EFFECT OF HAPTEN, N-ACETYL-D-GLUCOSAMINE, ON THE BIOLOGICAL ACTIVITY OF WHEAT LECTIN

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Received 3 October 2007

Summary. Modulation of the effect of wheat germ agglutinin (WGA) with hapten, N-acetyl-D-glucosamine (GlcNAc), in case of pre-sowing treatment of spring wheat (*Triticum aestivum* cv Rannyaya 93) seeds, on the biological components of the system ''plant – soil – microorganisms'' was studied. The application of WGA (100 nM) stimulated root formation, biomass production, total chlorophyll content and wheat grain productivity. A positive effect of WGA on the amount of soil nitrogen-fixing microorganisms, their nitrogenase activity and the growth-activating ability of rhizosphere soil was also established. The stimulatory effect of 100 mM GlcNAc on the plants and the soil nitrogen-fixing microorganisms was lower in comparison to wheat lectin. The binding of hapten to the active centers of wheat lectin inhibited the effect of WGA in relation to *T. aestivum* development parameters and nitrogen-fixing microorganisms in the rhizosphere soil.

Key words: spring wheat, wheat germ agglutinin, N-acetyl-D-

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glucosamine, total chlorophyll, soil nitrogen-fixing microorganisms, nitrogenase activity, grain productivity.
Abbreviations: WGA - wheat germ agglutinin; GlcNAc - N-acetyl-D-glucosamine, FW - fresh weight, DW – dry weight, NA - nitrogenase; Chl - chlorophyll; LC - liquid chromatography.

INTRODUCTION

Plant lectins are polyfunctional molecules participating in the formation and functioning of plant-microorganisms symbioses. Lectins are molecules exhibiting a wide range of biological activities (Singh et al., 1999; Peumans et al., 2001; Rudiger and Gabius, 2001). Phytohemagglutinins are multifunctional compounds that can play a role of receptors by binding microsymbiont polysaccharides at the stage of host-plant recognition (Singh et al., 1999; Laus et al., 2006), the molecular signal, or the factors controlling development and functioning of the plant-microbe symbiosis (Brencic and Winans, 2005; Lodeiro et al., 2000; Antonyuk and Evseeva, 2006).

Lectins belong to a class of (glyco)proteins which are capable of binding carbohydrates selectively and reversibly without causing their chemical transformation (Bouckaert et al., 1999; Loris, 2002). Carbohydrate specificity is a fundamental characteristics of phytohemagglutinin molecules. Wheat germ agglutinin (WGA) is one from series of N-acetyl-D-glucosamine-binding plant lectins as hevein, pseudohevein, *Urtica dioica* agglutinin and has four selective carbohydrate binding centers for hapten (Komath et al., 2006). Additionally, WGA is a protein with two primary and two secondary independent sugar-binding sites and has sugar binding specifity for 2 types of N-acetylated sugars, N-acetyl-D-glucosamine (GlcNAc) and N-acetylneuraminic acid (Karpati et al., 1999). Interaction of hapten with the active centers of WGA can reduce or completely block the lectin effects (Yegorenkova et al., 2001; Kyrychenko, 2006).

It has recently been shown that the inducing effects of WGA on endogenous lectin activity of wheat plants as well as on the functional activity of *Triticum aestivum* genome are partially inhibited by GlcNAc

(Kyrychenko and Tyshchenko, 2005).

Little information is available on the modulation of the biological activity of WGA by GlcNAc in relation to the components of the system "plant - soil - microorganisms". The aim of the present investigation was to study the growth parameters of *T. aestivum* plants, total chlorophyll content, grain productivity and the growth-activating ability of rhizosphere soil, the amount of soil nitrogen-fixing microorganisms and their nitrogenase activity under greenhouse conditions.

MATERIALS AND METHODS

Spring wheat (*Triticum aestivum* L.) cv. Rannyaya plants were grown on soil with components of Pryanishnikov nutrient medium (Grodzinsky and Grodzinsky, 1973) containing 0.12 g NH_4NO_3, 0.269 g $CaSO_4$, 0.172 g $CaHPO_4$, 0.16 g KCl, 0.025 g $FeCl_3.6H_2O$, 0.123 g $MgSO_4.7H_2O$, microelements: 0.007 g $(NH_4)_2MoO_4$, 0.007 g H_3BO_3, 0.0012 g $MnSO_4.5H_2O$, 0.0025 g $CuSO_4.5H_2O$ L^{-1} as a 0.5 mineral nitrogen norm which was added to the substrate for plant growth. The experiments were performed in Wagner pots (7 per variant, 20 seeds per one pot) in a greenhouse.

Preparations of wheat germ agglutinin (WGA, 100 nM), aminosaccharide N-acetyl-D-glucosamine (GlcNAc, 100 mM) and hapten of the wheat lectin ("Lectinotest", Lviv, Ukraine) were applied. The composition of lectin with hapten was 1:1, where lectin was pretreated with hapten for h and water was used as control for pre-sowing treatment of wheat seeds for 1 h.

The biomass of plants, total chlorophyll content in wheat leaves and grain productivity, amount of rhizosphere diazotrophe microorganisms with their nitrogen-fixing activity (NA), and growth-activating ability of rhizosphere soil were estimated at various periods of wheat vegetation (different developmental phases of wheat plants) (Kirby et al., 1999). Plants and rhizosphere soil were sampled during the following phases: tillering (30-day-old plants), boot (55-day-old plants), flowering (70-day-old plants) and ripening (110-day-old plants). The harvest index (HI) was calculated as the ratio of grains DW to plant DW.

Total Chl content in wheat flag leaves was determined according to Arnon after extraction with dimethylsulfoxide (Hiscox and Israelstam,

1979). Total Chl was measured in four replications.

Nitrogenase (NA) activity measured by acetylene reduction was detected in wheat root with rhizosphere soil according to Hardy et al. (1968) by gas chromatography using a Chromatograph 504 device (Mera Elwro, Poland) equipped with a flame-ionization detector. Analyses were conducted using a column (1.3 m by 4 mm) packed with the Paropak N at 80 °C. The column was calibrated against ethylene. The carrier gas was nitrogen at a flow rate of 50 ml/min. Acetylene-reducing activity (ARA) was registered after 1 h incubation of roots of 30- and 55-day-old wheat plants with acetylene injected in hermetic sealed flasks. The final acetylene volume was 10 % of flask volume. ARA was determined in three-four replications and expressed in nM ethylene (C_2H_4) produced by the enzyme from added acetylene per g FW of plant root.

Microbial density of rhizospheric diazotrophes and oligonitrophiles was expressed as colony forming units (CFU). Microbial cultures were grown in the agar selectivity nutrient Eshbi medium containing 0.2 g K_2HPO_4, 0.2 g $MgSO_4$, 0.2 g NaCl, 0.1g K_2SO_4, 5.0 g $CaCO_3$, 20.0 g saccharose, 16.0 g agar and 1 ml microelements l^{-1}, pH 7.3.

The growth activating ability of rhizosphere soil was determined by the phytotest using watercress (*Nasturtium microphyllum*) seedlings as a test object grown in the rhizosphere soil (Grodzinsky and Grodzinsky, 1973).

Experiments were performed in three replications. Statistical evaluation of the results was performed according to Statgraphics software statistical package 5.0.

RESULTS

The obtained results demonstrate that pre-sowing treatment of *T. aestivum* seeds with wheat lectin, hapten and their composition stimulated growth processes and formation of biomass of plants at all developmental phases under greenhouse conditions (Table 1). Treatment of wheat seeds with the above preparations led to increased plant biomass of the above-ground parts and roots of *T. aestivum* plants, but 100 mM GlcNAc reduced the stimulatory effect of 100 nM WGA concerning the FW of shoots (by 15-6 %) and DW of shoots (by 19 %), FW of roots (by 2-19 %) and DW of

Table 1. Effect of treatment of *Triticum aestivum* (cv. Rannyaya 93) seeds with WGA and N-acetyl-D-glucosamine on shoot and root weight and total chlorophyll content of wheat plants.

Treatment	Shoot weight per plant [g FW]	Root weight per plant [g FW]	Shoot weight per plant [g DW]	Root weight per plant [g DW]	Total Chl in leaves [mg g^{-1} DW]
		Tillering			
Control	0.78±0.04	0.31±0.01	0.32 ±0.02	0.12 ±0.01	0.24 ±0.01
100 mM GlcNAc	0.97±0.06	0.34±0.02	0.38 ±0.02	0.14 ±0.01	0.32 ±0.01
100 nM WGA	0.95±0.05	0.33±0.02	0.40 ±0.02	0.14 ±0.01	0.37 ±0.01
100 nM WGA with 100 mM GlcNAc	0.84±0.24	0.32±0.02	0.34 ±0.10	0.13 ±0.01	0.27 ±0.00
		Boot			
Control	3.49±0.14	1.56±0.03	0.74 ±0.03	0.33 ±0.01	0.72 ±0.02
100 mM GlcNAc	3.58±0.16	1.56±0.04	0.80 ±0.04	0.35 ±0.01	0.88 ±0.04
100 nM WGA	3.90±0.21	1.97±0.03	0.81 ±0.04	0.41 ±0.01	0.87 ±0.03
100 nM WGA with 100 mM GlcNAc	3.20±0.02	1.67±0.06	0.66 ±0.03	0.35 ±0.01	0.83 ±0.02

Table 2. Effect of treatment of *Triticum aestivum* (cv. Rannyaya 93) seeds with WGA and hapten on grain productivity.

Treatment	Spike number per pot	Spike weight per pot [g DW]	Weight of one spike [g DW]	Grain number per spike	Grain weight per spike [g DW]	Grain weight per pot [g DW]	Weight 1000 grains [g DW]	Harvest index
Control	18.8±0.5	21.00±1.09	1.13±0.05	21.5±0.3	0.80±0.02	14.76±0.38	395.3±9.9	0.40±0.02
100 mM GlcNAc	19.3±0.5	22.50±0.69	1.17±0.05	24.1±0.3*	0.91±0.02*	16.73±0.45*	412.0±6.7*	0.46±0.02*
100 nM WGA	19.0±1.1	24.15±0.93*	1.33±0.03*	24.3±0.1*	0.97±0.01*	18.39±1.21*	419.5±4.1*	0.47±0.03*
100 nM WGA with 100 mM GlcNAc	18.8±0.9	22.16±1.32	1.18±0.06	23.6±0.3*	0.91±0.01*	16.51±1.13*	397.5±8.4	0.46±0.02*

* – statistically significant difference with the control values

Table 3. Effect of treatment of *Triticum aestivum* (cv. Rannyaya 93) seeds with WGA and N-acetyl-D-glucosamine on nitrogen-fixing activity and amount of rhizosphere diazotrophic microorganisms.

Treatment	Nitrogenase activity [nmol C_2H_4 $h^{-1}g^{-1}$ FW]	CFU [cell g^{-1} soil]
	Tillering	
Control	1.877±0.223	49.6±5.6 · 10^{12}
100 mM GlcNAc	3.018±0.260	94.5±4.5 · 10^{12}
100 nM WGA	6.351±0.107	96.4±3.5 · 10^{12}
100 nM WGA with 100 mM GlcNAc	2.497±0.280	96.8±2.7 · 10^{12}
	Boot	
Control	0.672±0.078	3.0±0.4 · 10^{14}
100 mM GlcNAc	0.958±0.189	8.9±1.7 · 10^{14}
100 nM WGA	1.411±0.106	17.7±0.7 · 10^{14}
100 nM WGA with 100 mM GlcNAc	0.898±0.090	15.6±1.6 · 10^{14}
	Flowering	
Control	–	3.4±0.1 · 10^{14}
100 mM GlcNAc	–	17.5±1.1 · 10^{14}
100 nM WGA	–	38.0±1.7 · 10^{14}
100 nM WGA with 100 mM GlcNAc	–	43.7±1.4 · 10^{14}
	Ripening	
Control	–	74.2±6.8 · 10^{9}
100 mM GlcNAc	–	82.2±7.1 · 10^{9}
100 nM WGA	–	160.9±11.3 · 10^{9}
100 nM WGA with 100 mM GlcNAc	–	89.4±5.6 · 10^{9}

Note: CFU – colonies formation units; "–" – nitrogenase activity was not detected

Table 4. Effect of treatment of *Triticum aestivum* (cv. Rannyaya 93) seeds with WGA and N-acetyl-D-glucosamine on growth-activating ability of rhizosphere soil.

Treatment	*Nasturtium microphyllum* seedlings, [mg FW]		
	Tillering	*Flowering*	*Ripening*
Control	11.49±0.18	23.35±0.94	25.42±0.78
100 mM GlcNAc	11.62±0.25	23.15±0.45	29.99±0.90
100 nM WGA	12.60±0.13	23.43±0.66	26.16±0.56
100 nM WGA with 100 mM GlcNAc	11.06±0.41	22.95±0.87	25.81±1.33

roots (by 4-18 %) for two different phases of plant development. The trend of an increase in the FW of wheat roots was observed in all variants.

Treatment of seeds with WGA increased total Chl content in the wheat leaves (DW) at the phases of tillering and boot by 54 % and 21 %, respectively whereas GlcNAc led to increased total Chl content by 33 % and 22 %, respectively (Table 1). Hapten had a significant modulating effect on wheat lectin in the phase of tillering and caused a reduction in total Chl content by 21 % compared with WGA, however, no difference was observed at the boot phase.

Analysis of the yield structure demonstrated that the grain yield was enhanced due to the increase in the weight of spikes by 6–20 %, number and weight of grains per spike by 10–12 % and 14–21 %, respectively (Table 2). DW of grains per pot exceeded the control values by 12–24 % in all treatments. The increase in the DW of 1000 grains was insignificant (by 1–6 %). The harvest index (HI) increased by 15–18 %. This effect suggests that the potential maximum of wheat plants formed in the presence of WGA, hapten and the composition was directed rather towards the production of wheat grains, but not to plant vegetative biomass.

Studies on the nitrogen-fixing activity of the rhizospheric microbe complex in association with wheat roots demonstrated high activity of the diazotrophic microorganisms in the rhizosphere soil (Table 3). In the

tillering and boot phase of the wheat development these values were 1.4–3.6- and 1.4–2.6-fold higher, respectively. WGA had a stronger stimulatory effect on the nitrogen-fixing microorganisms. Soil microbiological analyses showed that the maximum stimulatory effect on the amount of nitrogen-fixing microorganisms in the rhizosphere soil was observed after treatment of seeds with WGA. Hapten decreased the effect of wheat lectin on the NA of rhizosphere microorganisms as well as their amount. The amount of diazotrophs and oligonitrophils in the wheat rhizosphere zone in all variants was 1.2 –12.0-fold higher during the vegetative period.

The growth-activating ability of the rhizosphere soil after treatment of seeds with WGA and GlcNAc was increased by 3–10 % and 18 %, respectively, as compared to controls and did not change under lectin modulation effect exerted by hapten (Table 4).

DISCUSSION

Our results demonstrated that pretreatment of WGA with the carbohydrate hapten modulated the effect of wheat lectin. Hapten inhibited the positive effect of WGA in relation to *T. aestivum* development parameters as root formation, growth, grain productivity and physiological activity of nitrogen-fixing microorganisms of rhizosphere soil due to binding to the active centers of lectin. WGA applied to the culture of *Azospirillum brasilense* sp. 245 at concentrations of 1–10 nM enhanced indole-3-acetic acid production, dinitrogen fixation and ammonium excretion by bacterial cells. Lectin promoted also the synthesis of proteins, both new and those already present in bacterial cells (Antonyuk and Ignatov, 2001). WGA was found to serve as a molecular signal for the rhizobacteria and the bacterial response to the plant lectin was pleiotropic: WGA applied at concentrations from 0.1 nM to 1 mM exerted a dose–dependent effect on a number of processes in the bacterium that were important for the establishment and functioning of symbiosis (Antonyuk and Evseeva, 2006).

Data exist in literature confirming our results that pretreatment of WGA with GlcNAc lowered the effects of wheat lectin. It was shown that two haptens of the wheat lectin, GlcNAc and chitotriose, blocked the WGA effects on metabolism activity, growth in pure culture of microorganisms

and plant biochemical parameters, like enzyme activities, membrane potential or the effect of polysaccharide-containing complexes from the *Azospirillum* capsule on bacterial adsorption kinetics by *T. aestivum* roots. *A. brasilense* cells deprived of capsular exopolysaccharides completely lost their ability to bind WGA and much of their ability to attach to wheat seedling roots (Yegorenkova et al., 2001).

It was reported that treatment of *Nicotiana tabacum* pollen grains with lectin concanavalin A (Con A) applied at concentrations of 10 – 1000 μg ml^{-1} induced plasma membrane hyperpolarization in the vegetative cells and enhanced pollen grain germination. Con A at a concentration of 100 μg ml^{-1} increased the intracellular pH by 0.3 units. These effects of Con A were blocked with sugar methyl-mannopyranoside at a concentration of 100 mM (Matveeva et al., 2004).

Pretreatment of the bacterial lectins with hapten and L-fucose decreased the effects of lectins (Alenkina, 2006). It was shown that incubation of bacterial lectins isolated from the nitrogen-fixing soil microorganisms *A. brasilense* sp. 7 and its mutant defective in hemagglutinating activity *A. brasilense* sp. 7.2.3 with the exocomponent, membrane and apoplast fractions of wheat roots increased the enzyme activities of α-glucosidase, β-glucosidase and β-galactosidase. Both wild-type and mutant lectins were most stimulatory to the activities of all exocomponent fraction enzymes studied and to the apoplast fraction of β-glucosidase.

Root-rhizosphere communication is an important process that characterizes the underground zone and local changes within the rhizosphere can include the growth and development of microorganisms (Martin and Plassard, 2001; Walker et al., 2003). The attachment of nitrogen-fixing microorganisms to the roots is essential for the establishment of an efficient association with the host plant (Bashan and Holguin, 1997; Barea et al., 2005; Vande and Vanderleyden, 1995). Our data showing a stimulatory effect of WGA on the functioning of the population of soil nitrogen-fixing microorganisms, their nitrogen-fixing activity and the growth-activating ability of rhizosphere soil are in agreement with previously demonstrated results. Legume lectins affect positively the realization of symbiotic properties of soil microorganisms (nodulation, nitrogen-fixing activity and effectiveness) during the formation and functioning of the legume-

rhizobium symbiosis (Lodeiro, 2000; Kyrychenko and Titova, 2005). Presowing treatment of soybean seeds with soybean lectin stimulated growth of soil microorganisms under conditions of pure culture and the development of soil nitrogen-fixing population. The number of diazotrophs and oligonitrophils in the soybean rhizosphere soil, their nitrogen-fixing activity and the nitrogen-fixing activity of soybean root nodules increased up to 2.7-fold, 1.3–2.0-fold and 1.3–3.0-fold, respectively (Kyrychenko and Titova, 2005). Kim et al. (2005) reported that there was no correlation between pure culture and plant-associated nitrogenase activity of the different nitrogen-fixing bacteria isolated from the rhizosphere of different crops, but the isolates of *Azospirillum brasilense* CW301, *Azospirillum brasilense* CW903, and *Azospirillum lipoferum* CW1503 showed higher nitrogenase activities associated with wheat roots. Literature data confirm the stimulatory effect of WGA on nitrogenase activity in *Azospirillum lipoferum* cells (Karpati et al., 1999). The capability of *A. lipoferum* for nitrogen fixation was efficiently increased in the presence of WGA. The highest (3.4-fold) nitrogenase activity was obtained with WGA and lower effect was observed after treatment with WGA together with N-acetylneuraminic acid (1.3-fold).

Our data showed that maximum wheat yield was obtained after treatment of *T. aestivum* seeds with WGA. This result confirm again the modulating function of plant lectins during growth and development of plants which is directed to the realization of plant potential leading to increased grain yield. Pretreatment of lectin with hapten led to an inhibition of WGA biological activity resulting in decreased grain productivity and harvest index of *T. aestivum* plants.

Thus, our results suggest that pretreatment of WGA with wheat hapten, GlcNAc, led to a reduction of all stimulatory effects of wheat lectin on the components of the system “plant – soil – nitrogen-fixing microorganisms”.

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