключові слова:** *Triticum aestivum* L., система генів *PPD*, розчинний білок, проростки, органоспецифічність, калюсна культура *in vitro*.