
GROWTH RESPONSE OF NEAR-ISOGENIC BREAD WHEAT (*TRITICUM AESTIVUM* L.) LINES CARRYING DIFFERENT *PPD* ALLELES UNDER *IN VIVO* AND *IN VITRO* CONDITIONS

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INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the world's most important cereal crops¹, accounting for more than one-third of global grain production and serving as a major source of plant-derived protein and carbohydrates² for populations worldwide. Rapid population growth, global climate change, and increasingly adverse agricultural conditions have intensified the need to develop new wheat cultivars capable of maintaining stable productivity across a wide range of environmental conditions. Consequently, one of the major objectives of modern wheat breeding is to investigate the genetic systems regulating plant growth, development, and adaptation to environmental factors³.

Among the most stable and evolutionarily conserved environmental signals is the photoperiod, defined as the daily duration of light and darkness at a given geographical location. In bread wheat, photoperiod sensitivity is an evolutionarily conserved and genetically determined trait controlled by the Photoperiod (*PPD*)⁴ gene system. The *PPD* genes play a pivotal role in the regulation of wheat ontogeny and are represented by three homoeologous loci, *PPD-A1*, *PPD-B1*, and *PPD-D1*, located on chromosomes 2A, 2B, and 2D,

¹ Erenstein, O., Jaleta, M., Mottaleb, K. A., Sonder, K., Donovan, J., Braun, H.-J., et al. (2022). Global Trends in Wheat Production, Consumption and Trade. Wheat Improvement. Springer, Cham. C. 47–66. DOI: 10.1007/978-3-030-90673-3_4

² Gul K., Yousuf B., Singh A. K., Singh P., Wani A. A. Wheat quality: A review on chemical composition, nutritional attributes, grain anatomy, types, classification, and function of seed storage proteins in bread making quality. *Frontiers in Nutrition*. 2023. DOI: 10.3389/fnut.2023.1053196

³ Dubrovna O. V., Mykhalska S. I., Komisarenko A. G. Modern advancements in Agrobacterium-mediated wheat transformation technology. *Фізіологія рослин і генетика*. 2025. Т. 57, № 4. С. 279–307. DOI: 10.15407/frg2025.04.279

⁴ Pérez-Gianmarco T. I., Slafer G. A., González F. G. Photoperiod-sensitivity genes shape floret development in wheat. *Journal of Experimental Botany*. 2019. Vol. 70, No. 4. P. 1339–1348. DOI: 10.1093/jxb/ery449

respectively. These genes constitute key components of the molecular pathway underlying photoperiod perception and determine the timing of the transition from vegetative to reproductive development. Dominant *PPD-1a* alleles confer photoperiod insensitivity by accelerating heading regardless of day length, whereas recessive *PPD-1b* alleles maintain long-day photoperiod sensitivity. Therefore, allelic variation at the *PPD* loci is a major determinant of the adaptation of wheat cultivars to diverse agro-climatic environments.

Over the past decade, substantial evidence has accumulated regarding the influence of *PPD* genes on heading time, productivity, and grain yield⁵. However, numerous studies have demonstrated that the functions of these genes extend far beyond the regulation of photoperiodic responses. They exert pleiotropic effects on plant height, growth rate, root system development⁶, biomass accumulation, photosynthetic efficiency, nitrogen use efficiency, productive tillering, and individual yield components⁷. These effects are believed to result from complex interactions between *PPD* genes, components of the circadian clock⁸, hormonal pathways regulating plant growth, and the vernalization (*VRN*)⁹ genes, which together constitute an integrated regulatory network controlling plant development.

Particular attention has recently been directed toward the early stages of plant ontogeny¹⁰, during which the fundamental morphological organization of the plant is established and the balance between shoot and root development is determined. Seedling growth, root system development, and the accumulation

⁵ Alvarez M. A., Slafer G. A., González F. G. et al. Photoperiod-1 regulates the wheat inflorescence transcriptome to influence spikelet architecture and flowering time. *Current Biology*. 2024. Vol. 34, No. 11. P. 2330–2343.e4. DOI: 10.1016/j.cub.2024.04.029

González F. G., Slafer G. A., Miralles D. J. et al. Features of Ppd-B1 expression regulation and their impact on the flowering time of wheat near-isogenic lines. *BMC Plant Biology*. 2017. Vol. 17. Article 107. DOI: 10.1186/s12870-017-1126-z

Royo C, Dreisigacker S, Alfaro C, Ammar K, Villegas D. Effect of Ppd-1 genes on durum wheat flowering time and grain filling duration in a wide range of latitudes. *The Journal of Agricultural Science*. 2016. 154(4) P. 612–631. DOI:10.1017/S0021859615000507

⁶ Makhoul M., Schlichtermann R.-H., Ugwuanyi S. et al. Novel PHOTOPERIOD-1 gene variants associate with yield-related and root-angle traits in European bread wheat. *Theoretical and Applied Genetics*. 2024. Vol. 137. Article 125. DOI: 10.1007/s00122-024-04634-9

⁷ Kitagawa S., Shimada K., Murai K. Effect of Ppd-1 on flowering time in wheat is associated with activation of FT1 and genes related to development of the inflorescence. *Breeding Science*. 2012. Vol. 62, No. 2. P. 184–190. DOI: 10.1270/jsbbs.62.184

⁸ Faure S., Turner A. S., Gruszka D. et al. Mutation at the ELF3 gene disrupts the circadian clock and causes barley photoperiod insensitivity. *Nature Genetics*. 2012. Vol. 44. P. 123–130.

⁹ Alvarez M. A., Tranquilli G., Lewis S., Kippes N., Dubcovsky J. Genetic and physical mapping of the photoperiod-insensitive gene Ppd-A1 and interaction with Vrn-1 alleles in wheat. *Molecular Breeding*. 2016. Vol. 36. Article 57. DOI: 10.1007/s10142-016-0490-3

Diallo A., Kane N, Agharbaoui Z, Badawi M, Sarhan F. Heterologous expression of wheat VERNALIZATION 2 (TaVRN2) gene in Arabidopsis delays flowering and enhances freezing tolerance. *PLoS One*. 2010 Jan 13;5(1):e8690. DOI: 10.1371/journal.pone.0008690

¹⁰ Carrera-Castaño G., Calleja-Cabrera J., Pernas M. et al. An updated overview on the regulation of seed germination. *Plants*. 2020. Vol. 9, No. 6. Article 703. DOI: 10.3390/plants9060703

of fresh and dry biomass largely determine the subsequent adaptation of plants to environmental stresses, including drought, salinity¹¹, nutrient deficiency, and temperature extremes¹². Despite numerous studies addressing the productivity of wheat cultivars carrying different *PPD*¹³ alleles, the influence of these genes on early vegetative growth remains insufficiently understood, and the available data are often inconsistent.

Near-isogenic lines (NILs)¹⁴ represent one of the most informative genetic models for assessing the contribution of individual genes to phenotypic expression. These lines differ only in one or a few target loci while sharing an otherwise nearly identical genetic background. Consequently, the influence of background genetic variation is minimized, allowing the direct evaluation of the role of a particular gene in the expression of a given trait. One of the best-characterized NIL collections was developed using the winter wheat cultivar Myronivska 808 and has been widely employed to investigate the functional roles of *PPD*, *VRN*, and other developmental regulatory genes in wheat.

In parallel with *in vivo* studies, *in vitro* tissue culture techniques have become increasingly important for investigating the expression of genetic characteristics at the cellular level¹⁵. Callus culture, consisting of a population of isolated cells that develop independently of the regulatory influences operating within the intact plant, represents a versatile experimental system for studying cellular dedifferentiation, proliferation, morphogenesis, and plant regeneration¹⁶. It is well established that callus induction, callus growth rate, and morphogenetic competence are strongly genotype-dependent. Consequently, plant tissue

¹¹ Iyem E., Yıldırım M., Kızılgöçü F. Comparative study on germination and seedling growth of wheat cultivars under salt stress regimes. *International Journal of Agriculture, Environment and Food Sciences*. 2020. Vol. 4, No. 4. DOI: 10.31015/jaefs.2020.4.8

¹² Hussain S., Chachar Q., Chachar S. et al. Temperature regimes on seeds germination and growth parameters of wheat (*Triticum aestivum* L.) genotypes at early seedling stage. *Pakistan Journal of Scientific and Industrial Research*. 2020. Vol. 63, No. 3. P. 214–225. DOI: 10.52763/PJSIR.BIOL.SCI.63.3.2020.214.225

¹³ Seki M., Chono M., Matsunaka H. et al. Distribution of photoperiod-insensitive alleles *Ppd-B1a* and *Ppd-D1a* and their effect on heading time in Japanese wheat cultivars. *Breeding Science*. 2011. Vol. 61, No. 4. P. 405–412.

Song T., Shi C., Wang Y. et al. Molecular characterization of a novel photoperiod-insensitive allele *Ppd-B1a.3* and its effect on heading date in Chinese wheat (*Triticum aestivum*). *Journal of Plant Research*. 2025. DOI: 10.1007/s10265-024-01609-1

¹⁴ Ning S., Li S., Xu K. et al. Development and characterization of near-isogenic lines derived from synthetic wheat revealing the 2 kb insertion in the *PPD-D1* gene responsible for heading delay and grain number improvement. *International Journal of Molecular Sciences*. 2023. Vol. 24, No. 13. Article 10834. DOI: 10.3390/ijms241310834

¹⁵ Kumléhn J. et al. Opportunities and challenges of *in vitro* tissue culture systems in the era of crop genome editing. *International Journal of Molecular Sciences*. 2023. Vol. 24, No. 15. Article 11920. DOI: 10.3390/ijms241511920

¹⁶ Wijerathna-Yapa A., Ramtekey V., Ranawaka B., Basnet B. R. Applications of *in vitro* tissue culture technologies in breeding and genetic improvement of wheat. *Plants*. 2022. Vol. 11, No. 17. Article 2273. DOI: 10.3390/plants11172273

culture has become an indispensable tool not only in plant biotechnology but also in fundamental studies of the genetic mechanisms regulating plant growth and development.

An important characteristic of callus tissue is its cytological organization. Cell density, cell size, the proportion of meristematic and highly vacuolated cells, and the degree of cellular differentiation reflect the physiological status of the culture and largely determine its regenerative capacity. Cytological analyses therefore provide valuable insight into the realization of genetic programs under *in vitro* conditions and facilitate the understanding of the relationships between growth processes at both the organismal and cellular levels.

Although numerous investigations have addressed the functional significance of *PPD* genes, most have focused primarily on heading time, photoperiodic responses, or yield formation. Comparatively little attention has been paid to the integrated evaluation of growth processes during early ontogeny, biomass accumulation, shoot-to-root relationships, and the manifestation of these genetic effects under tissue culture conditions. Moreover, studies integrating analyses of plant growth *in vivo* with callus formation and the cytological characteristics of callus cells *in vitro* using the same genetic material remain scarce. Such an integrated approach provides a deeper understanding of the pleiotropic effects of *PPD* genes and the mechanisms through which they regulate plant growth and development.

Therefore, the present study aimed to investigate the growth responses of near-isogenic lines of the bread wheat cultivar Myronivska 808, differing in the dominant alleles *PPD-A1a*, *PPD-B1a*, and *PPD-D1a*, using a comprehensive assessment of plant morphometric traits under *in vivo* conditions, fresh biomass accumulation, callus induction, and the cytological characteristics of callus cells cultured *in vitro*. The findings of this study will contribute to a better understanding of the pleiotropic functions of photoperiod sensitivity genes and may facilitate the development of highly productive wheat cultivars with improved growth performance, enhanced environmental adaptability, and superior biotechnological potential.

MATERIALS AND METHODS

Plants materials

A set of near-isogenic lines (NILs) of bread wheat (*Triticum aestivum* L.) differing in photoperiod sensitivity (*PPD*) genes and developed in the genetic background of the winter wheat cultivar Myronivska 808 was used as the experimental material. The use of NILs minimized the influence of background genetic variation and enabled the direct assessment of the contribution of individual *PPD* alleles to plant growth responses.

Table 1

**Characteristics of the near-isogenic lines (NILs)
of the wheat cultivar Myronivska 808**

Near-isogenic line (NIL)	Genotype	Growth habit	Developmental rate
Ppd-D1a	<i>Ppd-D1a Ppd-B1b Ppd-A1b</i>	Winter	Rapid
Ppd-B1a	<i>Ppd-D1b Ppd-B1a Ppd-A1b</i>	Winter	Slow
Ppd-A1a	<i>Ppd-D1b Ppd-B1b Ppd-A1a</i>	Winter	Rapid
Myronivska 808	<i>ppd-D1b ppd-B1b ppd-A1b</i>	Winter	Slow

In vivo experiments

Plant growth responses were evaluated under controlled pot-culture conditions. Plants were grown in soil substrate under the following environmental conditions: light intensity of 15 kLux, day/night temperatures of 22/18 °C, a 16 h/8 h light/dark photoperiod, and soil moisture maintained at 70% of field capacity. Plants were cultivated for 21 days, corresponding to the tillering stage. At the end of the experiment, plant height, shoot length, root length, shoot-to-root length ratio, and the fresh biomass of shoots and roots were determined.

In vitro experiments

To investigate growth responses at the cellular level, explants were introduced into *in vitro* culture and primary callus formation was induced using standard plant tissue culture techniques. Mature embryos isolated from surface-sterilized grains of the NILs served as explants. The embryos were cultured in Petri dishes containing Murashige and Skoog (MS) basal medium supplemented with 2 mg L⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D). Cultures were incubated in darkness at 26 °C for 21 days. Following cultivation, primary callus induction frequency and the callus growth index (GI) were determined. Callus induction frequency was calculated as the percentage of explants producing callus relative to the total number of cultured explants. The growth index was determined from the increase in callus surface area between the first and third weeks of cultivation using ImageJ software.

The cytological characteristics of callus tissue were evaluated by determining cell density (number of cells per milligram of tissue) and mean cell length. For cell density analysis, calli were macerated in 10% chromic acid for 30 min, after which the cells were counted using a Goryaev counting chamber. Cell length was measured using an ocular micrometer under a LOMO Mikmed-1 light microscope. At least 100–150 cells were analyzed for each experimental variant.

Statistical analysis

All experiments were performed in three biological replicates. In the *in vivo* experiments, 25–30 plants of each NIL were analyzed, whereas 21–28 primary calli were evaluated in the *in vitro* experiments. Statistical analyses were performed using Statistica 5.0 (StatSoft Inc., USA). Differences between mean values were assessed using the least significant difference (LSD_{0.05}) test. Graphs and tables were prepared using Microsoft Excel 2019 (Microsoft Corp., USA). Data are presented as mean $\pm$ standard deviation (mean $\pm$ SD).

1. Analysis of the Growth Response of Near-Isogenic Wheat Lines Carrying PPD Alleles during Early Ontogeny

Plant growth during the early stages of ontogeny is one of the most critical determinants of subsequent development, productivity, and adaptation to adverse environmental conditions. Early ontogeny encompasses seed germination, seedling establishment, root system development, and the formation of the first leaves. During this period, intensive cell division and elongation occur, accompanied by the activation of photosynthesis, water uptake, and mineral nutrition, thereby ensuring the establishment of a vigorous and viable plant.

The genetic regulation of wheat growth is governed by the coordinated action of numerous genes controlling the biosynthesis of enzymes, phytohormones, and regulatory proteins. Of particular importance are genes involved in the biosynthesis and signaling of gibberellins, auxins, and cytokinins, which regulate cell division, elongation, and differentiation. Equally important are the *Reduced height (Rht)* genes, which determine plant sensitivity to gibberellins and influence stem growth from the earliest developmental stages. In addition to genetic factors, environmental conditions—including temperature, soil moisture, light intensity, and nutrient availability—substantially affect gene expression and plant growth.

Thus, wheat growth during early ontogeny is the result of complex interactions between genetic mechanisms and environmental factors. Understanding the genetic regulation of these processes is essential for the development of high-yielding and stress-tolerant wheat cultivars capable of adapting to changing climatic conditions while maintaining stable productivity.

The early stages of wheat ontogeny are particularly important for the establishment of plant adaptive potential, as this period determines root system architecture, biomass accumulation, and the intensity of growth processes. It has been demonstrated that the photoperiod sensitivity genes *PPD-1* (*PPD-A1*, *PPD-B1*, and *PPD-D1*) regulate not only heading time but also influence early

growth rate, leaf area development, root system architecture, and the efficiency of resource utilization by the plant¹⁷.

1.1. Analysis of Linear Growth in Near-Isogenic Lines Carrying Different PPD Alleles

The analysis demonstrated that all near-isogenic lines (NILs) carrying dominant *PPD* alleles exhibited greater total plant length than the recurrent cultivar Myronivska 808 (Table 2).

Table 2

Growth response of near-isogenic wheat lines carrying different PPD alleles based on linear growth parameters at the tillering stage

Near-isogenic line	Plant length (cm)	Shoot length (cm)	Root length (cm)	Shoot/root ratio
<i>PPD D1a</i>	45,41	33,43	12,11	2,76
<i>PPD B1a</i>	46,43	32,72	13,82	3,34
<i>PPD A1a</i>	42,22	32,53	9,87	3,31
Myronivska 808	39,84	33,12	6,83	4,85
<i>LSD_{0.05}</i>	3,02	3,03	0,89	0,25

The greatest plant length was observed in the *PPD-B1a* NIL (46.43 cm), exceeding that of the recurrent cultivar by 6.59 cm (16.5%). A similarly high value was recorded for the *PPD-D1a* genotype (45.41 cm), which was 5.57 cm (14.0%) longer than the control. The *PPD-A1a* NIL also exhibited greater plant length (42.22 cm), exceeding the control by 2.38 cm (6.0%); however, this difference did not exceed the least significant difference ($LSD_{0.05} = 3.02$ cm) and therefore was not statistically significant.

The most pronounced genotypic differences were observed in root development. The longest roots were recorded in the *PPD-B1a* NIL (13.82 cm), representing nearly a twofold increase compared with the recurrent cultivar (6.83 cm). Root length reached 12.11 cm in the *PPD-D1a* line and 9.87 cm in the *PPD-A1a* line. In all NILs, root length significantly exceeded that of Myronivska 808, as the observed differences were greater than the corresponding $LSD_{0.05}$ value (0.89 cm).

In contrast, shoot length varied only slightly among the studied genotypes (32.53–33.43 cm), indicating that the principal effect of the dominant *PPD* alleles during early ontogeny was associated with enhanced root system development rather than shoot elongation.

Significant differences were also observed in the shoot-to-root ratio. The lowest values were recorded for the *PPD-D1a* and *PPD-B1a* NILs,

¹⁷ Hyles, J., Bloomfield, M.T., Hunt, J.R. *et al.* Phenology and related traits for wheat adaptation. *Heredity* 125, 417–430 (2020). <https://doi.org/10.1038/s41437-020-0320-1>

reflecting relatively greater investment in root growth. Conversely, the recurrent cultivar Myronivska 808 exhibited the highest shoot-to-root ratio (4.85), indicating considerably weaker root development relative to the aerial part of the plant.

These findings are consistent with current understanding of *PPD-1* gene function. In addition to regulating photoperiodic responses, *PPD* genes exert pleiotropic effects on plant growth by influencing meristem activity, developmental rate, and vegetative biomass accumulation from the earliest stages of plant development. The observed stimulation of root growth in NILs carrying dominant *PPD* alleles further supports the role of these genes in coordinating developmental processes beyond the regulation of flowering time.

1.2. Analysis of Fresh Biomass Accumulation

The analysis of fresh biomass accumulation demonstrated that the effects of different *PPD* alleles were manifested not only in linear growth but also in the distribution of biomass among individual plant organs (Table 3). The greatest total fresh biomass was recorded in the *PPD-B1a* NIL (4.95 mg), which significantly exceeded that of the *PPD-A1a* line (3.85 mg) and was slightly higher than that of the recurrent cultivar Myronivska 808 (4.63 mg). The *PPD-D1a* NIL also produced greater total biomass than the control, reaching 4.72 mg.

The most pronounced differences among the genotypes were observed in root biomass accumulation. The highest root fresh biomass was recorded in the *PPD-B1a* (1.11 mg) and *PPD-A1a* (1.05 mg) lines, whereas the recurrent cultivar accumulated only 0.54 mg. Thus, root biomass accumulation in the NILs was approximately twofold greater than that of the control genotype.

Table 3

Growth response of near-isogenic wheat lines carrying different *PPD* alleles based on fresh biomass accumulation at the tillering stage

Near-isogenic line	Plant fresh biomass (mg)	Shoot fresh biomass (mg)	Root fresh biomass (mg)	Shoot/root biomass ratio
<i>PPD D1a</i>	4,72	3,92	0,78	5,11
<i>PPD B1a</i>	4,95	3,64	1,11	3,32
<i>PPD A1a</i>	3,85	3,68	1,05	3,51
Myronivska 808	4,63	4,09	0,54	7,64
<i>LSD_{0.05}</i>	0,48	0,21	0,26	1,98

In contrast, the highest shoot fresh biomass was observed in Myronivska 808 (4.09 mg). However, the difference between the recurrent cultivar and the *PPD-D1a* NIL (3.92 mg) was smaller than the corresponding *LSD_{0.05}* value (0.21 mg)

and was therefore not statistically significant. These findings indicate that the principal effect of the dominant *PPD* alleles was associated with stimulation of root development rather than increased shoot biomass accumulation.

The shoot-to-root biomass ratio provided an integrated assessment of biomass partitioning within the plant. The highest ratio was observed in the recurrent cultivar (7.64), indicating preferential allocation of assimilates to shoot growth. In contrast, substantially lower values were recorded in the *PPD-B1a* (3.32) and *PPD-A1a* (3.51) NILs, reflecting a more balanced distribution of assimilates between aboveground and belowground organs and a relatively greater investment in root development.

Recent studies have demonstrated that photoperiod-insensitive *PPD-I* alleles, particularly *PPD-D1a* and *PPD-B1a*, accelerate plant development, modify plant architecture, and influence biomass accumulation well before the transition to the reproductive phase. The present results further support these observations, demonstrating that substitution of individual *PPD-I* alleles in the near-isogenic background of the cultivar Myronivska 808 substantially affects plant growth characteristics during the earliest stages of ontogeny.

Among the investigated genotypes, the *PPD-B1a* NIL exhibited the most pronounced positive effect on root system development, as evidenced by the greatest plant length, maximum root length and root biomass, and the lowest shoot-to-root ratios for both linear growth and biomass accumulation. The *PPD-D1a* NIL also displayed vigorous plant growth and a well-developed root system. Although the influence of the *PPD-A1a* allele was comparatively less pronounced, this genotype nevertheless outperformed the recurrent cultivar for most of the evaluated growth parameters.

Overall, the obtained results indicate that dominant *PPD* alleles determine not only the photoperiodic response of wheat plants but also influence assimilate partitioning among plant organs, promoting the development of a more vigorous root system during early ontogeny. Such modifications may enhance plant adaptation to unfavorable environmental conditions, particularly water deficit, thereby supporting the increasingly recognized pleiotropic role of *PPD* genes in regulating wheat growth, development, and stress adaptation.

2. Analysis of the Growth Response of Primary Calli of Near-Isogenic Wheat Lines Carrying Different *PPD* Alleles

2.1. Callus Induction from Mature Wheat Embryos

In vitro tissue culture is one of the most important tools of modern plant biotechnology and is widely used for cell selection, genetic transformation, production of somaclonal variants, and the rapid propagation of valuable

genotypes. In bread wheat (*Triticum aestivum* L.), mature embryos¹⁸ are among the most commonly used explants because they are available throughout the year, exhibit high viability, and are relatively easy to sterilize. Unlike immature embryos, the use of mature seeds does not require donor plants to be grown at specific developmental stages, thereby considerably simplifying biotechnological research¹⁹.

Callus induction begins with the dedifferentiation of scutellum cells or embryonic meristematic tissues in response to exogenous phytohormones. The most widely used induction medium is Murashige and Skoog (MS) basal medium supplemented with the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) at concentrations ranging from 1 to 3 mg L⁻¹. Auxins initiate intensive cell division, ultimately resulting in the formation of undifferentiated callus tissue.

The efficiency of callus induction is largely genotype-dependent. Numerous studies have demonstrated that wheat cultivars and breeding lines differ substantially in callus induction frequency, callus growth rate, and regeneration capacity. These differences are genetically determined through the regulation of cell division, morphogenesis, and hormonal signaling pathways. Genes involved in phytohormone responses, meristem activity, and photoperiod sensitivity may also influence the proliferative activity of cultured cells under *in vitro* conditions²⁰.

The quality of the induced callus is as important as its quantity. Compact, light-yellow or cream-colored embryogenic callus is considered the most suitable material for plant regeneration because it is characterized by high mitotic activity and consists predominantly of small, isodiametric cells with large nuclei. In contrast, loose, watery, or dark-colored callus generally possesses low morphogenetic potential and rapidly loses its capacity for organogenesis.

Callus induction is also strongly influenced by culture conditions. Optimal induction is generally achieved at temperatures of 24–26°C, under complete darkness during the induction stage, and on media containing elevated concentrations of sucrose as the primary carbon source. Furthermore, the efficiency of callus formation depends on the physiological status of the seeds, storage conditions, and the developmental stage of the embryos. The

¹⁸ Tamimi S. M., Othman H. Callus induction and regeneration from germinating mature embryos of wheat (*Triticum aestivum* L.). *Sains Malaysiana*. 2021. Vol. 50, No. 4. P. 889–896. DOI: 10.17576/jsm-2021-5004-01

¹⁹ Zhou Z., Xu Y., Zhao Y. et al. Overcoming genotypic dependency and bypassing immature embryos in wheat transformation by using morphogenic regulators. *Science China Life Sciences*. 2024. Vol. 67. P. 1535–1538. DOI: 10.1007/s11427-023-2565-9

²⁰ Fehér A. Callus, Dedifferentiation, Totipotency, Somatic Embryogenesis: What These Terms Mean in the Era of Molecular Plant Biology? *Frontiers in Plant Science*. 2019. Vol. 10. Art. 536. DOI: 10.3389/fpls.2019.00536

optimization of culture media and plant growth regulators can substantially increase the frequency of morphogenetically competent callus, even in genotypes exhibiting inherently low regeneration capacity.

Thus, mature wheat embryos represent a convenient and reliable explant source for callus culture. Although their efficiency is generally lower than that of immature embryos, they provide high experimental reproducibility, making them particularly valuable for genetic, breeding, and biotechnological studies. Investigation of callus formation in different genotypes, especially near-isogenic lines, provides an opportunity to elucidate the role of individual genes in the regulation of *in vitro* morphogenesis and contributes to the development of efficient systems for cell selection and plant regeneration.

2.2. Role of the PPD Gene System in the Regulation of Primary Callus Formation

During the experiments, all investigated near-isogenic lines were successfully introduced into *in vitro* culture, resulting in the production of primary calli. Based on their phenotypic characteristics, the calli of different origins did not differ morphologically (Fig. 1A). The primary calli were creamy-white in color, matte, opaque, compact, and homogeneous in structure (Fig. 1B).

Cytological analysis revealed typical callus cells, which were relatively large, elongated or curved, with thin cellulose cell walls, a large vacuole, and a clearly visible nucleus (Fig. 1C).

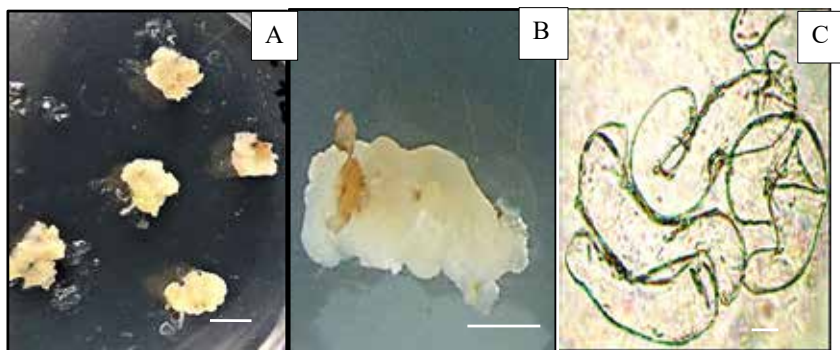


Fig. 1. Primary callus induction from mature wheat embryos. (A) General appearance of callus tissue after 21 days of cultivation; (B) primary callus formed from mature embryos; (C) typical mature callus cells (×400).

Scale bars: 1 cm (A, B) and 1 μ m (C)

Analysis of the experimental results demonstrated that the near-isogenic lines (NILs) of the bread wheat cultivar Myronivska 808, differing in their *PPD*

alleles, exhibited distinct capacities for callus induction and callus growth under *in vitro* culture conditions (Fig. 2). These differences indicate that photoperiod sensitivity genes, in addition to their established role in regulating the transition from vegetative to reproductive development, may indirectly influence cell proliferation, morphogenetic competence, and growth intensity in isolated tissue culture.

The most pronounced growth response was observed in the *PPD-B1a* near-isogenic line, which exhibited a callus induction frequency of 96.1% (Fig. 2A) and a callus growth index of 32.43% (Fig. 2B). Compared with the recurrent cultivar Myronivska 808, these values were approximately 1.6-fold and almost fourfold higher, respectively. These findings demonstrate the considerably greater morphogenetic potential of the *PPD-B1a* genotype and its enhanced capacity for intensive cell proliferation under artificial culture conditions.

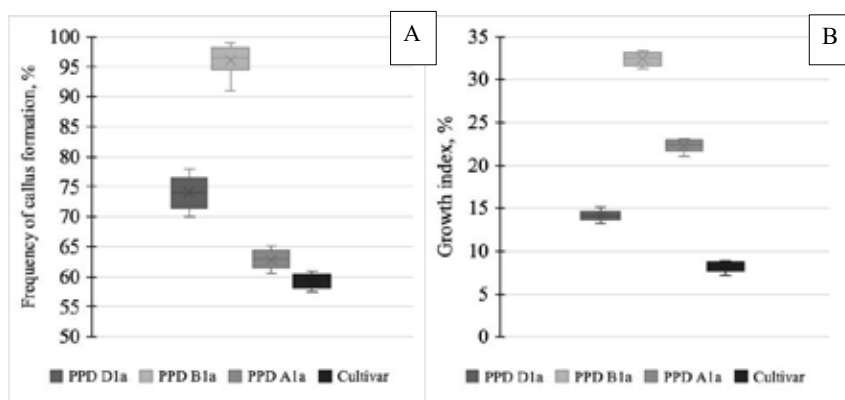


Fig. 2. Growth response of primary calli of near-isogenic wheat lines carrying different *PPD* alleles (A – callus induction frequency, B – callus growth index). Data are expressed as percentages

The high callus induction capacity observed in the *PPD-B1a* line may be associated with more efficient regulation of the cell cycle and enhanced mitotic activity. Callus formation is initiated through the dedifferentiation of somatic cells followed by their transition to intensive cell division. This process is controlled by a complex interaction between genetic factors and the phytohormonal system, particularly auxins and cytokinins. Altered expression of genes involved in photoperiodic regulation may affect the hormonal balance within cultured cells, thereby influencing both the rate of callus induction and the subsequent growth of callus tissue.

2.3. Cytological Analysis of Callus Tissues of Wheat NILs Carrying PPD Genes

The cytological analysis confirmed the results of the morphometric studies (Fig. 3). Among the investigated near-isogenic lines (NILs), the *PPD-B1a* isoline was characterized by the highest cell density in the callus tissue (4.23×10^6 cells mg^{-1}) (Fig. 3A), indicating a high level of mitotic activity. At the same time, the average cell length was lower ($1.05 \times 10^2 \mu\text{m}$) (Fig. 3B) than in the other experimental variants. This pattern is typical of tissues exhibiting intensive proliferation, where cell division predominates over subsequent cell growth and elongation. Smaller cells are physiologically younger and possess higher metabolic activity, thereby ensuring rapid accumulation of callus biomass.

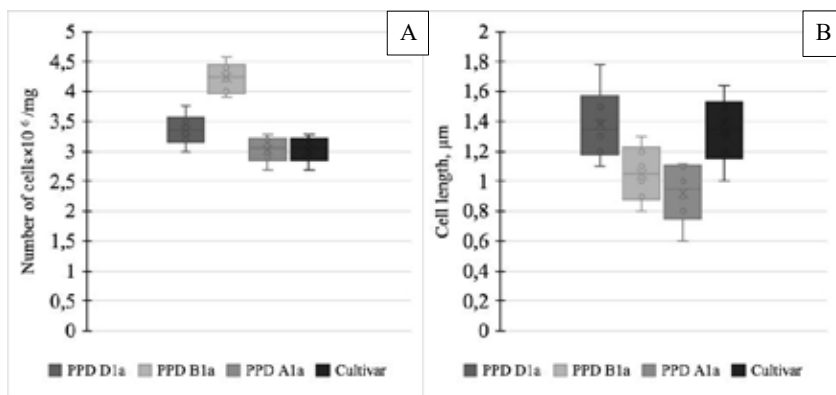


Fig. 3. Cytological characteristics of callus cells of wheat NILs carrying PPD genes in the cultivar Myronivska 808 (A – Cell density; B – Cell length)

In contrast to the *PPD-B1a* isoline, the *PPD-D1a* line exhibited a lower growth index (14.13%), although the number of cells remained relatively high (3.36×10^6 cells mg^{-1}). However, this isoline was characterized by the greatest average cell length (1.38 μm). This finding may indicate the predominance of cell elongation processes over active cell division. Such a growth pattern is commonly observed when mitotic activity declines, and tissue biomass increases primarily through the enlargement of already differentiated cells.

The *PPD-A1a* isoline occupied an intermediate position for most of the investigated parameters. The frequency of callus formation reached 62.9%, while the growth index was 22.3%, exceeding the corresponding values of the original

cultivar but remaining lower than those of the *PPD-B1a* isoline. Nevertheless, the callus tissue contained the lowest number of cells (2.57×10^6 cells mg^{-1}), and their average length was also the smallest (0.92 μm). This combination may indicate relatively low rates of both cell division and subsequent cell expansion, which is likely associated with the functional characteristics of the corresponding *PPD* allele.

The original cultivar Myronivska 808 exhibited the lowest indices of growth activity. The frequency of callus formation was only 59.3%, while the growth index reached 8.15%. These results indicate a considerably lower regenerative capacity compared with the investigated NILs. Therefore, the introduction of individual *PPD* gene alleles into the genetic background of the cultivar can substantially modify the response of plant tissues to *in vitro* culture conditions.

The obtained results are consistent with current concepts regarding the multifunctional role of photoperiod response (*PPD*) genes. Recent studies have demonstrated that *PPD* genes regulate not only heading time but also influence the intensity of growth processes, photosynthetic efficiency, hormonal status, adaptation to abiotic stresses, and meristem activity. Consequently, their effects may also be manifested under *in vitro* culture conditions, where tissue development is determined primarily by intrinsic physiological and biochemical mechanisms.

Thus, the results of the present study demonstrate that different alleles of the *PPD* genes exert distinct effects on the morphogenetic potential of wheat callus tissue. The *PPD-B1a* isoline exhibited the highest biotechnological value, providing the greatest frequency of callus formation, the highest growth index, and the largest population of actively proliferating cells. These characteristics make this genotype a promising candidate for biotechnological applications, including plant regeneration, cell selection, and genetic transformation of wheat.

3. Comparative Analysis of the Growth Response of Wheat NILs Carrying *PPD* Genes under *In Vivo* and *In Vitro* Conditions

A comparative analysis of the experimental results demonstrated that the effects of *PPD* genes are manifested both at the level of the whole plant (*in vivo*) and at the level of isolated cells and tissues (*in vitro*). Despite the substantial differences between these experimental systems, a common pattern was observed in both cases: the dominant alleles of the *PPD* genes promoted growth processes compared with the original cultivar *Myronivska 808*.

Under *in vivo* conditions, the most pronounced effect was observed in the *PPD-B1a* isoline, which exhibited the greatest plant height (46.43 cm), the longest root system (13.82 cm), and the highest fresh plant biomass (4.95 mg). Furthermore, this isoline showed the lowest shoot-to-root ratio, indicating more

intensive root system development. A similar trend was observed for the *PPD-D1a* isoline, although its values were slightly lower. The least pronounced positive effect among the NILs was recorded for *PPD-A1a*, whereas the original cultivar *Myronivska 808* exhibited the poorest root growth and a less efficient distribution of biomass between aboveground and belowground organs.

A similar pattern was also observed under *in vitro* conditions. The *PPD-B1a* isoline exhibited the highest frequency of callus formation (96.1%), the maximum growth index (32.43%), and the greatest cell density in the callus tissue (4.23×10^6 cells mg^{-1}). These results indicate a high proliferative activity of callus cells and a considerable morphogenetic potential of this genotype. In contrast, the original cultivar *Myronivska 808* was characterized by the lowest levels of callus induction and callus growth, consistent with its lower growth performance under natural conditions.

At the same time, certain differences were observed between the *in vivo* and *in vitro* systems. In the intact plant, growth is determined by the integrated interaction of the photosynthetic apparatus, the root system, assimilate transport, hormonal regulation, and environmental factors. In contrast, under *in vitro* culture conditions, cell development occurs in a controlled environment on an artificial nutrient medium, where intrinsic genetic mechanisms, hormonal balance, and the capacity of cells for dedifferentiation and re-entry into the cell cycle play the predominant roles. Consequently, individual parameters do not always show complete correspondence between the two systems. For example, the *PPD-D1a* isoline displayed vigorous plant growth *in vivo*, whereas under *in vitro* conditions its callus exhibited a relatively lower growth index despite possessing the largest average cell size. This observation suggests that cell expansion predominated over intensive cell proliferation.

Particular attention should be given to the *PPD-B1a* isoline, which exhibited the best overall performance in both experimental systems. Plants of this genotype showed intensive root system development and the greatest biomass accumulation, while under *in vitro* conditions they demonstrated the highest frequency of callus formation, the greatest growth index, and the highest cell density in the callus tissue. This correspondence indicates that the *PPD-B1a* allele positively affects not only whole-plant growth but also the fundamental processes of cell division and morphogenesis.

The obtained results support the current understanding of the multifunctional role of *PPD* genes. Although these genes are traditionally regarded as regulators of photoperiod sensitivity and heading time, their function is not limited to reproductive development. The findings suggest that *PPD* genes are also involved in the regulation of meristem activity, plant hormonal status, cell division, and growth processes during the early stages of ontogeny. The

consistency of the observed trends under both natural and tissue culture conditions indicates that the effects of *PPD* alleles are genetically determined and are expressed regardless of the level of biological organization.

In conclusion, the comparative analysis demonstrated that the results obtained under *in vivo* and *in vitro* conditions are complementary. Among the investigated genotypes, the *PPD-B1a* isoline proved to be the most promising, combining vigorous plant growth, a well-developed root system, and the highest morphogenetic potential of callus tissue. These characteristics make this genotype a valuable candidate for breeding programs, cell selection, and biotechnological research aimed at improving wheat adaptability and productivity.

CONCLUSIONS

The present study demonstrated that the substitution of individual dominant *PPD* alleles into the genetic background of the bread wheat cultivar Myronivska 808 significantly affected plant growth and morphogenetic responses under both *in vivo* and *in vitro* conditions. Although *PPD* genes are primarily recognized as regulators of photoperiod sensitivity and heading time, the obtained results provide further evidence of their pleiotropic effects on early plant development, root system architecture, biomass accumulation, and the proliferative activity of cultured cells.

Among the investigated near-isogenic lines, the *PPD-B1a* genotype consistently exhibited the most favorable performance. Under *in vivo* conditions, it showed the greatest plant height, the most developed root system, the highest fresh biomass accumulation, and a more balanced allocation of assimilates between shoots and roots. Under *in vitro* conditions, the same genotype demonstrated the highest frequency of callus induction, the greatest callus growth index, and the highest density of actively proliferating cells, indicating superior morphogenetic competence and regenerative potential.

The comparative analysis revealed a strong correspondence between plant growth under natural conditions and cellular behavior in tissue culture. Although the physiological mechanisms regulating development differ between *in vivo* and *in vitro* systems, the overall effects of the dominant *PPD* alleles remained consistent, suggesting that their influence is genetically determined and expressed across different levels of biological organization. The contrasting response of the *PPD-D1a* isoline, characterized by vigorous plant growth but relatively lower callus proliferation accompanied by larger cell size, further indicates that different *PPD* alleles may differentially regulate the balance between cell division and cell expansion.

Overall, the findings highlight the multifunctional role of *PPD* genes in the regulation of wheat growth and development beyond their established function in flowering-time control. The superior performance of the *PPD-B1a* isoline identifies this genotype as a valuable source of favorable alleles for wheat improvement. Its high growth vigor, enhanced root development, and elevated morphogenetic potential make it particularly suitable for breeding programs, cell selection, plant regeneration, and genetic transformation.

Thus, the results of the present study demonstrate that the dominant *PPD* alleles exhibit pleiotropic effects manifested at both the whole-plant and cellular levels. These findings indicate that the *PPD* gene system can be regarded as one of the key regulators of growth processes in wheat. These results contribute to a better understanding of the biological functions of *PPD* genes and provide a scientific basis for their application in modern wheat breeding and plant biotechnology.

SUMMARY

The study investigated the growth response of near-isogenic lines (NILs) of bread wheat (*Triticum aestivum* L.) cultivar Myronivska 808, differing in the allelic composition of the photoperiod sensitivity (*PPD*) genes, under *in vivo* and *in vitro* conditions. The aim of the study was to determine the effects of the dominant alleles *PPD-A1a*, *PPD-B1a*, and *PPD-D1a* on plant growth, biomass accumulation, root system development, callus formation, and the cytological characteristics of callus tissues. The results demonstrated that all NILs carrying dominant *PPD* alleles exceeded the original cultivar Myronivska 808 in growth performance during the early stages of ontogenesis. The most pronounced positive effect was observed in the *Ppd-B1a* isoline, which exhibited the greatest plant and root lengths, the highest fresh root biomass accumulation, and a more balanced shoot-to-root ratio. Under *in vitro* tissue culture conditions, this isoline also showed the highest callus induction frequency, the greatest growth index, and the highest cell density in callus tissue. These findings indicate that *PPD* genes exert a consistent genetic effect on growth regulation under both *in vivo* and *in vitro* conditions. The obtained results confirm the multifunctional role of *PPD* genes, which regulate not only photoperiodic response and heading time but also influence growth intensity, root system development, biomass accumulation, and cell proliferative activity. The *PPD-B1a* isoline can be recommended as a promising source material for wheat breeding programs and biotechnological research aimed at developing high-yielding and environmentally adaptable wheat cultivars.

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