

***Haploid plants production in triticale-wheat hybrids.***

O.V. Khomyakova, V.N. Anikina, T.I. Dyatchouk, S.V. Stolyarova, Yu.V. Italianskaya, N.F. Safronova, and L.P. Medvedeva.

Triticale-wheat hybrids developed using modern local wheat and rye cultivar are a valuable, initial breeding stock with introgressions of D-genome genetic material. Plants in the  $F_3$ - $F_4$  of two selfed hybrids were used for haploid plant production using anther culture. An induction medium with sucrose, maltose, and 2 mg/L 2,4-D was used to obtain haploid embryo-like structures. Responding anthers were transferred for callus development on a regeneration medium with 2% sucrose and 1 mg/L IAA. The number of green and albino plants was counted after about 30 days depending on plant development. Well-rooted regenerants were subjected to colchicine treatment.

Our results did not confirm the role of a cold pretreatment of the donor spikes prior to culturing as a trigger for sporophytic microspore development. Altogether, 128 viable green plants and 104 androgenetic albino plants were obtained from 527 embryo-like structures. The frequency of embryogenic anthers (the number of embryogenic anthers/100 anthers) was 8.9–10.5%. The rate of embryo-like structures (the number of embryo-like structures/100 anthers) was 15.5–15.7%. Molecular techniques for DNA, storage protein analysis, and FISH will be used to identify alien chromosome insertions or substitutions in the callus. The DH lines will be multiplied and investigated for resistance to biotic and abiotic stresses.

**INSTITUTE OF BIOCHEMISTRY AND PHYSIOLOGY OF PLANTS AND  
MICROORGANISMS, RUSSIAN ACADEMY OF SCIENCES  
13 ENTUSIASTOV AVE., SARATOV 410049, RUSSIAN FEDERATION.**

***Physiological-morphological changes in wheat seedlings inoculated with Azospirillum bacteria.***

N.V. Evseeva, L.Yu. Matora, G.L. Burygin, and S.Yu. Shchyogolev.

The physiological-biochemical bases for the functioning of plant-microbial symbioses is a topical problem in current agrobiology. With the known positive effects of interaction between the macro- and micropartners in symbioses, little attention has been paid to the functioning of root apical meristems, which serve as the formative and regulatory centers in the plant host (Ivanov 2004) and are a major site for the localization of associated bacteria (Bashan and Levanyon 1989). We have investigated the mitotic activity of root meristem cells and the morphological parameters of wheat (cv. Saratovskaya 29) seedlings after root inoculation with the associative bacteria *Azospirillum brasilense* Sp7 and Sp245.

Etiolated, 3-day-old wheat seedlings were inoculated for 24 h in suspensions of *A. brasilense* Sp7 and Sp245 and the enterobacterium *Escherichia coli* K12 (cell density, 108 cells/ml). Other seedlings were treated with *A. brasilense* Sp245 prefixed with 2% glutaraldehyde. After inoculation, the seedlings were placed in water. The control was uninoculated plants grown in hydroponic culture. Samples were taken 2 days after inoculation. The functional activity of the root meristem cells was assessed by using two parameters: (1) determining the cell mitotic index and (2) comparative estimates of the content of the proliferative antigen of initials (PAI), a molecular marker for wheat meristem cells (Evseeva et al. 2002). To determine the mitotic index, root apex meristems were fixed in acetic acid-ethanol (1:3), stained with acetohematoxylin, macerated with cytase enzyme, and visualized at 400 $\times$  magnification. PAI was revealed by enzyme immunoassay by using rabbit monospecific anti-PAI antibodies.

Inoculating wheat seedlings with live *Azospirillum* cells led to an approximately 2-fold increase in the mitotic activity of the root meristem cells and to an almost 1.5-fold increase in the PAI content in these cells. The effect of strain Sp245 did not differ essentially from that of strain Sp7. Shoot length increased by 30–40% and root length increased by 20–30%. The treatment of the seedlings with glutaraldehyde-fixed *A. brasilense* Sp245 did not substantially change the values for mitotic index of the root meristem cells, PAI content, or the morphological parameters. Our data agree with

those of Bashan et al. (1986), who showed that heat-killed azospirilla lose their adsorption ability, which indicates that active bacterial metabolism is needed for bacterial attachment to roots. *E. coli* K12 did not have a significant growth-promoting effect on wheat seedlings. The inoculation-induced enhancement of mitotic activity in root meristem cells is probably the main cause for the increase in the morphological parameters, although our results may indicate that the change in PAI content in root meristems is a parameter that characterizes the effectiveness of plant interactions with the soil microflora.

**References.**

Bashan Y and Levanony H. 1989. Factors affecting adsorption of *Azospirillum brasilense* Cd to root hairs as compared with root surface of wheat. *Can J Microbiol* 35:936-944.  
 Bashan Y, Levanony H, and Klein E. 1986. Evidence for a weak active external adsorption of *Azospirillum brasilense* Cd to wheat roots. *J Gen Microbiol* 132:3069-3073.  
 Evseeva NV, Tkachenko OV, Lobachev YuV, Mitrophanov AYu, Djatchouk TI, and Shchyogolev SYu. 2002. Studies on the embryogenic processes in the *in vitro* culture of wheat somatic tissues by using a proliferative antigen of initial cells. *Wheat Inf Serv* 94:1-4.  
 Ivanov VB. 2004. Meristem as a self-organizing system: maintenance and restriction of cell proliferation (review). *Rus J Plant Physiol* 51(6):926-941.

**PRYANISHNIKOV ALL  
 RUSSIAN RESEARCH  
 INSTITUTE OF  
 AGRICULTURE AND SOIL  
 SCIENCE  
 Pryanishnikova, 31. Moscow  
 127550, Russian Federation.**

***Growth activation testing of wheat cultivars for aluminum toxicity.***

N.V. Poukhalskaya, S.L. Ignatyeva, and N.I. Pavlova.

The toxic influence of Al ions on plants is real and urgent in breeding Al-resistant cultivars. Al-tolerance investigations have been conducted in solutions with 10, 30, 50, and 200 μM Al all over the world. Growth and changes in plants processes were researched in the investigations. At the genomic level, some cultivars have tolerance genes to Al ions, but the toxic effects of such ions appears in root shortening and chromosome aberrations.

At the same time, we discovered that Al ions cause root shortening as often as not and sometimes cause forcing of the vegetative part of wheat germ. Leaf length and area in several cultivars increase considerably Al (Tables 1 and 2). The length of the Lada seedlings in the Al variant increased by 74.74% over the control by the 12th growth day. Leaf area also increased in cultivars Voronezhskaya by 31.1 %, Omskaya 24 by 16.4%, and Kerba by 11.6% over the control. Stimulation of leaf growth could be caused by early sensitivity to Al toxicity.

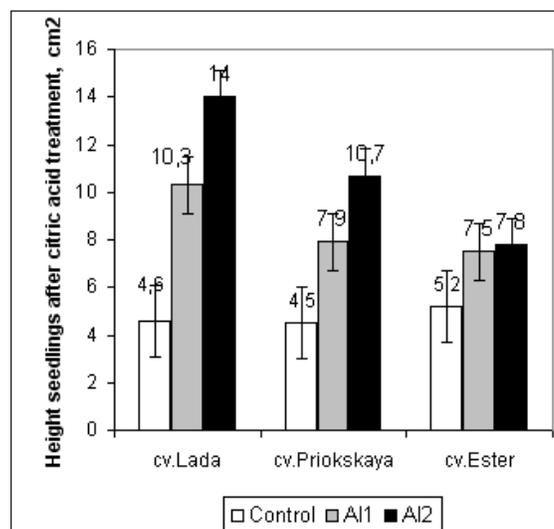
The Al solution stimulated leaf growth but to different degrees among the three cultivars tested (Fig.1). The greatest

**Table 1.** Increase of the vegetative part (cm) of the spring wheat cultivar Lada with the addition of Al (3 mg/L) into soil and solution.

Variant	Days-after-germination			
	6	8	10	12
Control	1.09 ± 0.35	1.76 ± 0.84	2.43 ± 0.81	3.16 ± 0.79
AlCl <sub>3</sub>	1.63 ± 0.50	3.49 ± 1.45	4.67 ± 1.25	5.52 ± 1.29

**Table 2.** Increase in the leaf area (cm<sup>2</sup>) of spring wheat cultivars with the addition of Al (0.72 mg/10 g soil) into soil and solution.

Variant	Cultivar		
	Voronezhskaya 14	Omskaya 24	Kerba
Control	51.98 ± 6.81	38.71 ± 7.65	38.18 ± 3.87
AlCl <sub>3</sub>	68.16 ± 14.84	45.07 ± 6.06	42.62 ± 3.78



**Fig. 1.** Area of assimilating leaf surface (cm<sup>2</sup>) of the spring wheat cultivars Lada, Priokskaya, and Ester after a preplanting seed treatment with citric acid.