The 4-year average for the different fertilizer backgrounds indicated that the least total grain damage by cereal bug larvae was in the block with organic-mineral fertilizer, 5.6 %. Damage in the blocks without fertilizer and with only organic fertilizer was 5.9 and 6.0 %, respectively. Total damage was 4.2–4.9 % in seed with a score of 2, which corresponded to 75–83 units of gluten quality (IDG, 2nd group). A reduction in grain damage, from 6.0 to 5.6 %, contributed to a reliable increase in crude gluten content in flour between 29.4 and 31.4 (LSD_{05} = 1.15 %), and protein content in the grain from 12.86 to 13.41 %, bread volume/100-g flour from 525 to 553 ml, and total bread-making estimate from 3.7 to 4.1 score. These analyses showed a negative correlation between the indices of grain quality and damage by the cereal bug (r = -0.8). However, higher gluten quality was found in grain from the organic fertilizer treatment, 83 alveograph units compared to 76 units for the block without fertilizer and 75 units for the block with organic-mineral fertilizer. Winter wheat grain grown without fertilizer and with organic fertilizers corresponded to the third class and that in the block with organic-mineral fertilizer to the 2nd class. On average, during 2001–05, the maximum grain yield was obtained in the organic-mineral fertilizer treatments, 6,44 t/ha, out-yielding the treatment without fertilizer by 0.43 t/ha (at LSD_{05} = 0.23 t/ha).

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Callus initiation and morphogenesis in in vitro culture of isogenic on gene type and rate of development in winter wheat lines.

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Methods of cultivating isolated cells, tissues, and organs for studying fundamental, plant physiology problems have found wide application in the development of unconventional approaches to various biological research areas and have a very wide spectrum of practical application (receiving of biologically active substances, transgeneration, selection, microcloning reproduction, and cryopreservation). The efficiency of cellular technology depends on many factors including the composition of nutrient medium, type of explant, age of a plant, and genotype (Machii et al. 1998; Stelmakh 1998; Tyankova and Zagorska 2001; Wang and Wei 2004). The search for genotypes with a high potential for callus formation and regeneration potential for the production of high-quality, fertile plant regenerants is a problem that depends on biotechnology (Tyankova and Zagorska 2001).

Systems of genetic monitoring of type (vernalization) and rates (photoperiod) developments determine a number of physiologico-biochemical processes of ability to vital activity of plants of wheat (Stelmakh 1998). These genetic systems also probably participate in the control of processes of callus initiation and morphogenesis *in vitro*.

Materials and Methods. Seven genotypes of soft winter wheat NILs for genes that control vernalization (*VRN1–VRN3*) and photoperiod *PPD1–PPD3*) were grown. The check cultivar Mironovskay 808 is completely recessive for all of these genes. Isogenic lines were produced by backcrossing with Mironovskay 808 by Stelmah (1998).

For production of callus and quality explants, we used the mature germ and apical meristems of aseptic roots. Seeds were sterilized in a 3% NaOCl solution for 15 min, washed for 5 min with sterile distilled water, and isolated germs transferred to a Petri dish with Murashige and Skoog medium (MS) with a full set of macro- and microsalts and containing 2,4 D (2 mg/l) as a growth regulator (Tyankova and Zagorska 2001). Explants were cultivated in the thermostat at 26°C in the dark. For apical root meristems, explants were grown for 4–5 days in on MS medium without phytohormone in the dark at 22°C. Isolated apical roots 1–1.5-cm long were transferred to MS medium with 2,4 D at 2 mg/l and cultivated in the dark at 26°C. At 14–21 days, explants isolated from apical meristems were sterilized with 3 % NaOCl solution for 15 min, washed 5 times in sterile distilled water, and placed on MS medium without phytohormone. Cultivation was at 22°C, with 3–4 lux of illumination and a 16-hour photoperiod at 70% humidity. For mature germ, seeds were sterilized and isolated germs were cultivated under the same conditions. The frequency callus induction and the efficiency of morphogenesis (%) was defined as the number of explants formed per callus or the number of plant regenerants to the initial number of explants. Results were from three independent experiments from not less than four Petri dishes or flasks (5–7 explants).

Results and Discussion. We investigated the influence of genotype on the efficiency of callus induction and morphogenesis *in vitro* on NILs for genes that control development (six lines and one cultivar that is recessive for all vernalization and photoperiod genes. We used mature germs and aseptic roots to produce primary callus. Mature germ is more effective at producing primary callus compared with apical meristems of aseptic roots.

All genotypes formed callus but with various frequencies (8–67%, Table 1). Using roots, the frequency of callus production was considerably below 20–30 %; line *PPD1* did not form callus in any experiment. Comparing the isolines for PPD and VRN, the PPD lines possess a greater potential for callus production. These lines differ in development; those with VRN are spring types and PPD and Mironovskay 808 (full recessive) are winter. Among the PPD lines, peak efficiency of callus production was found in line *PPD2* and the minimum in *PPD1*. Among the VRN lines, the minimum ability to generate callus was in line *VRN1* and the maximum in line *VRN3*.

Table 1. Callus initiation and morphogenesis in *in vitro* in NILs for the PPD and VRN genes of the wheat cultivar Mironovskay 808 (a cultivar fully recessive for genes PPD and VRN). Values expressed as frequency, % from number initial explants.

	Callus initiation		Morphogenesis *in vitro*	
Line	germ	roots	germ	apexes
Isogenic lines for PPD genes.				
PPD 1	8	0	75	31
PPD 2	67	30	50	13
PPD 3	58	20	100	50
Mironovskay 808	50	20	67	50
Isogenic lines for VRN genes.				
VRN 1	8	—	75	—
VRN 2	25	—	42	—
VRN 3	67	—	50	—
Mironovskay 808	50	—	67	—

Using various explants, we established differences between the types and rates of callus formation. Callus formation begins at apical sites in the roots 15–20 days earlier than in the mature germ. Differences were based on the degree of water and density to color of the callus. From aseptic roots, highly watery, friable, almost transparent, slightly whitish calli were obtained. From mature germ, the callus was more dense, less watery, and yellowish, which was characterized by differences confirmed by microscopic studies. Microscopically, callus tissue of the various isolines was shown to be typical for cereal callus cells; extended, with rounded ends, and not adjacent to each other. Cytological results showed that the various lines have calli that differ in sizes. The maximum length was in Mironovskay 808 and the minimum in cells of line PPD 3. Genotype influences the efficiency of callus formation and on the morphological features of cells of the callus tissues and also on their morphological potential.

Our results indicate that NILs for the *VRN* and *PPD* genes control the type and rate of development in wheat callus. More effective for growth *in vitro* are explants from mature germ, compared with those from apical meristems. Comparing the different isolines, those with *PPD* genes are easier to culture *in vitro* show higher morphogenetic potential than those with *VRN* genes. The maximum index of the efficiency of morphogenesis *in vitro* was for isoline PPD3 using mature germ and apical meristems. Among the VRN lines, peak regeneration efficiency *in vitro* was in line VRN1 and the minimum in isolines PPD2 and VRN2. Plants of line VRN2 have the longest period from shoot to heading. Plants of line PPD2 are the most sensitive to a short photoperiod and unfavorable day length conditions, followed by VRN2.

The efficiency of callus production and morphogenesis *in vitro* using mature germ to produce quality explants was shown. The processes of callus induction and morphogenesis depend on the genotype of the initial plant and are governed by different genetic systems. The maximum frequency of callus induction was in line PPD2 but also has the minimum indicators of efficiency of morphogenesis *in vitro*. Line VRN1 was shown to have maximum morphogenesis *in vitro* but the minimum frequency of callus generation. These results testify to the control of callus formation and morphogenesis by different, independent genetic systems. Genes that control type and rate of development, PPD and VRN, also help determine callus formation and morphogenesis *in vitro*.

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References.

Machii H, Mizuno H, Hirabayashi T, Li H, and Hagio T. 1998. Screening wheat genotypes for high callus induction and regeneration capability from anther and immature embryo cultures. Plant Cell, Tissue Organ Cult 53:67-74.

Stelmakh AF. 1998. Genetic systems regulating flowering in wheat. *In:* Wheat: Prospects for Global Improvement. Kluwer Academic Publishers, the Netherlands. Pp. 491-501.

Tyankova ND and Zagorska NA. 2001. Genetic control of in vitro response in wheat (*Triticum aestivum* L.). In Vitro Cell Dev Biol Plant 37(5):524-530.

Wang C and Wei Z. 2004. Embryogenesis and regeneration of green plantlets from wheat (*Triticum aestivum* L.) leaf base. Plant Cell, Tissue Organ Cult 77(2):149-153.

ITEMS FROM UNITED KINGDOM

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Genetic biodiversity for stripe and stem rust resistance in African wheat genotypes.

Lesley A. Boyd, Renee Prins, Zakkie A. Pretorius, and Ruth MacCormack.

A new program involves the genetic and phenotypic characterization of a large collection of African wheat genotypes for resistance to the new virulent stem rust *P. graminis* race Ug99. Stem rust resistance will be assessed in field trials in Kenya. The collection also will be assessed for resistance to stripe or yellow rust *P. striiformis* f.sp. *tritici* races in South Africa and the UK. DNA markers will be developed for useful sources of rust resistance and used as tools to determine the extent of biodiversity between the wheat genotypes. This program is a collaboration between Dr. L.A. Boyd at the JIC, UK, and Prof. Z.A. Pretorius and Dr. R. Prins at the University of the Free State, Bloemfontein, South Africa.

Publications.

Hysing SC, Hsam SLK, Singh RP, Huerta-Espino J, Boyd LA, Koebner RMD, Cambron S, Johnson JW, Bland DE, and Merker A. 2007. Agronomic performance and multiple disease resistance in T2BS·2RL wheat-rye translocation lines. Crop Sci 47:254-260.

Smith PH, Hadfield J, Hart NJ, Koebner RMD, and Boyd LA. 2007. STS markers for the wheat yellow rust resistance gene *Yr5* suggest a NBS-LRR-type resistance gene cluster. Genome 50(3):259-265.