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SHORT COMMUNICATIONS

# Assessment of the Effect of Azospirilla Lectins on c-AMP Level in Plant Cells

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The search for the receptor structures of the micro- and macropartners and study of responses developing in the course of associative interactions are an important area of investigation in molecular mechanisms of the functioning of plant–microbial associations. In the wheat–*Azospirillum* association, bacterial lectins are of interest, due to their known informational functions in various biological systems [1].

The lectin previously isolated from the surface of *A. brasilense* Sp7 cells was a 36-kDa glycoprotein with specificity to *L*-fucose and *D*-galactose [2]. A mutant strain was obtained, with the lectin exhibiting structural and functional differences with the lectin of the parent strain [3]. The lectins of azospirilla, together with other surface structures, were shown to participate in adhesion (attachment of bacteria to plant roots)[4] and, moreover, affect the metabolism of plant cells. For example, they stimulate seed germination and exhibit mitogenic and enzyme-modifying activity against the plant cell [5, 6]. The issue of involvement of adenylate cyclase signal system in the changes in plant cell metabolism induced by azospirilla lectins remains unclear. The adenylate cyclase signal system is known to play an important role in the functional and structural responses of plant cells to a number of biotic and abiotic external factors. Two enzymes, adenylate cyclase and phosphodiesterase, are important components of this system responsible for cAMP production and decomposition [7]. Calcium ions are known to have both a stimulatory and inhibitory effect on the activity of these enzymes [8].

The goal of the present work was investigation of the effect of *A. brasilense* Sp7.2.3 lectins on the dynamics of cAMP content in the root cells of the seedlings of Saratovskaya 29 wheat and assessment of the effect of exogenous calcium on this process.

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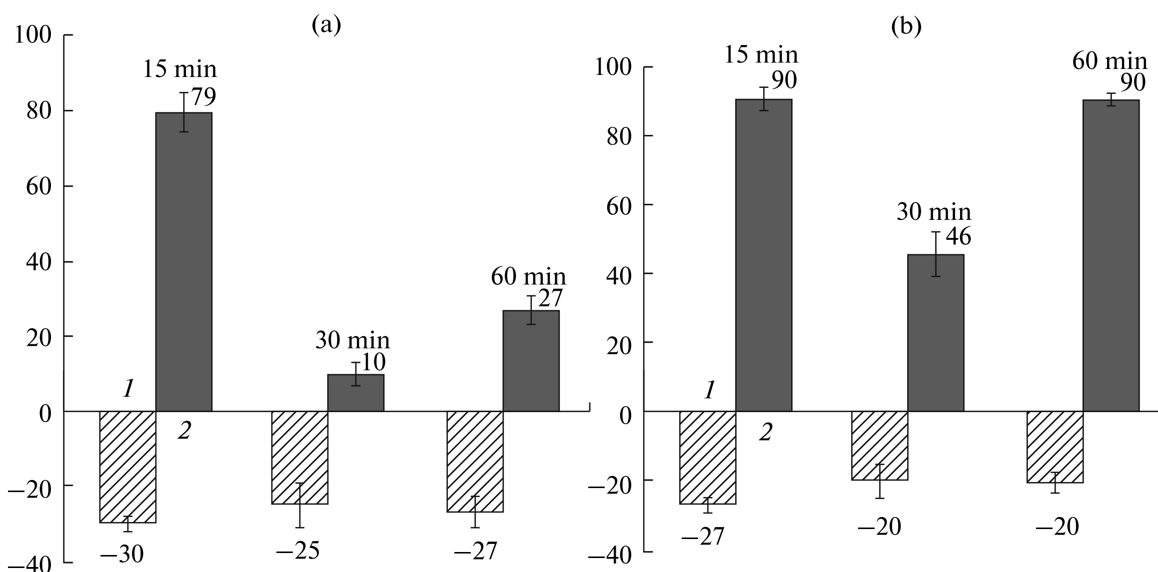
Lectins of two strains, *A. brasilense* Sp7 (collection of microorganisms, Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences) and *A. brasilense* Sp7.2.3, the mutant deficient in lectin activity [3]. The cultivation, as well as isolation and purification of the lectins were carried out as described previously [9, 10]. In the work, 3-day seedlings of Saratovskaya 29 wheat were used. The roots were incubated in lectin solutions (40 μg/ml). In one of the experimental series, these solutions contained $CaCl_2$ (0.1 mM). The roots were fixed in liquid nitrogen and homogenized in the isolation buffer (50 mM Tris-HCl, pH 7.4; 0.1 mM theophylline, 1 mM dithiothreitol, and 0.5 mg/ml polyvinylpyrrolidone). The mixture was filtered and centrifuged (10000 *g*, 40 min). For immune-enzyme analysis (ELISA), 96-well polystyrene plates were used (Medpolimer, St. Petersburg, Russia). For cAMP detection, root homogenate (0.1 g) was thoroughly resuspended in 0.5 ml of buffered saline (BS). Sequential twofold dilutions of the samples (50 μl) were introduced into the wells. The samples were immobilized by drying in an air flow at room temperature. To block the free bands on polystyrene, polyethylenglycol-20000 (100 μl of 0.05% solution) was added. The primary antibodies were the rabbit anti-cAMP antibodies (Sigma, United States, 0.1 mg/ml), the secondary ones were peroxidase-labeled goat anti-rabbit antibodies (Sigma, United States, 2 μg/ml). Peroxidase activity was determined in 0.1 M sodium citrate buffer (pH 4.5) with 0.03% *o*-phenylenediamine and 0.02% $H_2O_2$. The optical density of the samples was measured at 490 nm on an AIF-Ts-01S immune-enzyme analyzer (ILIP, St. Petersburg, Russia). The result was determined as a difference (%) between the optical density values for the experimental (incubated in the lectin solution) and control (untreated) roots. The results were statistically treated. The confidence intervals were determined for the 95% significance level.




Changes in cAMP content in the roots of wheat seedlings treated with the lectins of *A. brasilense* Sp7 (a) and Sp7.2.3 (b) in the absence (*1*) and presence (*2*) of calcium.

The effect of lectins from *A. brasilense* Sp7 and Sp7.2.3 on the content of the cyclic nucleotide in wheat root homogenates was determined after 15, 30, and 60 min of incubation in 40-μg/ml lectin solutions. Immune-enzyme analysis revealed that, in the absence of $Ca^{2+}$, the lectin of the parent strain decreased the cAMP content in plant cells. The results for the different times of incubation did not differ significantly (for 15, 30, and 60 min, the inhibition was 30, 25, and 27%, respectively) (Fig. a). The lectin of the mutant strain acted in a similar way, although the effect was less pronounced compared to the parent strain (Fig. b). Addition of $Ca^{2+}$ ions to the incubation solution with the parent strain lectin resulted in an increase in cAMP concentration by 79, 10, and 27% (compared to the control) for 15, 30, and 60 min of incubation, respectively. In the case of the lectin of the mutant strain, addition of calcium also resulted in increased cAMP concentration in the cells of wheat root seedlings. This increase was more pronounced than in the case of the parent strain lectin (figure).

Thus, lectins of azospirilla were shown to decrease the content of cAMP in plant cells. Considering the data obtained in the study of the role of the polysaccharide of the agent of potato ring rot in induction of the adenylate cyclase signal pathway [11], it may be concluded that the lectins have an inhibitory effect of adenylate cyclase in the roots due to lectin binding with $G_i$ protein, an inhibitor of this process. The lectins therefore act as suppressors. A drastic increase in cAMP content on addition of $Ca^{2+}$ to the incubation mixture was probably caused by the changed interaction between the lectins and the receptor, resulting in the activation of adenylate cyclase. Our results demonstrated that the lectins of the mutant and parent strains had different regulatory activity, probably due to the conformational differences between the lectin molecules, resulting in the differences of interaction with the surface of plant cells, determining the switching of the subsequent stages.

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