UDC 579.262:582. 736:631.461.5+631.348

Brovko I.,

Tltova L.,

Candidate of Biological Sciences

lutlnskaya G.

Corresponding Member of the National Academy of Sciences of Ukraine, Doctor of Biological Sciences, Institute of Microbiology and Virology. DK Zabolotny National Academy of Sciences of Ukraine

Formation of symbiotic systems at soya bean of different genotypes at pesticide load

The purpose. To study singularities of formation and operation of symbiotic systems at soya bean of different genotypes in conditions of pesticide loading.

Methods. Weight, acetylene-reductase and statistical.

Results. Negative Influence of herbicide is fixed on formation of nodules by industrial strain of Bradyrhizobiumjaponicum UKM B-6035, with simultaneous increase in nitrogen-fixing activity of symbiolic systems.

Conclusions. Formation of efficient sym-biosis of a strain with glyphosat-tolerant soya bean on chemozem soil which was multiply processed with glyphosat is shown. Thus productivity of grain was enlarged for 46%.

Key words: symbiotic systems, genotypes of soya bean, transgenic soya bean, glyphosat, nitrogen-fixing activity, productivity.

In agriculture, the problem of preserving the ecological stability of soils, increasing the yield and quality of crop production is relevant. Particularly acute is the issue of increasing the yield of leguminous crops, which is a source of valuable protein, balanced by the amino acid composition. To solve it, new biotechnologies and varieties adapted to environmental conditions and modern land use are being developed and introduced into production. Soybeans are considered as a valuable place in the agroindustrial complex of Ukraine as a valuable leguminous oilseed crop with a unique grain quality, high productivity and economical production. In addition to the development of promising agrotechnologies and the selection of new varieties (in particular, genetically modified), it is extremely important to study the peculiarities of soybean interaction of different genotypes with specific rhizobia, the sensitivity of new soybean varieties to inoculation, and the effect of pesticidal background on the efficiency of symbiotic systems. The purpose of the research is to study the peculiarities of the formation and functioning of symbiotic systems in soybeans of different genotypes under conditions of pesticide loading. Materials and methods of research. Objects of research - a highly effective production strain of tuberous soybean bacteria from the collection of the Department of General and Soil Microbiology of the Institute of Microbiology and Virology. DK Zabolotnoy National Academy of Sciences of Ukraine Bradyrhizobium japonicum УКМ B-6035 and soybean plants Podolsk 416 and the glyphosate-tolerant line 40-3-2. Soybean variety Podolsk 416 (Selection of the Institute of Agroecology and Natural Resources of NAAS and Vinnitsa State Agrarian University) - medium-high, high-yielding, resistant to precipitation, with high cold and drought tolerance. Favorable areas for its cultivation - Forest-steppe and Steppe. Line transgenic soybean 40-3-2 (glyphosate tolerant soybean, GTS) was created by Monsanto Canada Inc. Its main property is resistance to glyphosate, which is used to control weeds. The line GTS 40-3-2 was obtained by the technology of recombinant

DNA with the insertion of a bacterial glyphosate-stable gene of the enzyme 5-enolpyruvilshikmat-3-phosphatase (EPPSH, ES 2.5.1.19) [10]. Glyphosate (N-phosphonomethylglycine) - the active ingredient of the roundabout herbicide. It inhibits the activity of the plant enzyme, EPPSP, involved in the biochemical pathways for the synthesis of aromatic amino acids such as phenylalanine, tyrosine and tryptophan, which leads to inhibition of growth and subsequent death of plants. Thanks to the genetic modification of the GTS, it is insensitive to glyphosate and is able to synthesize aromatic amino acids under conditions of herbicide application. Experiments were carried out at the vegetation house of the Institute of Microbiology and Virology D.K. Zabolotny National Academy of Sciences of Ukraine. Soybeans were grown in ceramic vessels with a volume of 3 liters on 2 soil substrates: chernozem typical of deep, low-humus, large-peal-and-liver, selected on fields, for a long time treated with a roundabout herbicide (Bila Tserkva Research and Selection Department of the Institute of Bioenergetic Cultures and Sugar Beets, NAAS), and on peat drying (Joint-Stock Company Scientific-production enterprise of state enterprise "Gavrushchina peat"). The research scheme contained the following options: seed inoculation; seed inoculation and herbicide treatment of plants; treatment with herbicide of non-inoculated plants; control (without inoculation and without treatment with herbicide). Seed inoculation was carried out by production strain B. japonicum UKM B-6035 with a bacterial load of 107 kg / seed. In versions with herbicide treatment, a commercial roundup (Monsanto, USA) was used according to the manufacturer's instructions. The mass of root tubers and grains was determined according to generally accepted methods. The nitrogenase activity of soybean bulbs was evaluated in the budding phase - the beginning of flowering by the acetylene-reductase method [6]. Statistical processing of the results of experiments was carried out using standard computer programs. Research results. The influence of the roundabout on the formation of the symbiotic apparatus of the GTS on the soil sobs substrates under investigation (Table 1) is established. On the chernozem ground of the fields where technologies using glyphosate were used, the largest notational apparatus in GT plants was formed by inoculation of the strain B. japonicum strain UKM B-6035 without using the roundabout: the number of tubers was 4.4 times that of control.

1. *Formation and nitrogen fixation activity of the symbiotic apparatus of the glyphosate tolerant soybean line 40-3-2 (budding phase - the beginning of flowering)*

Варіант	Контроль	Інокуляція *B. japonicum*	Інокуляція, обробка раундапом	Обробка раундапом
Чорнозем типовий малогумусний				
Азотфіксувальна активність, мкмоль C_2H_2/росл./год	0,37±0,01	0,22±0,08	0,27±0,1	0,42±0,03
Кількість бульбочок, шт./росл.	5±0	22±1	15±0	17±2
Маса бульбочок, г/росл.	0,35±0,05	0,28±0,01	0,33±0,02	0,49±0,03
Урожай, г/росл.	1,3±0,19	1,9±0,28	1,7±0,25	1,4±0,21
Торфосуміш				
Азотфіксувальна активність, мкмоль C_2H_2/росл./год	0,73±0,02	0,19±0,02	0,74±0,24	0
Кількість бульбочок, шт./росл.	38±1	58±0	48±1	0
Маса бульбочок, г/росл.	0,49±0,01	0,37±0,01	0,39±0,01	0
Урожай, г/росл.	3,6±0,50	1,5±0,22	1,9±0,28	1,8±0,27

2. Formation and nitrogen fixation of symbiotic apparatus of Soybean variety Podolsk 416 (budding phase - the beginning of flowering)

Варіант	Контроль	Інокуляція *B. japonicum*	Інокуляція, обробка раундапом	Обробка раундапом
Чорнозем типовий малогумусний				
Азотфіксувальна активність, мкмоль C_2H_2/росл./год	0,39±0,06	0,60±0,10	0,42±0,06	0,60±0
Кількість бульбочок, шт./росл.	8±0	37±0	23±1	8±0
Маса бульбочок, г/росл.	0,38±0,05	0,58±0,02	0,60±0,03	0,40±0
Урожай, г/росл.	2,0±0,30	1,9±0,28	*	*

* Загибель рослин наприкінці вегетації.

For treatment of inoculated plants by roundup there was a suppression of bulbous formation in 1,5 times, however, while the actual nitrogen fixation activity of the symbiotic device increased by 1,2 times. In the conditions of spontaneous inoculation against the background of the application of the roundabout, the nodulation process increased in comparison with the control by 3.4 times, while simultaneously increasing the nitrogen-fixing activity of the tubers by 13.5%. In the variant with inoculation with a production strain without application of the roundup, the highest yield of grain was obtained, which was 46.2% higher than the soybean yield in the control variant. In the peat mix, in the control variant, the number of tubers formed in HT-plants by aboriginal strains was 1.5 times lower than in the variant with inoculation by the production strain (see Table 1). In both cases, the depressant effect of the roundabout has been recorded on the notation process. The number of bulbs in the variant with inoculation decreased for processing by a roundabout by 17.2%. In contrast to the results obtained in the experiment with chernozem, where there was marked the lapse stimulation of the endodontic activity of endemic populations of rhizobium, the cultivation of soybeans on peat mixing resulted in complete inhibition of spontaneous bulb formation on the pesticidal background. Perhaps this is due to the adaptation of aboriginal rhizobium to the roundabout in chernozem soils under the seeds of GT-soya. Thus, according to the authors [8], from the rhizomes grown on a medium enriched with glyphosate, the gene CP4-EPPSF, which is the basis of the genetic engineering of the GTS, was isolated. This suggests that in the selective conditions of glyphosate action, some representatives of bacterial populations to this herbicide can be resisted by spontaneous mutations or by horizontal transference of genes. A similar effect was observed during the study of non-target exposure of herbicides to rhizospheric microorganisms [2, 3, 5, 9]. The described processes are likely to affect the development of microbiocenoses for multiple use of glyphosate, which affects the effectiveness of microbial and plant systems. Nodulatory apparatus of inoculated plants on chernozem and on peat susbrastat was distinguished by the largest number of tubers, but the smallest nitrogen fixing activity, which increased by 3,9 times for processing by a roundup. Consequently, the roundabout inhibited the formation of tubers by the production strain in the GTS on both substrates. However, there was stimulation of the nitrogen-fixing activity of the tubers, which may be due to the high adaptive potential of the strain. In addition, some rhizobia use glyphosate or products of its degradation as a substrate [4]. Other organs of a soil biota are capable of destroying organophosphates [1, 7]. During the study of the effectiveness of the industrial strain Bradyrhizobium japonicum UKM V-6035, Podolsk 416 as a substrate used chernozem non-humus soil in the field of the Bila Tserkva Research and Selection Division of the Institute of Bioenergetic Cultures and Sugarbeet NAN (Table 2). In the variant with inoculation, against the background of the application of roundabout, the inhibition of bulbous formation and

nitrogen fixation activity was observed in 1.6 and 1.4 times, respectively, compared to inoculated plants on the natural background. At the same virulence endemic rhizobia in the control and in the version with a roundup, observed increase in nitrogen-fixing activity of the tubers in 1,5 times under the conditions of herbicide application.

Conclusions

For growth of the GTS on both substrates, the roundabout inhibited the formation of tubers by the production strain, but stimulated their nitrogen fixation activity. The productive strain Bradyrhizobium japonicum UKM B-6035 on the chernozem ground exposed to the multi-year influence of glyphosate formed an effective symbiosis with glyphosate-tolerant soybean, which contributed to a 46% increase in grain yield. Aboriginal rhizobia of chernozem soils were adapted to glyphosate and showed greater virulence and nitrogen fixation activity, while the physiological activity of endemic rhizobium of peat mix was completely suppressed by a roundabout.

Bibliography

1. Ermakova IT Microbial destruction of organophosphates by soil bacteria / I.T. Ermakova, T.V. Shushkova, AA Leontievsky // Microbiology. - 2008. - T. 77, No. 5. - P. 689 - 695.

2. Araujo A.S.F. Effect of glyphosate on the microbial activity of two Brazilian soils / A.S.F. Araujo, R.T.R. Monteiro, R.B. Abarkeli // Chaemosphaere. - 2003. - 52, No. 5. - P. 799 - 804.

3. Chakravarty P. Non-target effect of herbicides: I. effect of glyphosate and hexazinone on soil microbial activity. Microbial population and in-vitro growth of ectomycorrhizal fungi / P. Chakravarty, L. Chatarpaul // Issue Pesticide Science. - 1990. - V. 28, No. 3. - P. 233 -241.

4. Degradation of herbicide glyphosate by members of the family Rhizobiaceae / Liu c.-M., P.A. McLean, C.C. Sookdeo et al.//Appl. Environ Microbial - 1991. - V. 57. - P. 1799 - 1804.

5. Glyphosate affects microorganisms in rhizospheres of glyphosate-resistant soybeans / L.H.S. Zobiole, R.J. Kremer, Jr R.S. Oliveira, J. Constantin / / J. of Appl. Microbiol - 2011. - V. 110, No. 1. - P. 118 - 127.

6. Hardy R.W.F. Application of the acetyleneethylene assay for measuring nitrogen fixation / R.W.F. Hardy, R.C. Burns, R.D./Holsten // Soil. Biol Biochem - 1973. - 5, No. 1. - P. 41 - 83.

7. Metabolism of gluphosate in Pseudomonas sp. Strain LBr / G.S. Jacob, L.E. Garbow, L.E. Hallas et al.//Appl. Environ Microbiol - 1988. - V. 54, No. 12. - P. 2953-2955.

8. Molecular basis for herbicide resistance of Roundup Ready crops / T. Funke, H. Han, M.L. Healy-Fried et al .//Proc. Natl Acad Sci USA - 2006 - 103. - P. 13010 -13015.

9. Ratcliff A.W. Changes in the microbial community structure following herbicide (glyphosate) additions to forest soils / A.W. Ratcliff, M.D. Busse, C.J. Shestak // Applied Soil Ecology. - 2006. - 34, No. 2. - P. 114 - 124.

10. www.transgen.de/anbau/eu_international

Released 15.12.2014.