

ITEMS FROM THE RUSSIAN FEDERATION

AGRICULTURAL RESEARCH INSTITUTE FOR THE SOUTH-EAST REGIONS
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Evaluating spring bread wheat introgression lines of the Genetics and Cytology Laboratory at ARISER for resistance to stem rust race Ug99 + Sr24 (TTKST).

S.N. Sibikeev and A.E. Druzhin.

In 2013, a set of introgression lines was evaluated for resistance to stem rust race Ug99 + Sr24 (TTKST) in the experimental fields at the Kenyan Agricultural Research Institute, Njoro, Kenya, under natural epidemic conditions of the pathogens. One part of this material has been repeatedly screened; the other part has passed an initial screening. Among the 20 samples from repeated screening, 12 had a resistant type of reaction. These resistant lines were NILs with the translocation combinations *Lr19/Sr25+Lr24/Sr24* and *Lr19/Sr25+Lr26/Sr31*, and also lines with translocations from durum wheat and *Ae. speltoides*. Among the lines from the initial screening, except for lines with the above-stated gene combinations, resistant lines with 6Agi (6D) substitutions and 6Agi (6D) + *Lr26/Sr31* and *Lr19/Sr25 + Lr37/Sr38* combinations were detected.

Destruction of winter wheat cultivars by viral diseases in the Lower Volga Region.

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Lately, the spread of viral diseases to wheat is increasing in the Lower Volga Region, which is characterized by unusual injuries, especially in epidemics. The most common viral disease is winter wheat mosaic virus (WWMV), which damages both spring and winter wheat, rye, barley, panic grass, oats, and many other wild cereals. The symptoms of disease become visible in the autumn. Chlorotic spots appear on the leaves of damaged plants, which become yellow after time and then merge into lengthwise stripes. Winter wheat is bushing out redundantly with rosette. The most vivid symptoms are seen at the end of May and the beginning of June at spike formation stage as different degrees of bushiness, lagging growth, and some plant death.

The virus is vectored by the striped (*Psammotettix striatus* Fall.) and six-point (*Macrosfeles laevis* L.) leafhoppers. Both types overwinter in the egg stage on newly sown winter wheat. In autumn, especially in dry weather, the young, growing wheat leaves are inhabited by many types of leafhoppers; 40–50% of the general quantity. The probability of plant infection increases during a long, warm autumn, because species containing the virus often change place while feeding. In such cases, the loss of harvest from WWMV can reach 15–20%.

In 2010–12, we assessed the spread of winter wheat in the Lower Volga Region damaged by viral diseases. The results showed that highly resistant cultivars were not damaged by viral disease. The degree of damage depended on infection background strength. Saratovskaya 8, Saratovskaya 90, Levoberezhnaya 1, and Donskaya Awnless had a high percent damage (25–30% and more) due to viral disease spread; Lutescense 230, Guberniya, Mironovskaya 808, and Pearl of Volga Region were stable in 2010 and 2011 with moderate damage. In those years, damage did not exceed 5–10%. Victoria 95 and Smuglyanka were resistant during three years of research; only 1–5% was damaged. These conclusions suggest optimism in the direction of selecting cultivars resistant to viral disease.

Identifying wheat–*Aegilops columnaris* lines with addition, substitution, and translocation chromosomes.

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During 2007–12, *Ae. columnaris* (UX, 2n=28) was crossed with the spring bread wheat cultivars Saratovskaya 68, L503, and Dobrynya, and backcrossed 2–4 times with the cultivar recipients. Among the sets of wheat–*Ae. columnaris* lines, we observed lines with club ears, hairy ears, light green plants, reduced plant height, and resistance to leaf rust and powdery mildew. The C-banding pattern of these lines showed the addition lines with 2X and 6X chromosomes; substitution lines with 3U (3D), ? (5D) + ? (6D); monosomic 6X (6D); translocation T4BS-4BL-?L plus two additional 6X chromosomes; and the translocation T1DS-1DL-?L. In the future, studying these lines for agronomic performance and analyzing C-banding patterns will continue.

Effect of drought conditions on the change in wheat loose smut populations.

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We began to identify wheat loose smut races in the Laboratory of Genetics and Cytology in 2000. For differential fungus races, we used a Canadian test-cultivar set. In 2001, we found that race 18 was the predominant race in the local wheat loose smut populations. Further studies have shown that the local population of loose smut includes six races (Table 1). These races could not be identified from a key of known races. We found that the more virulent races of the pathogen in a population usually appear after dry years. Races I-S36 and I-S66 were identified after 2007, which was characterized as dry, and race XX was identified after the drought of 2010. Analysis of the data indicates that the dry conditions are more favorable for formation of new, more virulent races in the loose smut population.

Table 1. Reaction of different cultivars to loose smut of wheat (S = susceptible).

Cultivar differentiator	Year						
	2001	2005	2008	2008	2009	2009	2011
	Race 18	Race I-S60	Race I-S36	Race I-S66	Race I-SZ	Race I-UV2	Race XX
TD-1							
TD-2							
TD-3					S		
TD-4	S	S	S	S	S	S	S
TD-5	S	S	S	S		S	S
TD-6							
TD-7	S	S	S	S	S	S	S
TD-8	S	S	S	S	S	S	S
TD-9	S	S	S	S			S
TD-10							
TD-11							
TD-12				S			S
TD-13	S	S	S	S	S	S	S
TD-14							
TD-15				S		S	S
TD-16			S	S			
TD-17							
TD-18							
TD-19							

Morphological-anatomical changes in somatic wheat calli in vitro under the effect of bacterial lipopolysaccharide.

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We examined the effect of the lipopolysaccharide (LPS) of the associative, plant-growth-promoting bacterium *Azospirillum brasilense* Sp245 on the morphogenetic parameters of somatic tissues of spring wheat *in vitro*. For this purpose, we used a genetic model including two near-isogenic lines of wheat cultivar Saratovskaya 29 that differed in the *RhtB1c* gene and had contrasting embryogenic capacities. The work was conducted in accordance with the classic scenario of cellular transformations in a culture *in vitro* of immature (14-day-old) wheat embryos by way of indirect somatic embryogenesis. The control was a Linsmaier–Skoog medium containing 2 mg/L of 2,4-dichlorophenoxyacetic acid (2,4-D). In the experimental treatments, the standard medium, after being autoclaved, received 10 µg/mL of LPS isolated from the bacterial outer membrane. The resultant calli and regenerated plants were analyzed by morphometric and morphological-anatomical analysis. On day 30 of cultivation, analysis of the calli showed that in all experimental treatments, there was active callusing of somatic cells of the immature embryos. The callusing effectiveness was high (close to 100%) and did not depend on the genotype or the presence of LPS in the nutrient medium.

Yet in both lines, the formation of morphogenic calli in the control was less than 20% of the number of explants used. On the whole, the addition of LPS to the medium increased the activity of morphogenetic processes. Particularly noticeable was an increase in the yield of calli with meristematic activity loci in the tall line in the presence of 10 µg/mL of LPS in the nutrient medium. Consequently, the morphogenic potential of the calli of the lines under study leveled off. The yield of regenerated plants was influenced profoundly by the explant genotype. The regeneration capacity of the dwarf line was greater than that of the sister line, a fact evident in the control plants. In the experimental treatments, the yield of regenerated plants increased in the tall line under the effect of LPS. Morphological-anatomical analysis confirmed the positive effect of the *RhtB1c* gene at all stages of tissue culturing *in vitro*. LPS was found to promote the secondary differentiation of callus cells and the formation of morphogenesis loci as early as day 7 of embryo culturing, regardless of the genotype. Meristematic activity was observed mostly in the nodal plate, vascular bundles of the scutellum, and coleoptile of the embryos.

On the basis of this data, we established that the LPS of the associative bacterium *A. brasilense* Sp245 promotes the secondary differentiation and the regeneration capacity of wheat callus cells, thereby improving the effectiveness of culturing of genotypes with low embryogenic potential. A nutrient-medium concentration of LPS of 10 µg/mL has the greatest physiological activity toward callus cells. These results make possible a deeper understanding of the mechanisms of morphogenesis in tissue culture and can be used to increase the embryogenic potential of plant objects, specifically wheat, in an *in vitro* culture.

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The content of free amino acids and water-soluble carbohydrate in leaves of isogenic by VRN gene lines of wheat.

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Abstract. In a three-year experiment, we observed the effects of *VRN* genes in soft wheat on the development rate (shoot–booting period), content, and distribution of free amino acids and water-soluble carbohydrates in isogenic lines of Mironovskaya 808 and Olviya. Slowly developing isolines are characterized by a lesser amount of free amino acids and monosaccharides in the leaves. The correlation index C/N clearly correlates with the rates of the isoline development. We propose that *VRN* genes are involved in synthesis control, composition, and distribution of the basic photoassimilates of soft wheat.

Introduction. The transition of wheat plants from vegetative to generative is a major stage in the ontogeny, determining many economically valuable traits. The key genes in this process are the *VRN* genes, which define the reaction of wheat to vernalization and, thus, determine the rate of development. The reaction to vernalization in wheat is controlled by at least five genes, three of which, *Vrn-A1a*, *Vrn-B1a*, and *Vrn-D1a*, are localized on chromosomes 5A, 5B, and 5D, respectively. Winter-type plant development is manifested only when all three genes are recessive. If at least one of these genes is dominant, then the plants are spring type. *VRN* genes have been actively researched at the molecular-genetic level (Loukoianov A et al. 2005; Oliver SN et al. 2009) and have been cloned and several allelic variants for wheat described (Preston JC et al. 2009). Genes *Vrn-A1a* and *Vrn-B1a* are transcription factors (Distelfeld A et al. 2009; Trevaskis B 2010). These studies have deepened the notion of genetic and molecular-biological control of wheat development.

The primary products of photosynthesis are soluble carbohydrates and free amino acids. Their content, correlation, and distribution between the main organs, in many ways, define the rate of plant development, productivity, and protein content in grain. At present, nitrogen and carbonic metabolism of cereals is intensively studied, including the distribution of metabolites in plant organs and aspects of their reutilization of photoassimilates during grain formation (Amane M 2011), the connection between photosynthesis and general productivity (Champigny M-L 1995), and the increase of valuable proteins in grain (Marte P et al. 2003). Coruzzi and Bush (2001) have shown that low-molecular-weight nitrogen and carbohydrate compounds in plants in nature are not just metabolites, but signal molecules that activate and/or repress many genes.

Perhaps, the *VRN* genetic system, which determines the rate of plant development, reveals the mediated effects on the content of basic metabolites (photoassimilates) of the whole plant, their distribution to the organs of the main shoot, and the correlation of carbohydrate and nitrogenous metabolites. Our investigation researched the content and distribution, by plant organ, of the carbohydrates and amino acids in connection with the rate of wheat development.

Materials and methods. A proper study of the physical-biochemical process is possible only using near isogenic lines (NILs), which have minimal differences in all the genes besides the marked specific feature. We used NILs that were monogenic dominant for the *VRN* genes of soft wheat, which differed in their rate of development. These lines were created in the background of winter wheat cultivars Mironovskaya 808 (tall) and Olviya (short). Field experiments were made during 2010–12 at experimental plots of the Department of Physiology and Biochemistry, V. N. Karazin Kharkov National University. Observations were made on the duration of the shoot-to-boot period and biochemical analyses of the content of free amino acids in the different plants organs and the fractional composition of reduced saccharides in the leaves during the of booting–flowering period.