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## **Biological activity of wheat germ agglutinin relative to spring wheat plants under the influence of hapten N-acetyl-D-glucosamine**

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**Aim.** To study the biological activity of wheat germ agglutinin (WGA) relative to the spring wheat plants under the influence of N-acetyl-D-glucosamine (hapten of WGA, GlcNAc) at pre-sowing treatment of seeds. **Methods.** Inhibitory analysis as well as physiological (pot experiments, estimation of the plants growth and development, wheat grain productivity) and biochemical (chlorophyll *a+b* content) methods. **Results.** It was shown in the pot experiments that WGA (100 nM) at the pre-sowing of seeds stimulated the growth processes and the plants vegetative mass formation (shoot mass increased from 10 to 24 % in different ontogenesis phases) as well as the chlorophyll (*a+b*) accumulation in leaves (from 1.2 to 1.4 times), which led to an increased level of realization of wheat grain productivity (the grain number, weight per plant and 1000 grain weight increased by 26, 26 and 6 % respectively). Hapten of WGA N-acetyl-D-glucosamine (100 mM) inhibited the positive effects of wheat lectin on the plants: from 5 to 19 % (by the vegetative mass), from 0 to 18 % (by the level of green photosynthetic pigments), from 3 to 13 % (by the grain productivity). The maximum inhibitory effect of hapten was noted in the tillering and boot development phases of wheat ontogenesis. **Conclusions.** The biological activity of WGA at the pre-sowing treatment of spring wheat seeds was expressed in the intensification of plant growth and development, the active accumulation of chlorophyll in leaves and an increase in the wheat grain productivity. Hapten had a significant reducing effect on the biological activity of wheat lectin resulted in inhibition of positive lectin's effects on physiological and biochemical parameters of the spring wheat plants development. It is one of the arguments in favour of the lectin nature of these effects.

**Key words:** wheat germ agglutinin, hapten, *Triticum aestivum* L., treatment of seeds, chlorophyll, grain productivity.

## Introduction

The use of ecological biotechnology elements (microbial inoculants and biologically active substances of natural origin) in the rural economy in order to increase the crop productivity of culture plants is the urgent issue of sustainable agriculture. Microbial biotechnology and biologically active substances of natural origin (plants and microbial metabolites) are employed in agriculture to fulfill environmental and industrial tasks [1, 2]. Phytolectins are natural substances, the products of plants metabolism [3, 4]. Lectins are the molecules with a wide range of biological activities [5–9]. They are glycoproteins or oligomeric proteins with one or more sugar-binding sites per subunit. Lectins bind reversibly with specific sugars [10]. Carbohydrate specificity is a fundamental characteristic of the phytohemagglutinin molecules [10, 11]. Wheat germ agglutinin (WGA) belongs to the group of N-acetyl-D-glucosamine (GlcNAc)-binding plant lectins. WGA has two primary and two secondary independent sugar-binding sites and possesses sugar binding specificity for two types of N-acetylated sugars – N-acetyl-D-glucosamine and N-acetylneuraminic acid [4].

Recently, it has been shown the inducing effects of WGA on the RNA amount, enzyme (peroxydase and catalase) activity of antioxidant system of plant protection, endogenous lectin activity and flavonoids content in wheat leaves and the content of plant hormones (cytokinins and IAA) as well as on the nitrogen fixation activity of rhizospheric microorganisms [7, 12].

The aim of the present investigation was to study the biological activity of wheat germ

agglutinin (WGA). It was investigated an influence of N-acetyl-D-glucosamine (hapten of WGA, GlcNAc) at the pre-sowing treatment of spring wheat seeds on the wheat plants characteristics: growth parameters, chlorophyll (Chl) *a+b* content and grain productivity of *Triticuma estivum* L. in greenhouse conditions.

## Material and Methods

Spring wheat (*Triticuma estivum* L.) cv Rannya 93 plants [13] were grown on sand soil with components of Pryanishnikov nutrient medium containing (g/kg): 0.12 NH<sub>4</sub>NO<sub>3</sub>, 0.269 CaSO<sub>4</sub>, 0.172 CaHPO<sub>4</sub>, 0.16 KCl, 0.025 FeCl<sub>3</sub>·6H<sub>2</sub>O, 0.123 MgSO<sub>4</sub>·7H<sub>2</sub>O and microelements – 0.007 (NH<sub>4</sub>)<sub>2</sub>MoO<sub>4</sub>, 0.007 H<sub>3</sub>BO<sub>3</sub>, 0.0012 MnSO<sub>4</sub>·5H<sub>2</sub>O, 0.0025 CuSO<sub>4</sub>·5H<sub>2</sub>O 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 pot) in greenhouse experiments during three years.

Preparations of wheat germ agglutinin (100 nM) and aminosaccharide N-acetyl-D-glucosamine (100 mM) (“Lectinotest”, Lviv, Ukraine) were used [14]. The ratio for composition of lectin with hapten was 1:1, where lectin was pretreated with hapten for 1 h. Water was used as control, while WGA and WGA+GlcNAc – as experimental variants for pre-sowing treatment of wheat seeds for 1 h.

The shoot weight (fresh weight, FW) and chlorophyll (Chl*a+b*) content in wheat leaves were estimated at seedling development (10 day-old plants), tillering (30 day-old plants), boot development (45–50 day-old plants) and head emergence – flowering (55–

60 day-old plants) of wheat vegetation phase [15]. Chl(*a+b*) content in wheat flag leaves was determined according to Arnon after extraction of leaves with dimethylsulfoxide[12]. The level of Chl was measured in four replications and expressed in mg g<sup>-1</sup> FW leaves. The grain productivity (dry weight, DW of plants and grains) of spring wheat was estimated at ripening phase (90–100 day-old plants) of plants vegetation [15]. Yield structure parameters (weight of one spike, grain number and weight per spike, weight of 1000 grains and harvest index) were estimated.

Statistical evaluation of the results was performed according to Statgraphics software statistical package 5.0. All data presented in the paper are Mean ± standard error of mean (SEM).

## Results

The obtained results demonstrate that pre-sowing treatment of spring wheat seeds with WGA has stimulated the growth processes and formation of plants biomass, Chl (*a+b*) content in wheat leaves and wheat yield, but GlcNAc has reduced the stimulatory effect of lectin at all developmental phases under greenhouse conditions (Table 1–3). Shoot mass formation

(Table 1) increased by 10-24 %, while GlcNAc has inhibited a positive biological effect of WGA at different phases of wheat plants ontogenesis by 5, 19, 12 and 6 %. Hapten had a significant reducing effect on the wheat lectin biological activity regarding growth of spring wheat plants in the phases of tillering and boot development. At the same time no difference was observed in the phases of seedling development.

Pre-sowing treatment of spring wheat seeds with WGA has increased Chl (*a+b*) content in the wheat leaves at the phases of seedling development by 21 %, of tillering by 38 %, of boot development phase by 26 %, of head emergence – flowering by 19 % (Table 2). Hapten had a significant inhibition effect on wheat lectin at the tillering and boot development phases of wheat plants: caused a reducer in Chl content by 18 and 11 % compared with effect of WGA, however, no difference was observed in the wheat at the seedling development and the head emergence – flowering phases.

The analysis of the yield structure has demonstrated that the grain productivity of spring wheat was enhanced due to an increase in the weight of spikes by 25 %, number and weight

**Table 1. Effect of N-acetyl-D-glucosamine on biological activity of wheat germ agglutinin: shoot formation of spring wheat plants (average for three years)**

Treatment of seeds	Phases of wheat plants ontogenesis			
	Seedling development [FW]	Tillering [FW]	Boot development [FW]	Ripening [DW]
Shoot weight per plant [g]				
Control	0.41±0.02	2.31±0.12	4.16±0.22	2.14±0.11
WGA	0.45±0.01 <sup>a</sup>	2.87±0.11 <sup>a</sup>	4.80±0.30 <sup>a</sup>	2.42±0.13 <sup>a</sup>
WGA+GlcNAc	0.43±0.0 <sup>a</sup>	2.33±0.14 <sup>b</sup>	4.24±0.24 <sup>b</sup>	2.27±0.10 <sup>b</sup>

Table 1–3: <sup>a</sup> – statistically significant difference (P≤0.5) with the control variant, <sup>b</sup> – statistically significant difference (P≤0.5) with the WGA variant, “–” – values were not detected.

**Table 2. Effect of N-acetyl-D-glucosamine on biological activity of wheat germ agglutinin: chlorophyll content of spring wheat leaves** (average for two years)

Treatment of seeds	Phases of wheat plants ontogenesis			
	Seedling development	Tillering	Boot development	Ripening
	Chl ( <i>a+b</i> ) content in leaves [mg g <sup>-1</sup> FW]			
Control	0.87±0.02	1.27±0.03	1.72±0.03	2.05±0.01
WGA	1.05±0.02 <sup>a</sup>	1.75±0.03 <sup>a</sup>	2.16±0.06 <sup>a</sup>	2.44±0.05 <sup>a</sup>
WGA+GlcNAc	1.05±0.04 <sup>a</sup>	1.44±0.01 <sup>a b</sup>	1.93±0.03 <sup>a b</sup>	2.51±0.02 <sup>a</sup>

**Table 3. Changes of biological activity of wheat germ agglutinin under the influence of its hapten N-acetyl-D-glucosamine: grain productivity of spring wheat** (structure of harvest, average for three years)

Treatment of seeds	Weight of one spike [g DW]	Grain number per spike [number]	Grain weight per spike [g DW]	Weight of 1000 grains [g DW]	Harvest index [HI]
Control	1.19±0.04	22.8±0.09	0.88±0.02	40.91±0.51	0.36±0.01
WGA	1.49±0.06 <sup>a</sup>	28.7±1.0 <sup>a</sup>	1.11±0.02 <sup>a</sup>	43.45±0.64 <sup>a</sup>	0.41±0.02 <sup>a</sup>
WGA+GlcNAc	1.31±0.08 <sup>b</sup>	26.3±1.5 <sup>a</sup>	0.97±0.07 <sup>b</sup>	41.87±0.67 <sup>b</sup>	0.40±0.01 <sup>a</sup>

of grains per spike by 26 and 26 % respectively (Table 3).

An increase in the DW of 1000 grains was insignificant (by 6 %). The harvest index increased by 14 %. This effect suggests that the potential maximum of wheat plants formed in the presence of WGA was directed rather towards the production of wheat grains but not to the plant vegetative mass. GlcNAc has inhibited the activation of biological effects of WGA regarding to spring wheat productivity by 12 % (weight of one spike), by 8 % and 13 % (number and weight of grains per spike respectively). However, no difference has been shown in the values of 1000 grains and harvest index in its variants (by 4 % and 3 % respectively).

The intensification of plant shoot formation (Table 1) and chlorophyll content in leaves (Table 2) as well as the spring wheat yield at WGA use for pre-sowing treatment of seeds

(Table 3) was shown in greenhouse experiments during three years.

## Discussion

Our recent results provide an evidence of the considerable role of exogenous phytolectins as regulators of plant growth and development and physiological activity of the soil nitrogen-fixing microorganisms [2, 7, 12]. The response of spring wheat and soil rhizospheric nitrogen-fixing microorganisms to the pre-sowing treatment of seeds by wheat germ agglutinin was expressed in the metabolic changes of plants: an increase in RNA amount and endogenous lectin activity in wheat seedlings and leaves, enzyme activity of antioxidant system of plant protection (peroxydase and catalase), endogenous lectin activity and flavonoids content and the amount of endogenous plant hormones cytokinine and auxine nature in wheat leaves [7, 12], enhanced accumulation of plant bio-

mass (Table 1) and an increase in Chl (a+b) content in the wheat leaves (Table 2) as well as the nitrogen-fixing capacity of the rhizospheric microorganisms [2, 7]. As a result, the wheat grain productivity increased (Table 3). Hapten of WGA, aminosaccharide GlcNAc, binds to the active centers of wheat lectin on the level of molecular interaction [14] and partially decreases the activation effects of WGA on the plants physiological and biochemical parameters. It is one of the arguments in favour of the lectin nature of these effects.

It is known that lectins are the molecules exhibiting a wide range of biological activities regarding micro- (virus, bacterium, fungi) and macroorganisms (insects, plants, animals) [2, 5, 7, 8, 16–19]. Both endogenous and exogenous lectins have many physiological functions in the plants [3, 4, 6–8, 16, 18]. Literature data confirms the results of our investigation, which have shown that pretreatment of WGA with GlcNAc has inhibited the positive effect of lectin on the wheat plants. It was shown that haptens of lectins blocked the lectin effects on the metabolism activity, physiological and biochemical parameters (like enzyme activities, membrane potential e. a.) of plants. It was reported that treatment of *Nicotiana tabacum* pollen grains with lectin concanavalin A (Con A) applied at concentrations of 10–1000  $\mu\text{g ml}^{-1}$  has induced hyper polarization of plasma membrane in the vegetative cells and has enhanced pollen grain germination. Con A at a concentration of 100  $\mu\text{g ml}^{-1}$  increased the intracellular pH by 0.3 units. These effects of Con A were blocked with saccharide methylmannopyranoside at a concentration of 100 mM [16]. Pretreatment of the bacterial lectins with L-fucose has decreased the effects of lectins

[17]. It was shown that incubation of bacterial lectins isolated from the microsymbiont of wheat plant – soil nitrogen-fixing microorganisms *Azospirillum brasilense* sp.7 and its mutant defective in lectin activity (*A. brasilense* sp.7.2.3) with the exocomponent, membrane and apoplast fractions of wheat root increased the enzyme activities of  $\alpha$ -glucosidase,  $\beta$ -glucosidase and  $\beta$ -galactosidase. Lectins of wild-type and mutant strains had stronger stimulatory effect on the activities of all exocomponent fraction enzymes studied and of the apoplast fraction of  $\beta$ -glucosidase.

Thus, our data (table 1–3) have demonstrated that pre-treatment of WGA with N-acetyl-D-glucosamine changes the biological activity of protein at the pre-sowing treatment of wheat seeds in relation to the spring wheat plants. It is in agreement with previously demonstrated literature data on the inhibitory effect of haptens on the biological activity of lectins. Our research completes and provides the additional evidences for the lectin nature of stimulation effect of the molecules as the biologically active ones on the growth, development and grain productivity of spring wheat plants.

## Conclusions

The biological activity of WGA at the pre-sowing treatment of spring wheat seeds was expressed in the intensification of plant growth and development, the active accumulation of chlorophyll in leaves and an increase in the wheat grain productivity. N-acetyl-D-glucosamine – hapten of wheat lectin had a significant reducing effect on the wheat lectin biological activity that resulted in inhibition of positive lectin effects on physiological and

biochemical parameters of the spring wheat plants development. It is one of the arguments in favour of the lectin nature of these effects.

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### Біологічна активність аглютиніну зародків пшениці щодо рослин пшениці ярої за дії гаптenu N-ацетил-D-глюкозаміну

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**Мета.** Дослідження біологічної активності аглютиніну зародків пшениці (АЗП) щодо рослин пшениці ярої за дії гаптenu N-ацетил-D-глюкозаміну за передпосівної обробки насіння. **Методи.** Інгібіторний аналіз, фізіологічні (вегетаційні досліди, оцінка росту й розвитку рослин, зернової продуктивності пшениці) та біохімічні (вміст хлорофілу в листках) методи. **Результати.** У вегетаційних умовах показано, що АЗП (100 нМ) за передпосівної обробки насіння стимулював ростові процеси й формування вегетативної маси рослин (надземна маса збільшилась від 10 до 24 % у різні фази онтогенезу), а також накопичення хлорофілу у листках рослин (від 1,2 до 1,4 рази), що обумовило підвищений

рівень реалізації зернової продуктивності пшениці (кількість і маса зерен у колосі та маса 1000 зерен збільшились на 26, 26 і 6 % відповідно). Гаптен АЗП N-ацетил-D-глюкозамін (100 мМ) пригнічував позитивні ефекти лектину на рослини: від 5 до 19 % (за вегетативною масою), від 0 до 18 % (за рівнем зелених фотосинтетичних пігментів), від 3 до 13 % (за показника мизернової продуктивності рослин). Максимальний інгібуючий ефект гаптену відзначений у фазі кушіння та трубкування пшениці. **Висновки.** Біологічна активність аглютинину зародків пшениці за передпосівної обробки насіння проявлялася в інтенсифікації росту й розвитку рослин, активному синтезі хлорофілу в листках і підвищенню зернової продуктивності пшениці ярої. Гаптен суттєво зменшував біологічну активність лектину, у результаті чого блокувались позитивні ефекти його дії на рослини, що є одним із доказів їх лектинової природи.

**Ключові слова:** аглютинін зародків пшениці, гаптен, *Triticum aestivum* L., обробка насіння, хлорофіл, зернова продуктивність.

#### **Биологическая активность агглютинаина зародышей пшеницы относительно растений яровой пшеницы под влиянием гаптена N-ацетил-D-глюкозамин**

Е. В. Кириченко

**Цель.** Изучение биологической активности агглютинаина зародышей пшеницы (АЗП) под влиянием гаптена N-ацетил-D-глюкозамина при предпосевной обработке семян относительно растений пшеницы яровой.

**Методы.** Ингибиторный анализ, физиологические (вегетационные эксперименты, оценка роста и разви-

тия растений, зерновой продуктивности пшеницы) и биохимические (содержание хлорофилла в листьях) методы. **Результаты.** В условиях вегетационных опытов показано, что АЗП (100 нМ) при предпосевной обработке семян стимулировал ростовые процессы и формирование вегетативной массы растений (надземная масса увеличилась от 10 до 24 % в разные фазы онтогенеза), а также накопление хлорофилла в листьях растений (от 1,2 до 1,4 раза), что обусловило повышенный уровень реализации зерновой продуктивности пшеницы (количество и масса зерен в колосе, а также масса 1000 зерен увеличилась на 26, 26 и 6 % соответственно). Гаптен АЗП N-ацетил-D-глюкозамин (100 мМ) ингибировал положительные эффекты лектина на растения: от 5 до 19 % (по вегетативной массе), от 0 до 18 % (по уровню зеленых фотосинтетических пигментов), от 3 до 13 % (по зерновой продуктивности). Максимальный ингибирующий эффект гаптена отмечен в фазы кушения и трубкувания пшеницы. **Выводы.** Биологическая активность АЗП при предпосевной обработке семян яровой пшеницы проявлялась в интенсификации роста и развития растений, активном накоплении хлорофилла в листьях и повышении зерновой продуктивности пшеницы. Гаптен существенно снижал биологическую активность лектина, в результате чего блокировались положительные эффекты его действия на растения, что является одним из доказательств их лектиновой природы.

**Ключевые слова:** агглютинин зародышей пшеницы, гаптен, *Triticum aestivum* L., обработка семян, хлорофилл, зерновая продуктивность.

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