

VAVILOV SARATOV STATE AGRARIAN UNIVERSITY
1 Teatrnaya Ploshchad, Saratov 410012, Russian Federation.

**INSTITUTE OF BIOCHEMISTRY AND PHYSIOLOGY OF PLANTS AND
MICROORGANISMS**
**Russian Academy of Sciences, 13 Prospekt Entuziastov, Saratov 410049, Russian
Federation.**

***Influence of bacterial lipopolysaccharide on the morphogenetic and morphometric parameters of
cultivation of wheat somatic callus.***

Yu.V. Lobachev and O.V. Tkachenko (Vavilov Saratov State Agrarian University) and L.Yu. Matora, G.L. Burygin, and N.V. Evseeva (Institute of Biochemistry and Physiology of Plants and Microorganisms).

A major challenge in cultivating plants is ensuring that the cells preserve their morphogenetic potential. The capacity of plant explants for morphogenesis depends on the plants' genotypic peculiarities and on nutrient-medium composition and culture conditions (Yezhova 2003). In addition, plant-associated methylobacteria promote accelerated seed germination and the further seedling growth *in vivo* (Fall 1996), and they also stimulate plant growth and morphogenesis *in vitro* (Kalyayeva et al. 2001). N_2 -fixing bacteria promote plant growth only *in vivo* (Steenhoudt and Vanderleyden 2000). Inoculation *in vitro* of plants with these bacteria is technically difficult (Korzhenevskaya 1990). In this context, it is important to treat explants not with a bacterial suspension but with bacterial-cell components that determine the plant-bacterium interaction. The outer-membrane lipopolysaccharide (LPS) of N_2 -fixing bacteria of the genus *Azospirillum* is an active bacterial component that not only determines contact bacterium-plant root interactions (Fedonenko et al. 2001) but also is involved in the processes inducing plant responses to these interactions (Matora et al. 1995). The aim of this work was to examine the influence of LPS on the morphometric and morphogenetic parameters of cultivation of wheat somatic callus.

Immature embryos of two model near-isogenic lines (genetic background of cultivar Saratovskaya 29), differing in the *Rht-B1c* alleles were placed on a callus-initiation nutrient medium that contained 2.5, 5, and 10 mg/l LPS. The resulting morphogenic callus were transferred to a regeneration medium with the same LPS content. A study of callus initiation and the cultures' regeneration ability showed that the LPS at the concentrations used did not have a significant effect on the formation of morphogenic callus or on the ability of the *Rht-B1c* gene to increase this parameter, found by us previously (Tkachenko and Lobachev 2008). LPS slightly increased the mass of morphogenic and nonmorphogenic callus. The regeneration ability of the callus and the dynamics of formation of regenerated plants did not change in the presence of the LPS. In summary, the LPS at 2.5, 5, and 10 mg/l did not have a significant effect on the morphogenetic parameters of *in vitro* cultivation of wheat somatic cells. A search further for effective concentrations or for a method of introducing LPS into a nutrient medium for cultivation of wheat somatic embryos will be necessary.

References.

- Ezhova TA. 2003. Genetic control of totipotency of plant cells in an *in vitro* culture. *Rus J Devel Biol* 34(4):197-204.
- Fall R. 1996. Cycling of methanol between plants, methylotrophs and atmosphere. In: *Microbial Growth on C₁ Compounds* (Lidstrom ME, Lidstrom FR, and Tabita FR, Eds). Kluwer Acad Publ, Dordrecht, the Netherlands. Pp. 343-350.
- Fedonenko YuP, Egorenkova IV, Konnova SA, and Ignatov VV. 2001. Involvement of the lipopolysaccharides of *Azospirilla* in the interaction with wheat seedling roots. *Microbiology (Moscow)* 70(3):329-334.
- Kalyaeva MA, Zacharchenko NS, Doronina NV, Rukavtsova EB, Ivanova EG, Alekseeva VV, Trotsenko YuA, and Bur'yanov YaI. 2001. Plant growth and morphogenesis *in vitro* is promoted by associative methylotrophic bacteria. *Rus J Plant Physiol* 48(4):514-517.
- Matora LYu, Solovova GK, Serebrennikova OB, Selivanov NYu, and Shchyogolev SYu. 1995. Immunological properties of *Azospirillum* cell surface: The structure of carbohydrate antigens and evaluation of their involvement in bacteria-plant contact interactions. In: *Azospirillum VI and related microorganisms: Genetics, physiology, ecology* (Fendrik I, del Gallo M, De Zamaroczy M, and Vanderleyden J, Eds). Springer, Berlin. Pp. 377-382.
- Steenhoudt O and Vanderleyden J. 2000. *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetics, biochemical and ecological aspects. *FEMS Microbiol Rev* 24:487-506.

Tkachenko OV and Lobachev YuV. 2008. Using isogenic analysis to study genotype effect in in vitro cell and tissue culture of wheat. *Ann Wheat Newslett* 54:122.

Influence of bacterial lipopolysaccharide on the functional activity of wheat-seedling-root meristems.

N.V. Evseeva, L.Yu. Matora, G.L. Burygin, and S.Yu. Shchyogolev (Institute of Biochemistry and Physiology of Plants and Microorganisms).

During the past several decades, plant-growth-promoting rhizobacteria of the genus *Azospirillum* have been used as a model object for study of plant-microbial associativeness owing to their abilities to fix atmospheric nitrogen, to synthesize phytohormones, and to influence plant water status and also owing to other positive factors. In studying associative symbioses, it is important to reveal the associated partners' active components that characterize the effectiveness of this interaction. The outer-membrane lipopolysaccharide (LPS) of gram-negative bacteria of the genus *Azospirillum* has an important role in the formation of associative bacterium-cereal root interaction. In particular, *Azospirillum* LPS induces specific deformation of the wheat-seedling root-hairs, as happens in the presence of whole bacterial cells (Fedonenko et al. 2001). In addition, *Azospirillum* LPS causes an increase in the synthesis of major proteins in the wheat-root cell apoplast, comparable with the action of intact bacterial cells (Matora et al. 1995).

Currently, few data exist on the functional activity of plant-root apical meristems during plant interaction with the associated micropartners, although it is these organs that serve as formative and regulatory centers in the host plant and are a major site of localization of associative bacteria. This work examined the functional activity of wheat-seedling-root meristems during treatment with the LPS isolated from the outer membrane of *A. brasilense* strain Sp245, as compared with inoculation with whole bacterial cells.

Etiolated 3-day-old wheat seedlings were incubated for 24 h either in a solution of 10 mg/l LPS or in a bacterial suspension (cell density, 10^9 cells/ml). The control was noninoculated plants grown in water culture. Samples were taken at 2 days after inoculation. The functional activity of the seedling-root-tip meristem cells was assessed by using two parameters: (1) the results of determination of the cells' mitotic index and (2) comparative estimation of the content of the proliferative antigen of initials (PAI) – a molecular marker of wheat-meristem cells (Evseeva et al. 2007). For determination of the mitotic index of the root-apex meristematic cells, the material was fixed in acetic-acid-ethanol (1:3), stained with acetohematoxylin, macerated with the cytase enzyme, and visualized at 400X magnification. PAI was revealed with an immunochemical test-system developed by us on the basis of the enzyme immunoassay using rabbit monospecific anti-PAI antibodies.

Inoculation of the wheat-seedling roots with whole bacterial cells led to a 2-fold increase in the root-cell mitotic index and to an approximately 1.5-fold increase in PAI content in the cells, as compared with the noninoculated plants. When the wheat-seedling-root system was treated with the isolated LPS, the mitotic index of the root-meristem cells was increased 2.4-fold and PAI content was increased 1.4-fold.

In summary, the increase in PAI content recorded after root inoculation with whole bacteria and also after root treatment with the isolated bacterial LPS is associated with the fact that the cell divisions in the root meristems of inoculated plants proceed more intensively. This possibly facilitates the formation of new adventitious roots and leads to the well-known growth-promoting effects exerted by associative bacteria. Possibly, LPS may be considered to be an active component of the *Azospirillum* cell surface that determines contact bacterium-wheat root interactions and also is involved in the processes inducing plant responses to these interactions.

References.

- Evseeva NV, Tkachenko OV, Lobachev YuV, Fadeeva IYu, and Shchegolev SYu. 2007. Biochemical evaluations of the morphogenetic potential of wheat callus cells *in vitro*. *Rus J Plant Physiol* 54(2):273-277.
- Fedonenko YuP, Egorenkova IV, Konnova SA, and Ignatov VV. 2001. Involvement of the lipopolysaccharides of *Azospirilla* in the interaction with wheat seedling roots. *Microbiology (Moscow)* 70(3):329-334.

Matora LYu, Solovova GK, Serebrennikova OB, Selivanov NYu, and Shchyogolev SYu. 1995. Immunological properties of *Azospirillum* cell surface: The structure of carbohydrate antigens and evaluation of their involvement in bacteria-plant contact interactions. In: *Azospirillum* VI and related microorganisms: Genetics, physiology, ecology (Fendrik I, del Gallo M, De Zamaroczy M, and Vanderleyden J, Eds). Springer, Berlin. Pp. 377-382.

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UNIVERSIDAD POLITÉCNICA DE MADRID

Departamento de Biotecnología, E.T.S.I. Agrónomos.- C. Universitaria, 28040, Madrid, Spain.

A. Delibes, I. López-Braña, and E. Simonetti.

UNIVERSIDAD DE LLEIDA

Departamento de Producción Vegetal y Ciencia Forestal, Institut de Recerca i Tecnologia Agroalimentaries (UdL-IRTA), Rovira Roure, 191-25198 Lleida, Spain.

J.A. Martín-Sánchez and E. Sin.

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS

Departamento de Protección Vegetal, Centro de Ciencias Medioambientales, Serrano, 115, 28006, Madrid, Spain.

M.F. Andrés.

Localization of two class-III peroxidase genes expressed in the roots of a *Heterodera avenae*-resistant wheat line.

The cereal cyst nematode is a pest that seriously affects cereal crops in many of the world's wheat-growing areas. The *H. avenae* resistance gene *Cre2* from *Ae. ventricosa* present in the *Ae. ventricosa*/wheat introgression line H-93-8, was shown to confer a high level of resistance to the Spanish pathotype Ha71 (Delibes et al. 1993). The infection of H-93-8 line with *H. avenae* resulted in a hypersensitive reaction, with syncytial cells deteriorating in a few days. Following nematode infection, peroxidase, esterase, and superoxide dismutase activities increased in H-93-8 roots compared with the parental, susceptible cultivar Almatense, H-10-15 (Andrés et al. 2001). Twenty peroxidase genes were characterized from 211 ESTs and 259 genomic DNA clones of this resistant line. The alignment of deduced amino-acid sequences and phylogenetic clustering with peroxidases from other plant species showed that these enzymes fall into seven different groups (designated TaPrx108 to TaPrx114) that represent peroxidases secreted into the apoplast by a putative N-terminal peptide signal. The expression levels of groups TaPrx112 and TaPrx113 in roots of the H-93-8 resistant line increase in response to nematode infection. The maximum peroxidase levels were reached four days post-inoculation. Moreover, the expression of groups TaPrx112 and TaPrx113 always was much higher in H-93-8 line (4- and 100-fold, respectively) than in their susceptible parental. This fact may be related to a constitutive mechanism of defense in this resistant line. The chromosomal assignment of peroxidases of both groups was done using Sears' aneuploid wheat lines (Sears 1954; Kimber and Sears 1968) and PCR-specific primers from peroxidases. Two PCR fragments obtained from peroxidases TaPrx112-F and TaPrx113-F were absent in nulli-tetrasomic and ditelosomic lines N2BT2D and Dt2BL, respectively. Therefore, both peroxidase genes would be located in 2B short arm chromosome of wheat.