

Bacterial lipopolysaccharides in a culture of wheat calli.

O.V. Tkachenko and Yu.V. Lobachev; and L.Yu. Matora, N.V. Evseeva, V.V. Dmitrienko, G.L. Burygin, and S.Yu. Shchyogolev (Institute of Biochemistry and Physiology of Plants and Microorganisms Russian Academy of Sciences, 13 Entusiastov Ave., Saratov 410049, Russian Federation).

For several years, we studied the influence of outer-membrane lipopolysaccharide (LPS) in the nitrogen-fixing bacterium *Azospirillum brasilense* Sp245 on the cultivation of somatic wheat calli *in vitro*. Our working hypothesis for the possible effect of LPS on callus cells was based on their known ability to induce plant cell responses *in vitro* (Evseeva et al. 2011).

LPS at 1, 10, and 100 $\mu\text{g/mL}$ was included in the composition of a nutrient medium for callus initiation from immature embryos of two model near-isogenic soft spring wheat lines (genetic background of cultivar Saratovskaya 29) differing in the *Rht-B1c* gene. The resultant morphogenic calli were transferred to a regeneration medium with the same LPS content.

No direct effect of the LPS on cellular proliferation was found, because callus formation occurred at a frequency close to 100% in all treatments. Likewise, no differences in callus weight were observed. LPS is known to stimulate the process of secondary differentiation of callus cells and the formation of morphogenic loci. In the line with the *Rht-B1c* gene, whose morphogenic ability is greater than that of the sister line (Tkachenko and Lobachev 2008), the yield of morphogenic calli was found to increase in all treatments involving LPS. Yet the morphogenesis ability of the sister line increased significantly only on induction medium containing 1 $\mu\text{g/mL}$ of LPS. During plant regeneration, no significant effect of LPS on the growth or weight characteristics of shoots has been recorded. According to our experimental data, the measures of shoot regeneration from morphogenic calli were associated with the genotype of the donor plants to a greater extent than they were with the LPS content in the nutrient medium.

In summary, the effect of *Azospirillum* on plant cells can be determined by components of the bacterial outer membranes (in this case, LPS). LPS not only can promote the mitotic activity of plant cells (which has been found in experiments *in vivo*) but also can regulate the differentiation of proliferating cells *in vitro*.

References.

- Evseeva NV, Matora LYu, Burygin GL, Dmitrienko VV, and Shchyogolev SYu. 2011. Effect of *Azospirillum brasilense* Sp245 lipopolysaccharide on the functional activity of wheat root meristematic cells. Plant Soil 346(1-2):181-188.
- Tkachenko OV and Lobachev YuV. 2008. Using isogenic analysis to study genotype effect in *in vitro* cell and tissue culture of wheat. Ann Wheat Newslet 54:122.