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CONFERENCE PROCEEDINGS

Wheat Lectin as a Factor in Plant–Microbial Communication and a Stress Response Protein

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Abstract—Wheat lectin (wheat germ agglutinin, WGA), a representative of a broad group of cereal lectins, is excreted by plant roots into the surrounding medium and interacts with both pathogenic microflora and growth-stimulating rhizobacteria. WGA was found to serve as a molecular signal for the rhizobacterium *Azospirillum brasilense*, which forms endophytic and associative symbioses with wheat plants. The bacterial response to the lectin was pleiotropic: WGA at concentrations from 10^{-10} to 10^{-6} M exerted a dose-dependent effect on a range of processes in the bacterium that are important for the establishment and functioning of symbiosis. Plants with different WGA content differed in their responses to severe nitrogen starvation and to seed treatment with *Azospirillum.*

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It is common knowledge that plant–microbial symbioses and partner interactions in these supraorganismal communities have been under study for more than a century, and microbial preparations have been used for increasing crop yields and improving crop quality in various regions of the world for 110 years. Despite the obvious progress made in this field, the accumulated knowledge is still insufficient; biotechnological rhizobacterium-based products (biological fertilizers and pesticides, etc.) are therefore not widely used, and their practical effects are not always positive [1]. Our failure to understand which molecular signals produced by micro- and macrosymbionts control the formation of effective symbiosis is considered to be one of the factors restraining progress in this field. This problem is especially urgent in the case of phytosymbioses of the associative type, where, unlike legume–rhizobial symbioses, no morphological structures are formed on root colonization.

Associative of symbioses have been studied for only two decades, the researchers' most popular model being the wheat–azospirillum supraorganismal ensemble. Azospirilla have a large genome, flexible metabolism, and interact with the host plant fairly closely: strains of *A. brasilense* actively colonizing not only the root surface but also the root hairs and intercellular spaces (including the intercellular spaces of the root conducting system) are known [2].

Wheat lectin (the historically established name of this protein is wheat germ agglutinin, WGA) is a typical and very well-studied representative of the family of cereal lectins specific to *N*-acetyl-*D*-glucosamine oligomers and polymers [3]. The role of lectins (proteins capable of reversibly and specifically binding to carbohydrate-containing biopolymer fragments) in intercellular and interorganismic interaction is universally recognized; these proteins are currently the subject of rather intense investigation, with WGA being one of the representatives of chitin-binding lectins with well-studied physicochemical and biological properties [3–5]. WGA, like other plant lectins, has a marked biological activity towards a number of animal cells. For example, low concentrations of WGA, on addition to insulin-competent cells, completely reproduce the effect of insulin [4]; apart from its insulin-simulating activity, WGA can mimic the action of antibodies (in antibody-dependent lysis of tumors) and of thrombin; it also exhibits a membrane-tropic activity [5].

The possible role of wheat lectin in plant–microbial interaction attracted attention as early as the mid 1970s, after the hypothesis that this protein may protect plants against fungal infection was published. This suggestion triggered a thorough study of WGA localization at different stages of wheat ontogenesis. The main lectin pool in the seed appeared to be concentrated in the germ, specifically, in those cell layers which, on germination, come into contact with soil [6]. Both seedlings and adult plants excrete lectin into the environment.

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The sites of excretion of this biologically active substance by the cells of the root system practically coincide with the sites of azospirilla localization [2, 6–8]. Thus, lectin is secreted by the meristem cells; it is revealed in the root cap, in the tips of lateral roots (on the surface and in the intercellular spaces), as well as in the stem base [6–7]. Azospirilla are also detected in the stem base and the root segments mentioned above [2] and, as the root system develops, they penetrate into the soil [8]. The surface population, at least in the cases of azospirilla and pseudomonads, remains associated with the root portion containing meristematic cells and excreting WGA during the whole wheat vegetation period [6, 8].

Approximately until the mid 1990s, nothing was known concerning the functions of WGA and of the immunologically related chitin-binding cereal lectins. The substantial experimental material accumulated to date indicates that (i) wheat lectin is a polyfunctional protein and (ii) this protein is important both for the formation of an effective symbiosis with growth-stimulating rhizobacteria and for an adequate bacterial response to stress, including the stress caused by phytopathogens. Although other suggestions exist about the role of WGA in the plant vital functions, the relevant experimental data are so far fragmentary. This lectin is probably an essential protein, because, to our knowledge, all attempts to obtain wheat mutants with knocked out WGA genes have proven unsuccessful, supposedly due to the lethality of this mutation.

The question of whether this wheat lectin exhibits biological activity in relation to azospirilla was investigated in a series of works [7, 9–12]. WGA was found to affect the metabolism of azospirilla, and its effect was pleiotropic, similar to what occurs in the case of mitogenic stimulation of lymphocytes with plant lectins and simulation of the action of insulin and other hormones on competent cells in mammals. Azospirilla form nitrogen-fixing symbioses with cereals, and bacterial production of the phytohormone indole-3-acetic acid (IAA) is important for the stimulation of plant growth. Therefore, we primarily tried to determine whether wheat lectin influences these metabolic pathways of this bacterium. The experiments showed that WGA stimulates not only nitrogen fixation by azospirilla, but also the transport of its product, ammonium, out of the bacterial cell [7, 11]; lectin also caused an increase in IAA production [7]. It is important to point out that these effects were markedly pronounced; they were recorded in the range of WGA concentrations between 10^{-8} and 10^{-9} M and were caused by the ability of lectin to bind to N-acetyl-D-glucosamine-containing fragments of the bacterial cell surface [7, 11].

Apparently, a sufficiently profound restructuring of the azospirilla metabolism occurs under the influence of lectin. Thus, 1 h incubation of *A. brasilense* Sp245 with lectin led to a threefold increase in the protein concentration in crude cell extracts. The comparison of the polypeptide profiles of activated and inactivated cells revealed 64 bands in the activated cells and only 26 bands in the control [7]; immunoelectrophoresis with antibodies specific to glutamine synthetase, the key enzyme of cell nitrogen metabolism, indicated an increase in its biosynthesis under the influence of WGA [7].

It is well-known that in the bacterium–host macroorganism interaction, the surface polymers of both micro- and macroorganism cells play an important role. The comparative analysis of the surface properties of *A. brasilense* Sp245 cells activated with wheat lectin and not subjected to activation showed that a change took place in the cell surface of the bacterium influenced by WGA [10]. Thus, the growth of the bacterium in the presence of lectin led to a considerable increase in the hemagglutination activity of the azospirilla, probably because of the induction of the bacterial hemagglutinin triggered by WGA. In azospirilla, hemagglutinins, the known factors of adhesion of pathogenic bacteria, are glycoproteins with a molecular mass of 36–43 kDa [10, 13] and, in the case of *A. brasilense*, are also important for the successful colonization of the host plant.

Apart from hemagglutination, the growth of *A. brasilense* Sp245 in the presence of 10^{-8} M WGA also resulted in the induction of the surface-associated hemolytic factor [10]. In pathogenic bacteria, hemolysins are important factors of pathogenicity [14]. Among the growth-stimulating rhizobacteria, hemolysin has been revealed, to our knowledge, only in the endophyte *A. brasilense* Sp245. It is exposed on the surface of the bacteria growing under nitrogen fixation conditions and is not revealed by tests when nitrogen fixation is blocked by an excess of oxygen in the growth medium. The hemolytic activity is not related to hemagglutinin (a 42-kDa glycoprotein in *A. brasilense* Sp245); the chemical nature of the hemolytic factor remains so far unknown [10].

For a macroorganism to be successfully colonized, the bacteria have to attain a certain population density. Since the ability of pathogenic bacteria to use the host microorganism's functionally significant proteins for growth stimulation has been reported [15, 16], we assessed the influence of WGA on *Azospirillum* growth and revealed that lectin stimulated the growth of *A. brasilense* Sp7 and Sp245, i.e., it acted as a growth factor [9]. Like the stimulation of lymphocyte proliferation, this effect of wheat lectin was a prolonged one; it was not revealed until after several days (66–72 h) of cultivation in the presence of WGA [9–10].

Recent experiments have revealed that WGA and two other chitin-binding lectins (potato lectin and the agglutinin of the leguminous plant *Ulex europaeus* UEA II) at a concentration of 10^{-10}–10^{-6} M affected the motility of *A. brasilense* Sp245 and Sp107. Azospirilla are characterized by complex behavioral reactions: in planktonic cultures they swim, using the polar flagel-

lum for this purpose, while in semiliquid media, they propagate collectively [17]. In viscous media, the dominant phenotype for *A. brasilense* is swarming (Swa^+ phenotype), and only a small part of the population propagates by aggregates (Gri^+ phenotype; the term is derived from "granular inclusion") [17]. It was established that WGA modifies the proportion of the types of collective motility in the azospirilla population, increasing the number of cells propagating by aggregates [10; Sheludko et al., 2006, submitted for publication]. The effects described were specific: lectins with an alternative carbohydrate-binding specificity (concanavalin A, phytohemagglutinin) did not cause changes in *Azospirillum* motility; blocking the carbohydrate-binding WGA sites led to the elimination of the lectin effects.

Considering that wheat lectin is bound to *Azospirillum* cells [10, 11, 18], accessible for contact with the bacteria [2, 6], and activates processes significant for their action on the plant, the following notions of one of the WGA functions appear, in our opinion, to be logical. **In the wheat–*Azospirillum* symbiosis, the plant lectin is bound to bacterial cells and restructures their metabolism and behavior in the direction that enables successful colonization and the formation of an effective symbiosis.**

As for the colonization of the plant root system by azospirilla, the experimental data obtained lead us to believe that the lectin exposed at the root tip surface is involved in attachment of the bacteria [19]. Thus, the data accumulated to date indicate that wheat lectin is a molecular signal of the host plant, which is important for the formation of the wheat–azospirilla symbiosis.

Many aspects of the WGA–azospirilla interaction remain obscure, both at the molecular, organism, and population levels. Thus, it remains to be clarified whether all the cells in the population are competent to a given stimulus; whether the thansmembrane signal transmission occurs or WGA penetrates into the bacterial cell; whether the two-component regulatory systems are involved in the development of cell response to lectin; whether the response of *A. brasilense* to the plant molecular signal depends on the culture density. One of the intriguing questions is whether the ability to respond to WGA and other acetyl–glucosamine-binding lectins by changing metabolism and behavior is a unique feature of azospirilla or this phenomenon is widespread in symbioses of the associative type. While WGA is a growth factor for *Azospirillum* vegetative cells, it is important to determine whether it affects their resting stages, as occurs in other cases presently under investigation [16, 20].

Obtaining direct evidence for the role of wheat lectin in the formation of associative symbioses appears to be a difficult task. This is due not only to the absence of wheat WGA mutants but also to the impossibility of employing the inhibition of lectin carbohydrate-binding sites in plant experiments: wheat plants respond immediately to the inhibition by expressing this functionally significant protein. The already mentioned wheat lectin polyfunctionality additionally complicates the task. A considerable mass of experimental data on WGA involvement in the plant adaptive response to stress has been accumulated to date. Thus, wheat plants respond by increasing the root lectin to all the stress types studied: infection with phytopathogens, hyperthermy, drought, osmotic shock, moisture deficiency, injury, salt stress, and the presence of heavy metals in the environment [21–23, 12].

Despite the complexity of the system studied, we attempted to assess in plant experiments all the roles of WGA in the interaction with azospirilla and in plant response to severe nitrogen starvation. Both WGA polyfunctionality and the fact that wheat forms nitrogen-fixing symbioses with azospirilla were the reasons to choose this approach; one of the purposes of organismal cooperation is to provide the host plant with fixed nitrogen.

Of 16 varieties of soft spring wheat bred in the Saratov Selection Center and characterized earlier for the seed WGA content, four varieties were chosen: two with a low WGA level in the roots of four-day old seedlings (the varieties Saratovskaya 42 and Saratovskaya 38, S42 and S38) and two with a high WGA level (Saratovskaya 29 and Saratovskaya 52, S29 and S52). In our earlier vegetation experiments [12], we demonstrated that only in the case of the variety with the maximal WGA level (S52) were the azospirilla introduced into the system able to protect the 10-day old wheat seedlings against cadmium stress and to positively influence plant growth under contamination conditions.

The seeds of the high-lectin variety S52 and of the low-lectin variety S42 (with 12-fold differences in WGA content both in the seeds and in the seedlings) were used in the experiments described below. In order both to reveal the possible influence of the seed autochthonous microflora [24] and to avoid blocking the WGA involvement in reviving the dormant microflora of the seeds, which was expected based on the published data [16, 20], the seeds were not sterilized. It is important to note that azospirilla could have been present among the seed autochthonous microflora: *Azospirillum* strains from the seedlings of S52 and S42 were isolated, identified, and maintained at the Institute of Biochemistry and Physiology of Plant Microorganisms, Russian Academy of Sciences. The plants were grown in thoroughly washed and calcined river sand (each plant in a separate plastic pot); in the control variant, they were grown in complete Pryanishnikov medium; in the remaining cases, nitrogen-containing compounds were excluded from the medium. In the variant involving bacterial inoculation, the seeds were treated with *Azospirillum* (10^6 cells per seed). Throughout the whole vegetation experiment, the plants were watered every three to four days with an equal amount of distilled water.

Table 1. The yield (the number of grains per plant) of the high-lectin and low-lectin wheat varieties (Saratovskaya 52 and 42, respectively) grown under conditions of severe nitrogen starvation with and without seed treatment with *Azospirillum brasilense*

Experimental variant	Grain number in the group of 12–15 plants (average per 1 plant)	
	Saratovskaya 52	Saratovskaya 42
Control (complete Pryanishnikov medium)	51(3.6)	48(3.2)
Nitrogen-free (n-f) Pryanishnikov medium	67(5.2)	36(2.4)
N-f Pryanishnikov medium + *A. brasilense* Sp245	13(0.9)	36(3.0)

Note: The number of plants in the groups by the experimental variants (*n*): control, S52 (14), S42 (15); n-f Pryanishnikov medium: S52 (13), S42 (15); variants with azospirillum treatment: S52 (15), S42 (12).

Table 2. Assessment of the modification variability of the Saratovskaya 29 wheat plants by two meristemic cell proteins: wheat germ agglutinin (WGA) and the initial proliferative antigen (PAI)

Protein content	Field conditions for plant growth during seed formation		
	Boghara	Irrigation	Irrigation + Fertilizers
WGA, μg/ml	0.22*	0.50*	3.0*
PAI, %	100**	83**	100*

Note: The conditions are indicated for the growth of plants on which the 1997 crop grains were formed.

Four-day seedlings were obtained from the grains of the 1997 reproduction. The WGA content was assessed in the root extracts and the PAI content in the extracts containing the stem apex and the leaf basal meristems. WGA was assessed in the hemagglutination reaction [18]; a homogeneous WGA preparation (Lektinotest, Ukraine) was used for the calibration curve. PAI was assessed with immunodot assay [25]; the maximal value obtained in the experiments was taken to be 100%.

* The differences are statistically significant.

** The differences are not statistically significant.

The plants survived severe nitrogen starvation, underwent the whole vegetation period, and most of them developed grains (Table 1). This testifies to the capacity of wheat plants to form an effective nitrogen-fixing symbiosis under experimental conditions, because after the completion of the autotrophic period, atmospheric nitrogen fixed by bacteria was the only nitrogen source available to them in all the experimental variants. In the first half of the vegetation period, the high-lectin S52 surpassed the low-lectin S42 in the plant growth values in all the variants; however, by the end of vegetation, the picture had changed somewhat. Thus, the control variants had almost the same average amount of grains per plant (Table 1: 3.6 and 3.2 for S52 and S42, respectively). The high-lectin variety had considerable advantages when reviving the dormant seed microflora and the formation of an effective symbiosis with it was the only way for the plant to survive. In this case, the high-lectin variety S52 surpassed more than twofold the low-lectin variety S42 in the average number of grains per plant (Table 1: 5.2 and 2.4, respectively). And, finally, the varieties differed dramatically in response to inoculation with the highly effective endophytic *Azospirillum* strain Sp245. Thus, treating the seeds with azospirilla did not inhibit plant growth in the case of the low-lectin S42; we even observed a certain increase in the number of grains per plant in response to the presence of strain Sp245 (from 2.4 to 3.0, Table 1). On the contrary, in the case of the high-lectin variety, the introduction of azospirilla into a natural microbial–plant system resulted in a drastic decrease in the number of grains per plant (from 5.2 to 2.4). The fact that among wheat plants the maximum survival and tolerance to stress were observed not in the control variants, but in the high-lectin variety, whose survival is ensured by its natural microflora leads us to believe that bacteria play an essential role in the adaptation of the plants to stress.

The data obtained allow us to suggest that one of the possible causes of universal instability and non-reproducibility of the results of a biotechnological technique such as inoculation [1] is the fact that the level of lectin is not taken into consideration when the seeds are treated with azospirilla or other growth-stimulating rhizobacteria, although, according to our experimental results, this protein plays an important role in the formation of the associative type of symbiosis.

The complexity of the problem consists in the fact that the WGA level is a dynamic parameter determined not only by the plant genotype; this feature is characterized by an exceptionally high level of modification variability (Table 2). Thus, in the case of the high-lectin variety Saratovskaya 29, the growing of plants under field conditions in three groups with consistently improving growth conditions led to a consistent

increase in the WGA level in four-day old seedlings obtained from these seeds. The WGA level increased as the growth conditions improved (Table 2). The initial proliferative antigen (IPA) was used as the control because its localization in meristem zones is similar to that of WGA, although its function is different [25]. The data shown in Table 1 demonstrate that the IPA level in the seedlings did not change, while the WGA level increased (at most by an order of magnitude) with improving conditions for the formation of the dormant form of the plant (seeds).

The experimental data accumulated to date testify that wheat lectin is important as a factor of communication in plant–microbial interactions and as a factor of plant adaptation to stress, which seems to be accomplished with the involvement of microflora. The complexity and multi-component character of natural symbioses of the associative type, together with the polyfunctionality of lectin, the dynamism of its plant content, and the high level of its modification variability do not presently allow us to use the available knowledge in practical biotechnology.

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