ITEMS FROM UKRAINE

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The characteristics of primary callus NILs for PPD genes of winter wheat, Triticum aestivum L.

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Abstract. We studied primary callus isogenic for the *PPD* genes, controlling photoperiodic sensitivity, in lines of winter wheat. Genotypic dependence on the processes of callus formation, the rate of formation of callus tissue, size and number of cells, biomass accumulation, and water content was established. The genotype of the isolines, determining photoperiodic sensitivity *in vivo*, has an impact on the process of induction and on the characteristics of primary callus NILs *in vitro*.

Introduction. Methods of cultivating isolated plant cells are a unique tool for studying fundamental biological problems. Despite numerous research in cereal morphogenesis *in vitro*, many questions remain unresolved (Tyankova and Zagorska 2001; Wang and Wei 2004). The role of a particular genetic system or separate genes in the regulation of explants ability to cultivation *in vitro* is possible to study. To solve these problems, using near-isogenic lines (NILs) with a known connection between gene expression and its phenotype are a convenient system to use. Two basic gene systems in soft wheat define the type and rates of development (Stelmakh 1998). VRN genes (requiree for vernalization) determine spring/winter type, and *PPD* genes determine photoperiodic sensitivity. NILs of PPD genes differ in development rates, which are shown in short-day conditions (photoperiod). Isoline *PPD B1* shows the maximum sensitivity; *PPD D1a* and *PPD A1a* have a low degree of sensitivity, so they are almost photoperiodically neutral. These genetic systems are actively investigated at the molecular level in cereal flowering regulation (White et al. 2008; Wang et al. 2009; Bentley et al. 2010). The *PPD* system of genes also seems to take part in the control of callus initiation *in vitro*. This research investigated the influence of the *PPD* gene system on callus genesis processes and the cytological and morpho-physiological characteristics of primary callus from NILs of the wheat cultivar Mironovskay 808.

Materials and methods. Genotypes of soft winter wheat NILs for photoperiod sensitivity genes *PPD D1a*, *PPD B1a*, *PPD A1a*, and *PPD D1b PPD B1b PPD A1b* were used as objects of study. For callus production and quality explants, we used mature germs, which were cultivated on a nutrient Murashige-Skoog (MS) medium with a full set of macro and micro salts and containing 2,4 D (2 mg/l) as a growth regulator, 0.7% agar, 3% sucrose, and 10 mg/L $AgNO_3$. Explants were cultivated in an incubator at 26°C in the dark for 1.5 months. Growth rate was measured as the area of callus tissues per unit of time. Cytohistological observations of crushed preparations were made using a light microscope PZO (Warszava). Crude and dry biomass were defined at the end of cultivation, 45 days after the first passage. The quantity of soluble protein was calculated by the Loury method, allocating these fractions with tris-HCl buffer pH 5.6 and further photocolorimetrical analysis (730 nm) after reaction of the protein with Folin reactant. Results were from 4–5 independent experiments in not less than 4–5 Petri dishes or flasks (6–7 explants). Mean values and the least significant differences (LSD 0.5) are presented in the tables.

Results and discussion. Al investigated genotypes were capable of inducing primary callus, which we had shown previously (Avksentyeva et al. 2008). Primary callus from mature germ was dense, less watery, yellowish, and characterized by some elements of differentiation that were confirmed microscopically (Fig. 1A, p. 216). The most effective growth *in vitro* was detected in *PPD B1a*, 93.12%, less effective was *PPD A1a*, 61.30% (Table 1). The speed of growth was determined by the increase of callus area for a

Table 1. Growth of primary calluses NILs for *PPD* genes of wheat cultivar Mironovskay 808.

Genotype	% of callus genesis	Callus growth rate mm^2/day
PPD D1a	73.30	0.74
PPD B1a	93.12	0.85
PPD A1a	61.30	0.56
PPD D1b PPD B1b PPD A1b	84.55	0.41
LSD 0.5	19.30	0.11

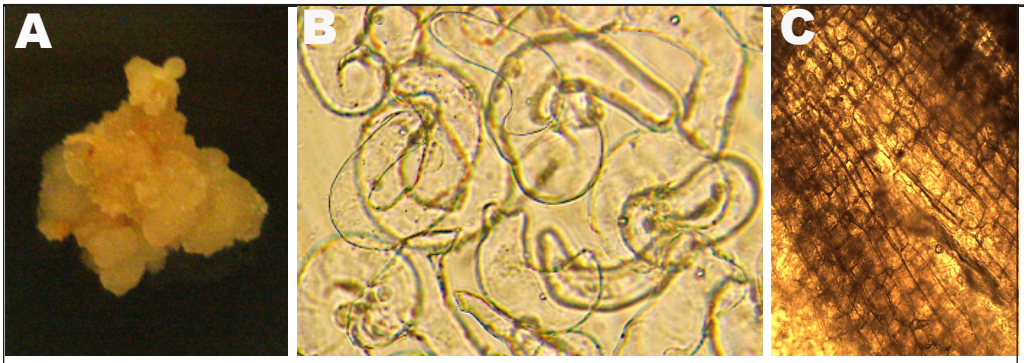

Fig. 1. Morpho-physiological characteristics of primary callus of wheat isogenic lines: A – callus general view, B – typical callus cells, and C – differentiation in callus tissue.

period of four weeks (mm^2/day). The maximum index for growth speed was detected in isoline *PPD B1a*, which also showed the maximum efficiency of callus genesis. Minimum values were found in Mironovskay 808.

Cytological investigations showed appreciable heterogeneity of callus tissues generated from mature germs. In addition to the typical callus cells of cereals, which are extended with round extremities, nontypical, bent cells, strongly vacuolated cells, meristematic cells, and large parenchymal cells were elements of differentiation (Fig. 1B and 1C).

The growth of primary callus tissues can occur either due to cell proliferation or to their growth by tension. Cytological results showed that among isolines, the minimum cell length and maximum quantity were detected in isoline *PPD B1a* (Table 2). Consequently, the growth of callus tissues of a given line seems to be defined only at the expense of their intensive division. Isoline *PPD D1a* had the opposite situation; a maximum cell size and a minimum number. We assume that the growth of primary callus in a given isoline is defined by the process of a vacuolization or growth by 'stretching'. In callus tissues of isoline PPD A1a, proliferation and vacuolization processes seems to be equal.

Table 2. Cytological characteristics of primary callus in NILs for *PPD* genes of the wheat cultivar Mironovskay 808.

Genotype	Cell length (μ)	Cell number (x 10^6/mg)
PPD D1a	167.1	2.0
PPD B1a	111.2	4.4
PPD A1a	113.9	1.9
PPD D1b PPD B1b PPD A1b	106.3	2.7
LSD 0.5	5.3	1.2

Biomass accumulation can be one indicator that characterizes the process of neoplasm in primary callus. Our results showed that the maximum biomass was detected in the primary callus of *PPD A1a* and the minimum in isoline *PPD B1a* (Table 3). Calculating callus tissue aqueousness showed that isoline *PPD B1a,* which accumulated the minimum biomass during the experiment also had the minimum aqueousness. The greatest aqueousness, 85.55%, was detected in isoline *PPD A1a*. Synthetic (metabolic) activity can aid in the maintenance of soluble protein in vegetative tissue. The fraction of soluble protein is mainly enzymes, which define metabolic activity. The maximum values were in isoline *PPD D1a* and *PPD A1*a callus. The callus tissue of these isolines was characterized by a minimum gain and maximum aqueousness. The genotype of a given isoline defines the fast transition from growth processes to develop the minimum gain and maximum synthetic activity.

Table 3. Morpho-physiological characteristics of primary callus from NILs for *PPD* genes of the wheat cultivar Mironovskay 808.

Genotype	Biomass (mg)		Aqueousness (%)	Protein (mg/g)
	crude	dry		
PPD D1a	45.75	6.50	84.38	5.70
PPD B1a	32.31	5.51	83.80	3.17
PPD A1a	47.02	7.25	85.55	8.84
PPD D1b PPD B1b PPD A1b	39.01	6.13	84.24	4.01
LSD0,5	6.30	0.80	0.35	2.20

Minimum maintenance of soluble protein fractions was shown for calluses of the isoline *PPD B1a*. Callus of this isoline were characterized by the minimum amount of crude and dry biomass accumulation, which also indicates a lower level of synthetic activity. Thus, our experiments showed that the genotype of an isoline determines photoperiodic sensitivity *in vivo* influences the callusogenesis processes and the characteristics of primary callus growth *in vitro*.

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ITEMS FROM THE UNITED STATES OF AMERICA

INDIANA

USDA–ARS AND PURDUE UNIVERSITY

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Wheat production. According to the USDA National Agricultural Statistics Service, harvested wheat acreage in Indiana in 2011 totaled 400,000 acres. Acreage seeded to wheat in fall of 2010 was 420,000 acres. Total production was estimated at 24.8 x 10^6 bushels, with an average yield at 59 bu/a. Winter survival of wheat during the winter of 2009–10 was excellent, but average temperatures from February to mid-April were significantly below normal and soil moisture was higher than normal due to frequent rainfall, resulting in delayed growth and development of wheat and limited uptake of nitrogen. Growth stage of wheat was 1 week later than normal at mid-April. However, from mid-April through June, temperatures were above normal and frequent rainfalls continued, so that wheat matured 1 week earlier than normal. Grain yields were below average, likely due to reduced plant development during the fall of 2009 and early spring of 2010.

Weather conditions were excellent for harvest of soybeans and corn in fall 2010, resulting in timely seeding of wheat and a return to typical acreage of wheat, estimated at 450,000 acres for the 2010–11 season. However, fall 2010 continued unusually dry throughout much of Indiana, especially southern Indiana; resulting in delayed and erratic emergence of wheat in some fields. Luckily there was good snow cover during cold weather periods resulting in little winterkill throughout Indiana, even given the late fall emergence and lack of wheat growth going into winter. Beginning in late January, the spring and summer through wheat harvest was unusually wet; and the unusually cool temperatures through April, together with the continually wet soil conditions resulted in loss of and limited uptake of nitrogen, causing