BIOLOGY

Growth and morphogenetic reactions in near-isogenic lines of PPD genes of winter wheat *Triticum aestivum* L. under *in vivo* and *in vitro* conditions

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Abstract. The paper presents the results of the study of the effects of PPD genes that determine the photoperiodic sensitivity of soft wheat, on the growth response and morphogenetic processes in near-isogenic lines (NILs) of winter wheat cultivar Mironivska 808 (M-808) under *in vivo* and *in vitro* conditions. Our results show that the genotype of NILs determines the heading date, the dynamics of the biomass accumulation and the formation of shoots *in vivo*. The effects of PPD genes on the induction of callus genesis, the growth rate of the calli and the efficiency of the direct and indirect pathways of morphogenesis in culture *in vitro* are revealed. It is shown that the effects of the genes on the growth response manifest in the same way in *in vivo* and *in vitro* conditions.

Keywords: *Triticum aestivum L., PPD genes, NILs, development rate, shoots, callus genesis, morphogenesis in vitro.*

Introduction. Winter wheat is one of the most important food crops in the world, which is grown in different ecological and geographical zones under various temperature and photoperiodic conditions. These factors largely determine adaptability, resistance to abiotic and biotic stressors, and the productivity and quality of wheat crop [4]. In the soft wheat (*Triticum aestivum* L.) photoperiodic reaction is controlled by the system of genes PPD (photoperiod) located in the second homoeologous group of chromosomes: in chromosome 2D - Ppd-D1a, Ppd-B1a and Ppd-A1a [10]. The reduced sensitivity to the photoperiod is controlled by the dominant alleles of PPD genes, and the strong reaction to the photoperiod is manifested in genotypes with the presence of only recessive alleles ppd of all three genes [13]. Ppd genes, in addition to photoperiodic sensitivity, determine a number of agronomic features of winter soft wheat - development rate, heading date, individual productivity, frost resistance [5-7,12,13]. Nowadays molecular characteristics of the PPD gene system, the promoter sequences, protein products, genetic nets, the link with the VRN gene system and the genes of the floral morphogenesis, etc. are actively studied [9,11,13].

The use of *in vitro* culture methods becomes one of the most widely used research tools in plant physiology. The basis of the culture methods of isolated cells, tissues and organs of plants is the unique property of plant cells - totipotency [1]. *In vitro* culture methods are widely used in the study of the main agricultural crop - soft wheat *Triticum aestivum* L. Currently, these studies are aimed at improving wheat to increase yields and minimize its losses due to adverse environmental conditions [2]. However, today the studies of the influence of individual genes or the system of genes on the manifestation of the totipotency of cells in vitro in objects with severe regeneration, which includes soft wheat, are not numerous [14]. Since the PPD gene system determines the development rate of soft wheat plants *in vivo*, we hypothesized that these genes may have effects on morphogenetic processes, also *in vitro* culture conditions.

Study objectives. Proceeding from the above, the aim of the study was to investigate the effect of the state (dominant / recessive) of the PPD genes that determines the photoperiodic reaction of soft wheat on growth and the morphogenetic processes in these wheat genotypes (NILs) of winter wheat Myronivska 808 under *in vivo* and *in vitro* conditions.

Materials and methods. In the work as a plant material, monogenic dominant near-isogenic lines (NILs) were used. These lines were monogenic dominant of the genes controlling photoperiodic sensitivity of soft wheat - the PPD gene, and were created in the Myronivska 808 cultivar genetic background. Their genotypes were PPD-D1aB1bA1b (Ppd 11), PPD-D1bB1aA1b, (Ppd 22) PPD-D1bB1bA1a and (Ppd 33) PPD-D1bB1bA1b (cultivar). Under conditions of *in vivo* experiments plants were grown in field conditions on the experimental plot of the Department of physiology and biochemistry of plants and microorganisms of V.N. Karazin Kharkiv National University, also the plants were grown in vegetative chamber. During the experiments, the development rate of NILs was determined - the terms of transition to the generative phase of development (the length of the period from germination to heading), the dynamics of the formation of shoots and the accumulation of biomass of the aboveground part of plants during the vegetative phase of development of soft wheat plants. The experiments on *in vitro* cultures were conducted on the basis of the biotechnological laboratory of the Department "Morphogenesis of higher plants in vitro", using commonly accepted biotechnological methods [1]. The introduction of the NILs into the culture was carried out according to the developed protocol [8], using both mature embryos and aseptic sprouts as an explant. The callus cultures were grown on a Murashige-Skoog (MS) medium + 2 mg/L of 2,4-D, in a thermostat at +26 °C for 4-6 weeks. The frequency of callusogenesis and growth response were determined by measuring changes in the area, that was calculated using the Photo M 5.1 program. Then, the callus cultures of NILs were passaged to the regenerative medium MS + 0.5 mg/l of IAA +0.5 mg/l kinetin and MS + 0.5 IAA mg/l + 0.5 mg/l 2,4-D to stimulate morphogenetic reactions by indirect morphogenesis. Also, direct morphogenesis was investigated - isolated sterile mature embryos and apices of NILs seedlings were passaged to the non-hormonal MS me-

dium. The results obtained are statistically processed, the tables show the average values and their standard deviations.

Results and discussion. *Growth and morphogenetic reactions in vivo.* One of the integral indicators that reflects the development rate of wheat, is the length of the **p**eriod from **g**ermination to **h**eading (PGH) [5]. According to the results of our experiments (Table 1), the NILs and the cultivar differed in their rate of development - the duration of PGH both in the conditions of field and vegetativ In the order of increasing the duration of PGH lines should be ranked as follows: the cultivar> PPD B1a> PPD D1a> PPD A1a. The line with recessive alleles in all the loci, the winter wheat cultivar, was characterized by the maximum duration of the PGH.

Table 1. The duration of the **p**eriod from **g**ermination to **h**eading (PGH) in near-isogenic lines (NILs) of PPD genes of wheat, days

NILs genotype	PGH, days (field)	PGH, days (vegetative)
PPD-D1aB1bA1b (*Ppd 11)*	235 ± 3	130 ± 3
PPD-D1bB1aA1b (*Ppd 22)*	238 ± 2	134 ± 2
PPD-D1bB1bA1a (*Ppd 33)*	236 ± 2	125 ± 1
PPD-D1bB1bA1b (cultivar)	247 ± 3	137 ± 4

Thus, according to our experiments, the differences between the NILs in the rate of development are determined by the PPD genes. Similar pleiotropic effects of PPD genes have been described in literature [6,12].

The integral indicator of the intensity of biosynthetic and growth processes in plants is the dynamics of the biomass accumulation. The results of determining the mass of the dry matter of the aboveground part of the plants showed that it grew in all NILs during the experiment, regardless of the allelic state of the PPD genes (Table 2). Analysis of the results shows that the growth of biomass of the above-ground part of the plants is associated with the genotype of the lines. The largest increase in the mass of plants was in PPD-B1a NIL, smaller in the PPD-D1a NIL, and the smallest in the PPD-A1a NIL and plants of cultivar bearing only recessive ppd alleles. The lowest weight of dry matter and the rate of its accumulation distinguished NIL of PPD-A1a, that perhaps can be regarded as evidence of the impact of gene PPD-A1a on intensity of accumulation of assimilates in the leaves and their outflow to the meristems.

An important feature of the growth and development of cereal crops, including wheat, is the process of tillering, which is the development of lateral sprouts from the buds, located in the tillering nodes. Tillering is one of the most important processes that provides wheat resistance in ontogenesis to adverse environmental conditions, as well as an increase in leaves and roots [7]. The dynamics of shoots formation during tillering in cereals is an important indicator of growth processes, since it reflects the activity of tillering node, in which morphogenetic processes occur that provide the formation of new vegetative organs.

Table 2. Accumulation of plant biomass of near-isogenic lines (NILs) of PPD genes of wheat, during the vegetative phase of development

NILs genotype	Mass of the dry matter of the aboveground part of the plants, g			Growth,
	27.11.17	5.12.17	19.12.17	%
PPD-D1aB1bA1b	0,40 ± 0,01	0,66 ± 0,02	0,98 ± 0,02	145
PPD-D1bB1aA1b	0,35 ± 0,02	0,78 ± 0,02	0,92 ± 0,02	163
PPD-D1bB1bA1a	0,36 ± 0,02	0,55 ± 0,02	0,78 ± 0,02	117
PPD-D1bB1bA1b	0,42 ± 0,02	0,64 ± 0,02	0,86 ± 0,02	105

During the experiment, the number of tillers increased in all NILs, as indicated by the results of determining the dynamics of their formation (Table 3). Analysis of the data shows the dependence of the ability to form tillers from the genotype of the NILs on the PPD genes. Thus, in the fastest growing PPD-A1a NIL, the number of shoots was 1.5-2 times less than in other NILs and cultivar M-808 (Table 3), and the increase in the number of tillers during the experiment was minimal - 18%. In the cultivar M-808 (only recessive alleles of ppd genes), which shows the slowest development, the number of tillers was much smaller than in other NILs. The maximum number of tillers and their maximum growth was determined in PPD-B1a and PPD-D1a NILs, which were also characterized by maximum biomass accumulation rates.

Table 3. The dynamics of tillers formation of near-isogenic lines (NILs) of PPD genes of wheat, during the vegetative phase of development

NILs genotype	Tillers, pcs/plant			Increase,
	27.11.17	5.12.17	19.12.17	%
PPD-D1aB1bA1b	4,6 ± 0,2	8,4 ± 0,2	8,2 ± 0,2	78
PPD-D1bB1aA1b	4,0 ± 0,1	8,0 ± 0,2	8,1 ± 0,2	103
PPD-D1bB1bA1a	3,4 ± 0,1	4,2 ± 0,1	4,0 ± 0,1	18
PPD-D1bB1bA1b	3,8 ± 0,2	6,3 ± 0,3	6,3 ± 0,3	66

It is known that growth and development are coordinated in time and space [3]. This suggests that the PPD genes in the dominant or recessive state are involved in this coordination. Thus, in the fastest growing PPD-A1a, the formation of tillers and growth of biomass is the least intensive. At the same time, these processes in the cultivar M-808 (only recessive alleles of ppd genes) and the PPD-B1a NIL are most intensive coupled with retarded development rate.

It is possible that the dominant state of PPD-A1a and PPD-D1a genes leads to accelerated development indirectly, through inhibition of growth processes. At the same time, the dominant state of the PPD-B1a gene and the recessive of all these genes (in cultivar M-808) may inhibit the transition to a generative state by enhancing growth processes. The physiological mechanism of this may consist in the participation of PPD genes in the regulation of the attraction of assimilates (mainly sugars) to the apical cone of the leading shoot,

which may contribute to the accelerated transition from the vegetative to the generative stage of organogenesis coupled with the restriction of vegetative growth. According to modern concepts, sugars are involved in the expression of a number of genes, in particular, the ones of floral morphogenesis [3].

Growth and morphogenetic reactions in vitro. The influence of the genotype of the NILs on the frequency of callus genesis was investigated. The results of experiments (Table 4) showed that all genotypes formed callus, but with different frequency (62.9% - 96.1%). The maximum frequency of callus genesis was detected in NIL with the dominant PPD-B1a gene (Table 4). In experiments, different types of explants were used to obtain primary somatic callus: mature embryos, apical parts of aseptic roots and leaf explants.

Table 4. Frequency of the formation of primary callus from different types of explants from wheat NILs of PPD genes, %

NILs genotype	Frequency of callus formation, %		
	Mature embryos	Apical parts of roots	Leaf explants
PPD-D1aB1bA1b	74,0 ± 4,2	84,4 ± 2,0	13,0 ± 0,5
PPD-D1bB1aA1b	98,5 ± 1,2	86,4 ± 1,1	17,8 ± 1,1
PPD-D1bB1bA1a	62,9 ± 2,1	75,0 ± 1,5	10,7 ± 0,8
PPD-D1bB1bA1b	90,3 ± 1,1	85,6 ± 2,2	14,7 ± 0,9

It is shown that the type of selected explant influences the efficiency of formation of primary callus (Table 4). Mature embryos and apical parts of the aseptic roots were more effective explants for obtaining the primary callus, compared to leaf explants. The induction of callus formation with the use of leaf explants was minimal and ranged from 10.7 to 17.8%. When using apices of the roots it reached 75.0 - 86.4% and the maximum induction of callus formation was obtained using explants of mature embryos - 62.9 - 98.5%. Regardless of the type of explant, the callus formation rate was highest in the PPD-B1a NIL.

The rate of growth of the callus tissues was determined by the increase in the area of the primary calluses during four weeks (mm^2/day). Maximal growth rates of calli were observed in the PPD-B1a NIL(Table 5), which also had the highest rates of callus genesis for all types of explants. Minimum values of these parameters were observed in PPD-D1a NIL. All investigated NILs had the maximal growth of the callus tissue in the first week of cultivation, which corresponds to the logarithmic phase of the "growth curve" of the callus tissues, the second week of cultivation showed a significant inhibition of the growth of the calli, i.e., the transition to the stationary phase of the "growth curve" was determined, although during the third week of growth again somewhat increased in the primary calli of all NILs.

Table 5. The rate of growth response of the primary callus of near-isogenic lines (NILs) of PPD genes of wheat, mm^2/day

NILs genotype	Growth response of the primary callus, mm^2			Increase, mm^2/day
	7-14 days	14-21 days	21-28 days	
PPD-D1aB1bA1b	2,69 ± 0,11	0,21 ± 0,10	1,48 ± 0,12	0,35 ± 0,11
PPD-D1bB1aA1b	3,50 ± 0,12	0,86 ± 0,14	2,65 ± 0,15	0,48 ± 0,13
PPD-D1bB1bA1a	3,95 ± 0,18	0,47 ± 0,12	1,81 ± 0,11	0,44 ± 0,12
PPD-D1bB1bA1b	1,48 ± 0,17	0,86 ± 0,18	2,64 ± 0,13	0,38 ± 0,10

The NILs differed in rate of growth during the studied period. In the first week of observation, PPD-A1a NIL grew with maximal rates, then its growth was slowed down; the primary callus of the cultivar M-808 in the first week had the minimum growth rates and the maximum in the second and third weeks; NIL PPD-B1a had the most stable growth rates throughout the period of observations.

Morphogenesis is the process of initiation and differentiation of tissues and organs in multicellular organisms [1]. There are direct and indirect pathways of morphogenesis *in vitro*. Direct somatic morphogenesis is the process of forming a bipolar structure with an axis "root/stem" with a closed independent vascular system from the cells of the explant without prior dedifferentiation and stage of callus formation. Indirect morphogenesis necessarily involves the stage of formation of not differentiated callus tissue with the subsequent induction of initiation of somatic embryos (proembryos) and their development in bipolar structures with "root / stem" axis [1,2].

In further studies, we investigated the effectiveness of various pathways of morphogenesis *in vitro*, depending on the genotype of NILs and the type of selected explant. In the case of use as an explants of mature germs, the rates of morphogenesis are 3-5 times higher than in case when we use apical cones. However, the effect of the genotype, regardless of absolute indicators, manifested the same type and with the use of apices and mature embryos. The process in PPD-D1a and PPD-A1a NILs, as well as PPD-B1a NIL and cultivar M808, was the less effective.

Table 6. Frequency of morphogenesis in near-isogenic lines (NILs) of PPD genes of wheat 808, %

NILs genotype	Indirect morphogenesis, %		Direct morphogenesis,%	
	MS+0,5 IAA+0,5кin	MS+2,4 D+0,5кin	Mature embryos	Apices
PPD-D1aB1bA1b	48,5 ± 0,9	30,0 ± 0,7	75,0 ± 3,7	25,6 ± 0,8
PPD-D1bB1aA1b	26,2 ± 0,4	18,3 ± 0,5	70,8 ± 3,2	12,5 ± 0,5
PPD-D1bB1bA1a	35,9 ± 0,7	22,0 ± 0,9	77,8 ± 4,1	32,5 ± 1,3
PPD-D1bB1bA1b	33,1 ± 1,1	21,6 ± 0,8	72,2 ± 2,9	18,8 ± 0,6

In subsequent studies of the indirect pathway of morphogenesis *in vitro* after the formation of the callus tissues from various explants and their transfer to the medium for induction of morphogenesis, differences in the manifestation of the morphogenetic potential are shown, depending on the types of callus and genotype of the source NIL. Dense, yel-

lowish, morphogenic callus, formed from mature embryos, showed regenerative capacity. During 10-15 days of cultivation in the regenerative environment, processes of gemogenesis - formation of coleoptiles and rhizogenesis - formation of roots were recorded. The morphogenesis of a transparent, homogeneous, brittle callus, formed from aseptic roots occurs only through rhizogenesis. Many authors have shown that this type of morphogenesis is not regenerative, that is, in subsequent cultivation it is impossible to form complete fertile regenerated plants [1,2]. The study of morphogenetic potential was carried out using two modifications of the composition of the regenerative medium (Table 6). The most optimal was the medium of the following composition: MS + 0,5 mg/l IAA + 0,5 mg/l kinetin - the regeneration s frequency were on average 1,5 times higher than in the regeneration medium MS + 0,5 mg/l 2,4-D + 0.5 mg/l kinetin. The effect of the genotype of investigated NILs on the indirect route of morphogenesis was manifested uniformly, as well as in the case of direct morphogenesis. The most effective morphogenesis is shown for PPD-D1a NIL, the least effective - in PPD-B1a NIL. Thus, regardless of the pathway of morphogenesis (direct or indirect), regardless of the type of selected explant, the composition of the regenerative medium, the influence of the genotype of the investigated NILs manifested itself in a similar manner.

Conclusions. In our research, it has been shown that the dominant allele of the PPD-A1a and PPD-D1a genes leads to accelerated development indirectly, through inhibition of growth processes, and the dominant allele of the PPD-B1a gene may inhibit the transition to generative development by enhancing growth processes. Investigation of the processes of callus genesis of PPD NILs showed that the genotype and type of explants of the original line influence the frequency of callus formation, the maximum indices of callus genesis are found in PPD-B1a NIL, which *in vivo* develops the slowest among the investigated lines.

Thus, it has been established that the genetic system controlling development rate of wheat and its photoperiodic sensitivity - PPD genes, which determines the rate of growth and development of wheat plants *in vivo* conditions, also affects the processes of callus genesis and morphogenesis *in vitro*.

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