

## **INFLUENCE OF PRESOWING GAMMA – IRRADIATION OF AMARANTH SEEDS ON THE FREQUENCY OF MITOTIC DISORDERS IN ROOT MERISTEM**

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Studies of mitotic activity, frequency and range of chromosomal aberrations in cells of root meristem in the first post-irradiation mitotic cycles enable us to reliably assess levels of primary damage in the genetic system and activity of repairing processes.

**The aim and tasks of the study.** The purpose was to study effects of various doses of gamma-rays on the frequency of mitotic disorders in root meristem of different amaranth varieties.

The objectives were to determine the optimum temperature for germination of amaranth seeds and to select a dye for staining amaranth roots.

**Materials and methods.** Three amaranth varieties were used in the research: Students, Kharkov - 1 and Sem gamma-irradiated at the doses of 15 Gy, 30 Gy, 40 Gy, 150 Gy, 400 Gy and 700 Gy. The anaphase method was used to determine the frequency of mitotic disorders.

**Results and discussion.** In general, the frequency of mitotic disorders in amaranth root meristem after gamma-irradiation of Students, Kharkov – 1 and Sem varieties significantly varied. The varieties differed by the fragment and bridge numbers in cells, which were higher as doses of mutagen increased.

For example, 15 Gy of Students caused resulted in nine cells with disorders (five with bridges and four with fragments), and in Sem variety after exposure to the same dose eight damaged cells were found (three with fragments and five with bridges).

**Conclusions.** Thus, the research revealed that the varieties of amaranth, *A. hypochondriacus*, (Sem, Kharkov -1 and Students) were susceptible to gamma-rays. The research showed that the higher dose of gamma-rays was, the higher the frequency of chromosomal abnormalities became. 400 Gy and 700 Gy were lethal to amaranth. After 150 Gy, the percentage of disorders increased from 6.7% to 7.7% in Sem variety and in Kharkov - 1 variety.

**Key words:** *amaranth, mitotic activity, gamma rays, mitosis, cell, chromosomal abnormalities, bridges, fragments.*

UDK 635.655:575

## **EFFECT OF RHIZOBIUM BIOFERTILIZERS ON MAIN CHARACTERISTICS AND YIELD OF SOYBEAN**

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In this study, high-efficiency nitrogen-fixing rhizobium strains were prepared into rhizobium biofertilizers GF<sub>5</sub> and GF<sub>6</sub> for field trials to investigate the effect of rhizobium biofertilizers on yield of soybean. The results showed that fresh weight per plant, root nodule number per plant and fresh weight of root nodules per plant were improved after application of rhizobium biofertilizers; 100-grain weight was improved by over 3.9%. Moreover, rhizobium biofertilizer GF<sub>6</sub> exerted a more significant effect than GF<sub>5</sub>

**Key words:** *soybean, rhizobium fertilizer, yield*

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ISSN 1026-9959. Селекція і насінництво. 2016. Випуск 109.

**Analysis of publications, pose the problem.** Rhizobium is a species of rod-shaped bacteria in symbiosis with leguminous plants, which can fix nitrogen from the air and supply nutrients to plants. According to statistics, annual biological nitrogen fixation amount reaches  $1.75 \times 10^8$  t in the world [1], which is 4.34 times the annual nitrogen fertilizer production capacity [2]. The symbiotic nitrogen fixation of soybean and rhizobia can not only directly improve the nitrogen nutrition status, yield and quality of soybean [3, 4, 5, 6], but also release more fixed nitrogen into soil, thus improving soil fertility. Producing high-efficiency nitrogen-fixing rhizobium biofertilizers based on research and utilization of nitrogen fixation ability of nitrogen-fixing rhizobia can reduce applied amounts of chemical fertilizers, improve soil conditions, increase crop yields, decline environmental pollution and achieve sustainable development of agriculture, thereby protecting resources and ensuring environmental security [7, 8].

**The aim and tasks of the study.** Northeast of China is the main soybean producing areas, which account for about 70% of the national soybean planting area annually. For a long time, crop yield and production costs were increased at the same time and the plating soil was growing more harden by relying on traditional chemical fertilizer. Crop could only absorb limited fertilizer resources for growing, which could lead to natural ecological balance and agricultural sustainable development were destroyed by many unused nitrogen, phosphorus and potassium drained off into the groundwater and rive with rainfall. The new fertilizer of soil effective nitrogen fixing rhizobia, which isolated from nodules of soybean, was testing approved and applied in production. This new fertilizer could be able to improve soybean nitrogen fixation ability, reduce the environmental pollution by using long term chemical fertilizer, and produce more pollution-free products.

**Materials and methods.** Two high-efficiency nitrogen-fixing rhizobium strains were screened from multiple rhizobium strains of soybean and prepared into rhizobium biofertilizers GF<sub>5</sub> and GF<sub>6</sub>. In this study, on the basis of ecological and environmental characteristics of main soybean-producing regions in Heilongjiang Province, the effects of two rhizobium biofertilizers on yield of soybean cultivar Heinong 46 were analyzed.

**Rhizobium biofertilizers.** Rhizobium biofertilizers used in this study were prepared by the Institute of Biotechnology, Heilongjiang Academy of Agricultural Sciences.

**Soybean cultivar.** Soybean cultivar Heinong 46 was provided by the Institute of Soybean Research, Heilongjiang Academy of Agricultural Sciences.

**Experimental design.** The field trials were carried out in the experimental base of Heilongjiang Academy of Agricultural Sciences. The experimental soil was black loam containing 2.78% of organic matter, 0.139% of total nitrogen, 156.7 mg/kg soluble nitrogen, 48.17 mg/kg available phosphorus and 161.2 mg/kg available potassium, pH 6.65. A total of three treatments were designed. Treatment 1: rhizobium biofertilizer GF<sub>5</sub>; treatment 2: rhizobium biofertilizer GF<sub>6</sub>; treatment 3: control (CK). The applied amount of rhizobium biofertilizers was 45 kg/hm<sup>2</sup>. The rhizobium biofertilizers were applied at the depth of 1-2 cm below seeds. 160 kg/hm<sup>2</sup> diammonium phosphate was applied as seed fertilizer. A randomized block design was employed in three replicas. The area of each plot was 21 m<sup>2</sup>.

**Field survey.** At the initial flowering stage, peak flowering stage and seed filling stage, 10 seedlings in each treatment were surveyed to investigate plant height, fresh weight per plant, root nodule number per plant and fresh weight of root nodules per plant. At the mature stage, soybean seeds in each plot were tested and harvested to record the yield.

**Data processing.** Variance analysis of experimental data was performed. Experimental data were analyzed using Microsoft Excel and DPS7.05 statistical software.

**Results and discussion. Effect of rhizobium biofertilizers on main characteristics of soybean.** According to the survey results (Table 1), after application of rhizobium biofertilizer GF<sub>6</sub>, the plant height, fresh weight per plant, root nodule number per plant and fresh weight of root nodules per plant of soybean plants were higher than those in the control group. Specifically, the plant height, fresh weight per plant, root nodule number per plant and fresh weight of root nodules per plant were improved by 1.0%, 12.7%, 35.4% and 26.3% at the initial flowering stage, 3.0%, 4.2%, 23.7% and 52.6% at the peak flowering stage, 3.5%, 21.2%, 17.6% and 62.8% at the

seed filling stage, respectively. In treatment 1, at the initial flowering stage, plant height, fresh weight per plant, root nodule number per plant and fresh weight of root nodules per plant of soybean exhibited no significant differences compared with the control; at the peak flowering stage, the plant height, fresh weight per plant, root nodule number per plant and fresh weight of root nodules per plant of soybean were improved by 2.4%, 2.5%, 16.2% and 35.1% compared with the control, respectively; at the seed filling stage, plant height, fresh weight per plant, root nodule number per plant and fresh weight of root nodules per plant of soybean were improved by 2.1%, 16.0%, 1.2% and 59.0% compared with the control, respectively.

Table 1

**Effects of rhizobium biofertilizers on the main characteristics of soybean at different growth stages**

Growth stage	Treatment	Plant height, cm	Fresh weight per plant, g	Root nodule number per plant	Fresh weight of root nodules per plant, g
Initial flowering stage	1	30.5±0.1aA	26.9±0.1aA	38.3±0.1aA	0.43±0.01aA
	2	30.9±0.2aA	28.3±0.2bA	51.2±0.3bA	0.48±0.02bA
	CK	30.6±0.1aA	25.1±0.1aA	37.8±0.2aA	0.38±0.01aA
Peak flowering stage	1	67.3±0.1bA	48.9±0.2bA	70.2±0.2bB	0.77±0.03bB
	2	68.5±0.2cA	49.8±0.3cA	74.7±0.3cB	0.87±0.03cB
	CK	65.7±0.1aA	47.7±0.1aA	60.4±0.4aA	0.57±0.02aA
Seed filling stage	1	82.4±0.1bA	70.1±0.2bB	105.6±0.3bB	1.24±0.01bB
	2	83.5±0.1cA	73.2±0.1cB	109.1±0.4cB	1.27±0.02cB
	CK	80.7±0.1aA	60.4±0.1aA	92.8±0.3aA	0.78±0.01aA

As Table 1 shows, at the initial flowering stage, there were no significant differences in the plant height between different treatments; fresh weight per plant, root nodule number per plant and fresh weight of root nodules per plant in treatment 2 were significantly different from those in the control group, but these indices exhibited no significant differences between treatment 1 and CK. At the peak flowering stage, the plant height and fresh weight per plant in treatment 1 and treatment 2 varied significantly compared with those in the control group; the root nodule number per plant and fresh weight of root nodules per plant in treatment 1 and treatment 2 exhibited very significant differences compared with those in the control group. At the seed filling stage, the plant height in treatment 1 and treatment 2 varied significantly compared with that in the control group; the fresh weight per plant, root nodule number per plant and fresh weight of root nodules per plant in treatment 1 and treatment 2 exhibited very significant differences compared with those in the control group. In addition to the plant height at the initial flowering stage, the plant height, fresh weight per plant, root nodule number per plant and fresh weight of root nodules per plant in treatment 1 and treatment 2 varied significantly between the three different growth stages, which indicated that both rhizobium biofertilizers, GF<sub>6</sub> and GF<sub>5</sub>, could enhance nitrogen fixation ability of soybean roots and improve vegetative growth indices such as plant height and fresh weight per plant, resulting in long durations of nodulation and nitrogen fixation. Moreover, rhizobium biofertilizer GF<sub>6</sub> exerted a more significant effect than GF<sub>5</sub>.

**Effect of rhizobium biofertilizers on the grain yield and yield components of soybean.** As Table 2 shows, after application of rhizobium biofertilizers, the pod number per plant was improved by 8% and 11%, respectively; the grain number per plant was improved by 17% and 25%, respectively; the 100-grain weight was improved by 0.8 and 1.7 g, respectively; the yield was increased by 414.7 and 478.7 kg/hm<sup>2</sup> compared with the control, which corresponded to 14.5% and 16.8%, respectively. According to the results of variance analysis, there were very significant differences in the yield between treatment 2 and the control and between treatment 1 and the control; there were significant differences in the yield between treatment 1 and treatment 2, which indicated that applying rhizobium biofertilizers could very significantly improve the yield of soybean. In addition, rhizobium biofertilizer GF<sub>6</sub> exerted a more significant effect than GF<sub>5</sub>.

Table 2

**Effects of rhizobium biofertilizers on the grain yield and yield components of soybean**

Treatment	Pod number per plant	Grain number per plant	100-grain weight, g	Yield, kg/hm <sup>2</sup>	Yield increment compared with control, kg/hm <sup>2</sup>	Yield growth rate compared with control, %
2	52	124	22.0±0.3	3330.0cB	478.7	16.8
1	49	116	21.1±0.3	3265.6bB	414.3	14.5
CK	41	99	20.3±0.2	2851.3aA		

**Conclusions.** Most legumes are capable of symbiotic nitrogen fixation, which can be realized only through symbiosis with rhizobia. Practical experience in agricultural production shows that rhizobium inoculum inoculation can improve yields of leguminous crops and reduce environmental pollution by declining applied amounts of chemical nitrogen fertilizers, thus contributing to sustainable development of agriculture. The effect of inoculation on yield depends primarily on competitive nodulation ability and nitrogen fixation efficiency of rhizobia. Therefore, selection and application of rhizobium strains with strong competitive nodulation ability and high nitrogen fixation efficiency are the key to improve the effect of rhizobium inoculum.

In the present study, the results show that the fresh weight per plant, root nodule number per plant and fresh weight of root nodules per plant of soybean were improved at different growth stages after application of rhizobium biofertilizers; the 1000-grain weight of soybean was improved by over 3.9% at the late growth stage. Moreover, rhizobium biofertilizer GF<sub>6</sub> exerted a more significant effect than GF<sub>5</sub>. It suggests that rhizobium biofertilizer GF<sub>6</sub> has better affinity and adaptability, stronger competitive nodulation ability and higher nitrogen fixation efficiency. Rhizobium biofertilizers can significantly improve nitrogen fixation efficiency and promote growth, development and dry matter accumulation of soybean, thereby improving the yield.

In plot trials, yield growth rate is not only related to soil fertility and indigenous bacteria amount in experimental plots, but also associated with climatic conditions and field management measures. Therefore, during application of rhizobium biofertilizers, actual soil fertility should be considered, combined with rational use of chemical nitrogen, phosphate and potassium fertilizers. Moreover, intertillage, weeding, timely irrigation, pest control and other agrotechnical measures should also be strengthened for an effective role of rhizobia in fixation [9].

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#### **ВПЛИВ RHIZOBIUM BIOFERTILIZERS НА ОСНОВНІ ХАРАКТЕРИСТИКИ І ВРОЖАЙНІСТЬ СОЇ**

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**Мета і задачі дослідження.** Нові rhizobium biofertilizers з бульбочкових бактерій будуть покращувати азотфіксацію сої, сприяючи тим самим підвищенню врожайності, скороченню вимивання азоту та інших поживних елементів ґрунтовими водами в ріки, що покращить екологічне становище.

**Матеріали і методи.** Матеріалом для дослідження були rhizobium biofertilizers GF<sub>5</sub> і GF<sub>6</sub> та сорт сої Heinong 46. Варіанти дослідів: застосування rhizobium biofertilizers GF<sub>5</sub> і GF<sub>6</sub>, контроль. На початку цвітіння, на піку цвітіння та в стадії наливу насіння у 10 рослин у кожному варіанті дослідів було визначено висоту, вагу зеленої рослини, кількість бульбочок на корені, вагу бульбочок. Після дозрівання було визначено врожайність насіння сої.

**Обговорення результатів.** У результаті дослідження встановлено, що застосування rhizobium biofertilizers GF<sub>6</sub> істотно підвищило показники висоти рослин, ваги зеленої рослини, кількості бульбочок на корені, ваги бульбочок у порівнянні з контролем. Зокрема, висота рослин зросла на 1,0 % у фазі початку цвітіння, на 3,0 % на піку цвітіння, на 3,5 % у фазі наливу насіння. Вага зеленої рослини зросла на 12,7 %, 4,2 % і

21,2 %, кількість бульбочок – на 35,4 %, 23,7 % і 17,6 %, вага бульбочок – на 26,3%, 52,6 % і 62,8 % відповідно.

Застосування rhizobium biofertilizers GF<sub>5</sub> не покращувало показники на початковій стадії цвітіння. В інших фазах відмічено покращення у порівнянні з контролем, але менше, ніж у варіанті з rhizobium biofertilizers GF<sub>6</sub>. На кількість бобів і зерна застосування GF<sub>6</sub> також мало більш істотний вплив, ніж GF<sub>5</sub>.

**Висновки.** Rhizobium biofertilizers GF<sub>6</sub> на всі показники сої впливало більш істотно, ніж GF<sub>5</sub>. Таким чином, GF<sub>6</sub> має більш високу конкурентоздатність бульбочок і більш високу ефективність фіксації азоту. Це може значно підвищити врожайність сої.

*Ключові слова: соя, rhizobium fertilizer, урожайність*

## **ВЛИЯНИЕ RHIZOBIUM BIOFERTILIZERS НА ОСНОВНЫЕ ХАРАКТЕРИСТИКИ И УРОЖАЙНОСТЬ СОИ**

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**Цель и задачи исследования.** Новые rhizobium biofertilizers из клубеньковых бактерий будут улучшать азотфиксацию сои. Тем самым будут способствовать повышению урожайности, сокращению вымывания азота и других питательных элементов грунтовыми водами в реки, что улучшит экологическую ситуацию.

**Материалы и методы.** Материалом для исследования были rhizobium biofertilizers GF<sub>5</sub> и GF<sub>6</sub> и сорт сои Heinong 46. Варианты опытов: применение rhizobium biofertilizers GF<sub>5</sub> и GF<sub>6</sub>, контроль. В начале цветения, на пике цветения и в стадии налива семян у 10 растений в каждом варианте опыта были определены высота, вес зеленого растения, количество клубеньков на корне, вес клубеньков. После созревания была определена урожайность семян сои.

**Обсуждение результатов.** В результате исследования установлено, что применение rhizobium biofertilizers GF<sub>6</sub> существенно повысило показатели высоты растения, веса зеленого растения, количества клубеньков на корне, веса клубеньков по сравнению с контролем. В частности, высота растений улучшилась на 1,0 % в фазе начала цветения, на 3,0 % на пике цветения, на 3,5 % в фазе налива семян. Вес зеленого растения улучшился на 12,7 %, 4,2 % и 21,2 %, количество клубеньков – на 35,4 %, 23,7 % и 17,6 %, вес клубеньков – на 26,3%, 52,6 % и 62,8 % соответственно.

Применение rhizobium biofertilizers GF<sub>5</sub> не оказывало улучшения показателей на начальной стадии цветения. В других фазах отмечено улучшение по сравнению с контролем, но меньше, чем в варианте с rhizobium biofertilizers GF<sub>6</sub>. На количество бобов и зерна применение GF<sub>6</sub> также оказало более существенное влияние, чем GF<sub>5</sub>.

**Выводы.** Rhizobium biofertilizers GF<sub>6</sub> на все показатели сои оказало более существенное влияние, чем GF<sub>5</sub>. Таким образом, GF<sub>6</sub> имеет более высокую конкурентоспособность клубеньков и более высокую эффективность фиксации азота. Это может значительно повысить урожайность сои.

*Ключевые слова: соя, rhizobium fertilizer, урожайность*

## ***EFFECT OF RHIZOBIUM BIOFERTILIZERS ON MAIN CHARACTERISTICS AND YIELD OF SOYBEAN***

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**The aim and tasks of the study.** New rhizobium biofertilizers from nodule bacteria will improve nitrogen fixation of soybean. Thus, they will enhance yield capacity, reduce the groundwater leaching of nitrogen and other nutrients in rivers, improving the ecological situation.

**Materials and methods.** Rhizobium biofertilizers GF<sub>5</sub> and GF<sub>6</sub> and soybean cultivar Heinong 46 were the test material. The experimental variants: application of rhizobium biofertilizers GF<sub>5</sub> and GF<sub>6</sub>, control. Plant height, green plant weight, root nodule number per root, nodule weight were measured in 10 plants at the beginning of flowering, at the peak of flowering and at the seed filling stage for each experimental variant. Soybean seed yield was determined after ripening.

**Results and discussion.** The study found that rhizobium biofertilizer GF<sub>6</sub> significantly increased the plant height, green plant weight, nodule number per root, and nodule weight as compared to the control. In particular, the plant height was improved by 1.0% at the beginning of flowering, by 3.0% at the peak of flowering, and by 3.5% at the seed filling phase. The green plant weight was improved by 12.7%, 4.2% and 21.2%, respectively; the nodule number - by 35.4%, 23.7% and 17.6%, respectively; the nodule weight – by 26.3%, 52, 6% and 62.8%, respectively.

Rhizobium biofertilizer GF<sub>5</sub> did not improved performance at the initial stage of flowering. Some improvement was seen as compared the control at other phases, but it was smaller than that with rhizobium biofertilizer GF<sub>6</sub>. GF<sub>6</sub> also more significantly affected the pod and seed numbers than GF<sub>5</sub>.

**Conclusions.** Rhizobium biofertilizer GF<sub>6</sub> had more significant effects on all the parameters than GF<sub>5</sub>. Thus, nodules are more competitive, and the efficiency of nitrogen fixation is higher with GF<sub>6</sub>. This can significantly increase soybean yields.

*Key words: soybean, rhizobium fertilizer, yield*