THE CONTENT OF HYDROGEN PEROXIDE AND CATALASE ACTIVITY IN DIFFERENT ON EFFECTIVENESS OF SYMBIOTIC SYSTEMS *GLYCINE MAX – BRADYRHIZOBIUM JAPONICUM* UNDER DROUGHT CONDITIONS

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Aim. *To investigate the effect of prolonged drought on the changes in the content of hydrogen peroxide and catalase activity in the soybean roots and root nodules by inoculation Bradyrhizobium japonicum strains and Tn-5 mutants with contrasting symbiotic properties.* **Methods.** *Microbiological, physiological, biochemical, gas chromatography, spectrophotometry.* **Results.** *It has been established that effective symbiotic systems formed with the participation of soybeans and active strains of the B. japonicum 646 and Tn-5 mutant B1-20 differed for the manifestation of catalase activity and the content of hydrogen peroxide in the roots and root nodules during the effects of drought. However, these changes were accompanied by the development of stress – protective reactions and had an adaptive nature that led to the preservation of the effective functioning of their symbiotic apparatus in conditions of drought. In symbiotic systems formed with soybean and low-active Tn-5 mutant 107 and inactive strain 604k, increased hydrogen peroxide content and unstable activity of the enzyme under stress, as well as a poor recovery of their level to optimal in the post-stress period, were found.* **Conclusions.** *Efficiency of functioning of symbiotic systems Glycine max – B. japonicum for prolonged exposure to drought is marked by adaptive changes in the activity of catalase in the roots and root nodules that induce the regulation of the content of hydrogen peroxide and are accompanied by the preservation the work of symbiotic apparatus.*

Keywords: Glycine max (L.) Merr., Bradyrhizobium japonicum, hydrogen peroxide, catalase, reactive oxygen species, drought.

Plants are constantly exposed to the external environment, so they must quickly and effectively respond to environmental fluctuations. It is now consider that reactive oxygen species (ROS) take part in signaling the response of plant growth and development processes to biotic and abiotic stresses [1]. At the same time, the change in ROS level may be a signal of a change in the physiological and biochemical processes in cells [2, 3]. The ROS, especially hydrogen peroxide (H_2O_2), plays an important role in signaling processes in various cellular processes. It was revealed that H_2O_2 accumulation is indirectly associated with a cascade of reactions in the movement of calcium ions and activation of mitogen activated protein kinase (MAPKs) [4, 5]. In particular, it was investigated that the influence of different stressors on the soybean cells caused the formation of H_2O_2, which in turn induced a rapid influx of calcium ions and changes that led to apoptosis [6]. Another way is the MAPK-cascade, which can be activated by H_2O_2, and then transmit the signal to the nucleus for

the activation of specific transcription factors [7]. There is persuasive evidence that ROS play an important role in signaling processes for the establishment of legume-rhizobium symbiosis [8, 9, 10, 11, 12]. This interaction involves a complex molecular signaling between the host plant and the symbiont, which leads to infection of the root hairs with rhizobia and the formation of nodules in which nitrogen fixation occurs [13]. It is known that rhizobia (free-living forms) have a higher sensitivity to H_2O_2 than other types of bacteria [14, 15]. However, they are symbiotic microorganisms, and during differentiation in bacteroids, they can use the host plant's protective responses against toxic oxygen species, such as H_2O_2. In order to overcome oxidative stress during a symbiotic interaction, plants and rhizobia possess a complex of antioxidants, ROS-metabolizing enzymes such as catalase (CAT), superoxide dismutase and peroxidase [16, 17, 18].

It is consider that rhizobium infection of legumes substantially modifies the metabolism of host plants, which results in the creation of optimal conditions for infection and nodulation [19] and may be a precondition for the further effective functioning of legume-rhizobium symbiosis. In this aspect, special attention should be paid to the study of the role of ROS and antioxidant enzymes in the regulation of the symbiotic relationship between macro- and microsymbionts for optimal and adverse environmental factors.

The aim of work was to investigate the effect of prolonged drought on changes in the H_2O_2 content and the activity of CAT in the soybean root and root nodules by inoculation *B. japonicum* strains and Tn-5 mutants with contrasting symbiotic properties.

Materials and methods. The objects of the study are chosen symbiotic systems, formed with the participation of soybean (*Glycine max* (L.) Merr.) Diamond variety, various on the effectiveness of strains *Bradyrhizobium japonicum* 646 (active, virulent) and 604k (inactive, highly-virulent) and Tn-5 mutants B1-20 (active, virulent) and 107 (low-activity, virulent). We used *Bradyrhizobium japonicum* strains and Tn-5 mutants from the museum collection of symbiotic nitrogen fixation of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine.

Before sowing, soybean seeds sterilized with ethanol (70 %), then washed for 1 hour and inoculated with rhizobium culture (the titre of the suspension was 10^8 cells / ml). The culture of rhizobium was grown on solid mannitol - yeast medium for 9 days at 26 – 28 °C.

Plants were grown in sand pots in the application of a nutritional mixture of Gelrigel with 0,25 nitrogen rates from natural light and optimal water supply (60 % of full water capacity). The combined model drought was created by stopping the watering of the plants into the stage of three true leaves to 40 % of full water capacity and the budding stage to 30 % of full water capacity. After the termination of the drought, the moisture content of the substrate was brought to 60 % of full water capacity in the stage of mass flowering – the watering restoration. The control was provided by non-inoculated and inoculated rhizobium plants that grew for optimal watering (60 % of full water capacity). For research, soybeans roots and root nodules were selected in the stages of three true leaves, budding and mass flowering.

The nodulation ability of *B. japonicum* was determined by counting the number and weight of the root nodules. Nitrogen fixation activity (NFA) by acetylene method on gas chromatograph «Agilent GC system 6850» (USA) with flame-ionization detector [20]. The separation of gases was carried out on a column (Supelco Porapak N) at a thermostat temperature of 55 ° C and a detector - 150 ° C. The carrier gas was helium (20 ml per 1 minute). The volume of the analyzed sample of the gas mixture was 1 cm^3. As a standard, pure ethylene (Sigma-Aldrich, No. 536164, USA) was used.

To obtain the enzyme extract, the weight of the plant material (1:2) was homogenized with cooled 0,5 M tris-HCl buffer (pH 7,8) containing 5 mM β-mercaptoethanol and 0,1 % polyvinylpyrrolidone. The homogenate was centrifuged at 10,000 rpm (4 ºC) for 20 minutes. The supernatant used to determine the activity of the CAT (EC 1.11.1.6.) by the development of a color reaction with ammonium molybdate according to the modified method of Doliba et al. [21]. The enzyme activity was measured at optical density of 410 nm on the Smart Spec Plus spectrophotometer (USA). The results presented in units of activity of the enzyme (U) on the protein concentration (mg) in the supernatant. The content of total soluble protein in the enzyme extract was determined by Bradford [22].

The content of H_2O_2 was determined by ferrocyanic method [23]. Extraction of the plant material was carried out (ratio 1:3) with a cooled solution of 5 % trichloroacetic acid. The supernatant was obtained by centrifugation at 14,000 rpm for 5 minutes (4 °C). The concentration of H_2O_2 was determined by color reaction with potassium thiocyanate at optical density of 480 nm and calculated using a gauge curve with known concentrations of H_2O_2. The results are presented in μmol per gram mass of dry matter.

The results were statistically analyzed by Student's t-test. The data are presented as mean values and standard errors and are considered significant at P < 0,05. The tables and figures show the arithmetic mean values and their standard errors (x ± SE).

Results. It was found that with moderate drought effects in the stage of three true leaves in soybean, inoculated with the active strain of rhizobia 646, there was no decrease in the number of root nodules. However, their weight decreased almost twice. At the same time, in plants inoculated with active Tn-5 mutant B1-20, there was a decrease in both the mass of nodules (up to 57,1 %) and their number (up to 33,4 %) (Table 1).

For the subsequent influence of the moisture deficit in the stage of budding, in these symbiotic systems there was a slight inhibition of the nodulation ability of rhizobia. In particular, the mass of root nodules decreased by 34,4 and 37,8 % for inoculation by strain 646 and Tn-5 mutant B1-20 and their number even increased by 63,2 and 14,5 % respectively.

The inhibition of the nodulation processes in soybean root nodules, inoculated with the low-activity Tn-5 mutant 107 detected at the initial stages of dehydration, which was due to a significant reduction in the mass of root nodules (up to 92,2 %) and their number (up to 68,4 %). The same tendency to inhibition of nodules formation was remain also under strict conditions of drought in this symbiotic system.

Table 1

Influence of drought on the formation of soybean symbiotic apparatus by inoculated with *B. japonicum* strains and Tn-5 mutants (x ± SE, n = 8)

Variants	Stages of ontogenesis					
	three true leaves		budding		mass flowering	
	drought		drought		watering restoration	
	number, pc. / plant	mass, g / plant	number, pc. / plant	mass, g / plant	number, pc. / plant	mass, g / plant
Strain 646	31,03 ± 1,82	0,37 ± 0,021	28,04 ± 1,71	0,32 ± 0,021	40,01 ± 2,44	1,05 ± 0,061
Strain 646 + drought	32,02 ± 2,03	0,18 ± 0,012[aa]	45,73 ± 2,74[aa]	0,21 ± 0,012[a]	33,04 ± 2,01[a]	0,35 ± 0,023[aaa]
Tn-5 mutant B1-20	32,01 ± 1,91	0,42 ± 0,021	31,06 ± 1,81	0,37 ± 0,021	29,07 ± 1,74	0,72 ± 0,042
Tn-5 mutant B1-20 + drought	21,34 ± 1,32[bb]	0,18 ± 0,015[bb]	35,54 ± 2,15[b]	0,23 ± 0,013[bb]	42,21 ± 2,52[bb]	0,33 ± 0,021[bbb]
Tn-5 mutant 107	9,51 ± 0,64	0,09 ± 0,005	8,51 ± 0,51	0,17 ± 0,011	16,25 ± 0,91	0,64 ± 0,003
Tn-5 mutant 107 + drought	3,02 ± 0,21[ccc]	0,007 ± 0,0004[ccc]	5,03 ± 0,34[c]	0,04 ± 0,002[ccc]	2,08 ± 0,14[ccc]	0,05 ± 0,003[ccc]
Strain 646	72,04 ± 4,33	0,17 ± 0,014	124,61 ± 7,51	0,93 ± 0,051	103,01 ± 6,21	0,40 ± 0,023
Strain 604k + drought	67,31 ± 4,12[d]	0,01 ± 0,006[ddd]	50,22 ± 3,02[ddd]	0,05 ± 0,003[ddd]	71,72 ± 4,33[ddd]	0,26 ± 0,014[ddd]

Note: data compared to the control are reliable at [a b c d] – P < 0,05, [aa bb cc dd] – P < 0,01, [aaa bbb ccc ddd] P < 0,001 ([a] and [d] – relative to the variants by inoculated with strains 646 and 604к, [b] and [c] – relative to the variants by inoculated wiht Tn-5 mutants B1-20 and 107)

For moderate water stress, soybean plants in symbiosis with an inactive strain of rhizobium 604k did not undergo noticeable changes in the number of root nodules, however showed a significant decrease in their mass (by 94,1 %). For prolonged dehydration in the budding stage there was an inhibition of nodules formation – a decrease in the number (by 59,7 %) and mass (by 94,6 %) of nodules on plant roots, inoculated with inactive strain of rhizobium 604k.

It was investigated that moderate drought in the stage of the three true leaves resulted in a decrease in the total NFA of the root nodules (by 18,5 %) in the symbiotic system formed with the active strain of rhizobium 646 (Fig. 1). For prolonged exposure to stressor in the stage of budding NFA of symbiotic apparatus reduced by 54,8 %. In the symbiotic system formed with the active Tn-5 mutant B1-20, the total NFA of the root nodules was suppressed by 45,2 – 58,6 % during the effects of drought.
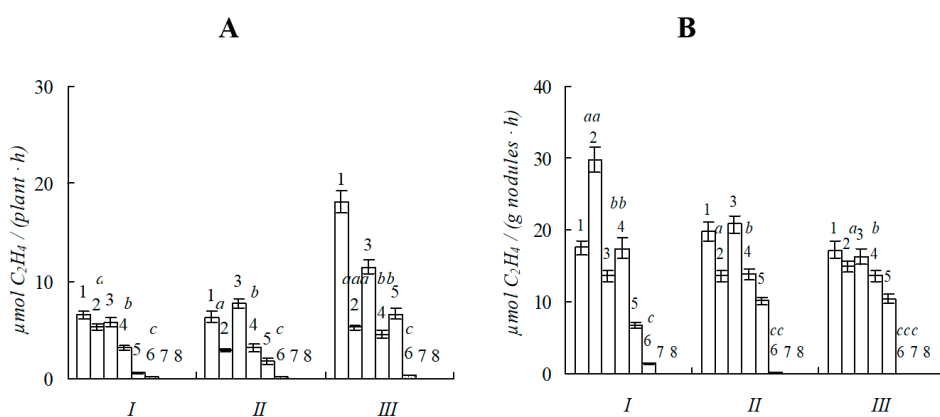

Fig. 1. Influence of drought on the total (A) and specific (B) nitrogen fixation activity of soybean root nodules by inoculated with *B. japonicum* strains and Tn-5 mutants
1 – strain 646, 2 strains 646 + drought, 3 – Tn-5 mutant B1-20,
4 – Tn-5 mutant B1-20 + drought, 5 – Tn-5 mutant 107, 6 – Tn-5 mutant 107 + drought,
(7 – strain 604k, 8 – strain 604k + drought)*. (x ± SE, n = 8), the data compared with the control are reliable at [a b c] − P < 0,05, [aa bb cc] − P < 0,01, [aaa bbb ccc] P < 0,001 ([a] – relative to the variant by inoculated with strain 646, [b] and [c] − relative to the variants by inoculated with Tn-5 mutants B1-20 and 107). Stages of ontogenesis: *I* – the three true leaves, *II* – the budding, *III* - the mass flowering (* – in variants 7 and 8 not detected nitrogen fixation activity)

At the same time, the specific NFA of the root nodules increased in plants, which were inoculated by *B. japonicum* strain 646 and Tn-5 mutant B1-20, respectively, at 69,8 and 28,7 % at the initial stages of dehydration and decreased by 31,1 and 33,4 % for prolonged dehydration (Fig. 1). This indicates the preservation of the functioning of the symbiotic apparatus in effective symbiotic systems in conditions of drought. Drought led to a significant decrease of total (by 98,3 – 99,7 %) and specific(by 78,5 – 98,9 %) NFA of root nodules in plants inoculated with a low-activity Tn5 mutant 107.

After the restoration of the watering of plants into the stage of mass flowering, the restoration of water balance and the functioning of the symbiotic apparatus was observed in plants, inoculated by the active strain of rhizobia 646 and the Tn-5 mutant B1-20. In spite of the fact that in effective symbiotic

systems, the processes of nodulation and total NFA were almost twice lower, but the specific NFA of the root nodules was reduced by only 30 %. This indicates the restoration of the symbiotic apparatus under the optimal conditions for water supply. In post-stress period, there was no restoration the processes of nodulation in plants by inoculated with an inactive strain of rhizobia 604k, as well as inhibition of the processes of nodulation and nitrogen fixation in plants inoculated with low-activity Tn-5 mutant 107.

In the symbiotic system formed with the participation of soybean plants and the active strain of rhizobia 646, the content of H_2O_2 in the root nodules increased during the drought effect by 73,4 % in the stage of the three true leaves and by 61,6 % in the stage of budding (Fig. 2). Obviously, this was a protective response of the symbiotic system to the action of a stressor (drought), which was accompanied by adaptive reorganization of the metabolism, since in the post-stress period the H_2O_2 level was restored to the optimum.

In the symbiotic system formed with the active Tn-5 mutant B1-20, there was a significant increase in the H_2O_2 content in the root nodules under the effects of drought by 26,9 and 16,2 % respectively in the stages of three true leaves and budding. After the restoration of watering, its contents was rising to a plants that grew for optimal water supply. Such data indicate a slight development of oxidation processes in the initial stages of dehydration and rapid adaptation of plants in symbiosis to growing conditions.

During the drought, accumulation of H_2O_2 in soybean root nodules, inoculated with low-activity Tn-5 mutant 107 (by 85,9 – 96,4 %) and its level decrease by 49,1 % in post-stress period was revealed. In the symbiotic system formed with the participation of soybeans and the inactive strain of rhizibia 604k, an increase in the content of peroxide (by 128,8 %) recorded during prolonged droughts, the content of which has not been restored to optimal in the post-stress period. Such changes were due to the significant development
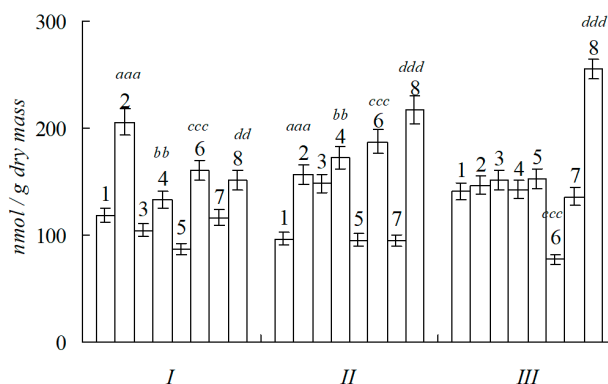

Fig. 2. Influence of drought on the content of hydrogen peroxide (H_2O_2) in soybean root nodules by inoculated with *B. japonicum* strains and Tn-5 mutants
1 – strain 646, 2 strains 646 + drought, 3 – Tn-5 mutant B1-20, 4 – Tn-5 mutant B1-20 + drought, 5 – Tn-5 mutant 107, 6 – Tn-5 mutant 107 + drought, 7 – strain 604k, 8 – strain 604k + drough. Stages of ontogenesis: *I* – the three true leaves, *II* – the budding, *III* – the mass flowering). (x ± SE, n = 5), the data compared with the control are reliable at [a b c d] – P < 0,05, [aa bb cc dd] – P < 0,01, [aaa bbb ccc ddd] P < 0,001 ([a] and [c] – relative to the variants by inoculated with strains 646 and 604к, [b] and [d] – relative to the variants by inoculated with Tn-5 mutants B1-20 and 107)

of oxidative processes and the inability of soybean plants in these symbiosis to mobilize their own protective systems and adapt to drought conditions.

In the symbiotic system, formed with the participation of soybean plants and the active strain of the rhizobia 646 observed a decrease in CAT activity in the root nodules during the drought and insignificant increase in its activity after the restoration of watering the plants (Fig. 3). This indicates the stability of the enzyme in this symbiotic system.
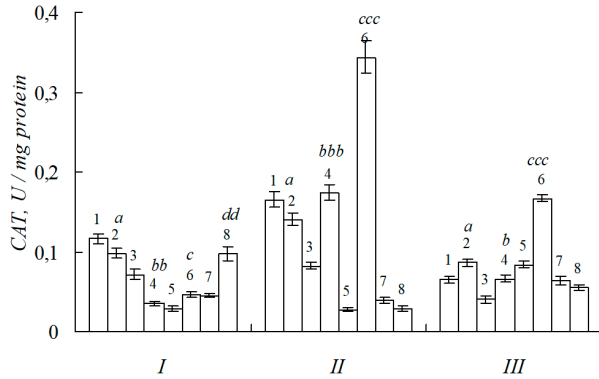

Fig. 3. Influence of drought on the catalase activity (CAT) in soybean root nodules by inoculated with *B. japonicum* strains and Tn-5 mutants
1 – strain 646, 2 – strains 646 + drought, 3 – Tn-5 mutant B1-20, 4 – Tn-5 mutant B1-20 + drought, 5 – Tn-5 mutant 107, 6 – Tn-5 mutant 107 + drought, 7 – strain 604k, 8 – strain 604k + drough. Stages of ontogenesis: *I* – the three true leaves, *II* – the budding, *III* - the mass flowering). (x ± SE, n = 5), the data compared with the control are reliable at [a b c d] − P < 0,05, [aa bb cc dd] − P < 0,01, [aaa bbb ccc ddd] P < 0,001   ([a] and [c] – relative to the variants by inoculated with strains 646 and 604к, [b] and [d] − relative to the variants by inoculated with Tn-5 mutants B1-20 and 107).

For moderate drought in the stage of three true leaves in soybean plants, inoculated with active Tn5 mutant B1-20, the activity of CAT in root nodules decreased by 50,7 %. For prolonged water stress in the budding stage, the enzyme activity increased by 112,1 % and remained at a high level in the post-stress period. This indicates the inclusion in the work of the enzyme with an increase in the action of stress (drought) and the reorganization of plant metabolism in symbiosis with nodule bacteria.

An increase in CAT activity by 12 times in soybean root nodules inoculated with low-active Tn-5 mutant 107 under conditions of prolonged drought was recorded. This intensification of the enzyme activity was accompanied by an elevated level of H_2O_2 in the root nodules (as described above) and indicates a significant development of oxidative stress in plants in this symbiosis. In the post-stress period, the activity of the CAT remained at an elevated level. In the symbiotic system of soybean and the inactive rhizobia 604k strain CAT activity in root nodules was increased by 117,7 % in the stage of three true leaves and decrease 28,2 % in the budding stages. This indicates the development of a protective reaction of enzyme for moderate dehydration and the depletion of its pool of stress. The obtained data are consistent with an increased level of H_2O_2 in root nodules. In the post-stress period, in the stage of mass flowering, CAT activity was practically restored to the level of plants with optimal watering.

It was shown that the initial stages of dehydration in a symbiotic system formed with the participation of soybean and the active strain of rhizobia 646, the H_2O_2 content in the roots increased by 34,1 % and remained almost at the same level with a prolonged water shortage, as well as after the restoration of watering the plants (Table 2). A moderate drought caused an increase in H_2O_2 level by 45,1 % in the roots of plants inoculated with the Tn-5 mutant B1-20. With prolonged exposure to water stress, its content raised by 34,4 %. In the post-stress period it was restored to the level of plants with optimal watering.

In a symbiotic system with a low-level Tn-5 mutant 107, an increase of H_2O_2 content by 47,1 % was found during long-term dehydration in the budding stage and a weak recovery of its level in the post-stress period. The content of H_2O_2 in the roots of plants, inoculated with an inactive 604k strain, increased twice during the period of drought, however was restored partly after the restoration of watering the plants. This suggests a disturbance in the balance of prooxidant-antioxidant processes in these symbiotic systems.

Table 2

Influence of drought on the content of hydrogen peroxide in soybean roots by inoculated with *B. japonicum* strains and Tn-5 mutants, nmol / g dry mass

Variants	Stages of ontogenesis		
	three true leaves	budding	mass flowering
	drought		watering restoration
Non-inoculated plants	241,71 ± 14,52	266,82 ±16,02	263,08 ± 15,81
Non-inoculated plants + drought	252,12 ± 15,14[e]	384,61 ± 23,01[e]	190,51 ± 11,44[e]
Strain 646	114,74 ± 6,82	120,74 ± 7,23	122,83 ± 7,32
Strain 646 + drought	153,91 ± 9,23[a]	171,51 ± 10,34[a]	156,32 ± 9,41[a]
Tn-5 mutant B1-20	100,32 ± 6,06	125,65 ± 7,51	133,44 ± 8,05
Tn-5 mutant B1-20 + drought	145,53 ± 8,71[b]	168,94 ± 10,12[b]	148,71 ± 8,91
Tn-5 mutant 107	115,86 ± 7,02	128,81 ±7,74	126,91 ± 7,62
Tn-5 mutant 107 + drought	147,34 ± 8,83[c]	189,42 ±11,42[c]	159,93 ± 9,61[c]
Strain 646	139,01 ± 8,34	137,13 ± 8,21	167,52 ± 10,04
Strain 604k + drought	216,12 ± 13,01[ddd]	205,35 ± 12,32[dd]	200,04 ± 12,02[dd]

Note: data compared to the control are reliable at [e a b c d] – P < 0,05, [ee aa bb cc dd] – P < 0,01, [eee aaa bbb ccc ddd] P < 0,001 ([e] − relative to non-inoculated plants with optimal watering, [a] and [d] – relative to the variants by inoculated with strains 646 and 604к, [b] and [c] − relative to the variants by inoculated wiht Tn-5 mutants B1-20 and 107).

It has been proven that prolonged drought in the budding stage induced an increase in the content of H_2O_2 in the root of non-inoculated plants by 44,1 % compared to plants that grew in optimal watering conditions. After watering restoration, the level of H_2O_2 decreased by 27,6 %. This is evidence of oppression in the functioning of plant metabolism and significant development of oxidative processes induced by insufficient water supply.

At the same time, CAT activity in the root of non-inoculated plants increased (by 228,1 %) even with moderate drought in the stage of three true leaves (Table 3). For prolonged drought in the budding stage, the enzyme activity decreased (by 75,1 %), compared to plants with optimal watering. Changes in CAT activity during the period of drought and restoration of its activity level up

to 48,5 % in the post-stress period were recorded. This is indicate a significant disruption of the antioxidant enzyme and low protective properties of plants that were not inoculated with rhizobia.

Influence of drought on the catalase activity in soybean roots by inoculated with *B. japonicum* strains and Tn-5 mutants, unit. act. / mg protein

Variants	Stage of ontogenesis		
	three true leaves	budding	mass flowering
	drought		watering restoration
Non-inoculated plants	$0,025 \pm 0,0011$	$0,036 \pm 0,0021$	$0,035 \pm 0,0022$
Non-inoculated plants + drought	$0,082 \pm 0,0052^{ee}$	$0,009 \pm 0,0013^{eee}$	$0,052 \pm 0,0031^{e}$
Strain 646	$0,075 \pm 0,0043$	$0,047 \pm 0,0032$	$0,052 \pm 0,0034$
Strain 646 + drought	$0,129 \pm 0,0074^{aa}$	$0,056 \pm 0,0031^{a}$	$0,072 \pm 0,0042^{a}$
Tn-5 mutant B1-20	$0,040 \pm 0,0021$	$0,025 \pm 0,0011$	$0,033 \pm 0,0021$
Tn-5 mutant B1-20 + drought	$0,095 \pm 0,0063^{bb}$	$0,019 \pm 0,0012^{b}$	$0,031 \pm 0,0014$
Tn-5 mutant 107	$0,037 \pm 0,0021$	$0,053 \pm 0,0031$	$0,096 \pm 0,0062$
Tn-5 mutant 107 + drought	$0,140 \pm 0,0081^{ccc}$	$0,042 \pm 0,0024^{c}$	$0,123 \pm 0,0071^{c}$
Strain 646	$0,061 \pm 0,0042$	$0,025 \pm 0,0017$	$0,050 \pm 0,0031$
Strain 604k + drought	$0,103 \pm 0,0063^{d}$	$0,009 \pm 0,0006^{dd}$	$0,028 \pm 0,0024^{d}$

Note: data compared to the control are reliable at $^{e\ a\ b\ c\ d}$ − P < 0,05, $^{ee\ aa\ bb\ cc\ dd}$ − P < 0,01, $^{eee\ aaa\ bbb\ ccc\ ddd}$ P < 0,001 (^{e} − relative to non-inoculated plants with optimal watering, ^{a} and ^{d} − relative to the variants by inoculated with strains 646 and 604к, ^{b} and ^{c} − relative to the variants by inoculated wiht Tn-5 mutants B1-20 and 107).

Soybean inoculation involving active strain of the rhizobia 646 resulted in the activation of protective systems in conditions of drought. In particular, CAT activity in roots increased by 72,1 % with moderate drought and 19,1 % under prolonged conditions of dehydration. In the post-stress period the enzyme activity was restored to an optimum level.

During the effects of drought on plants, inoculated with active Tn-5 mutant B1-20, the activity of CAT in the roots increased by 137,5 % in the stage of three true leaves and decreased by 24,1 % in the budding stage. After a stressful period, its activity achieved the level of plants with optimal watering. The obtained data testify to the different nature of the manifestation of the activity of the antioxidant enzyme – CAT due to the effects of drought in effective symbiosis formed with the participation of soybean plants and the active strain 646 and Tn-5 mutant B1-20. At the same time, changes in the activity of the enzyme in the roots of plants of both symbiotic systems were characterized by a protective effect and were aimed at regulating the content of H_2O_2 under stress.

In the symbiotic system formed with the participation of the low-level Tn-5 mutant 107, CAT activity increased by 278,4 % in the roots under moderate stress and the enzyme activity decreased by 20,7 % for a prolonged shortage of water. For exposed to drought in the stages of three true leaves, CAT activity was also increased by 68,8 % in the roots of plants inoculated with the inactive rhizobia strain 604k and its activity decreased by 64,0 % during the budding stage. After the restoration of watering the plants, the level of CAT activity in soybean roots the inoculated Tn-5 mutant 107 was increased by 28,1 %, whereas the enzyme activity was reduced by 44,0 % in the roots of soybean,

inoculated with inactive strain 604k. Such an unstable enzyme reaction was obviously associated with a disruption in its functioning due to the action of water stress.

Discussion. Changes in water balance induced by drought were accompanied by significant dislocation in the work of the symbiotic apparatus. This is evidenced by the decrease in nodulation ability and the nitrogen fixation activity of *B. japonicum* in symbiosis with soybean plants. The degree of negative impact of drought on the processes of nodulation and nitrogen fixation depended on the duration of the stressor and the adaptive potential of the formed symbiotic system. In particular, with moderate drought in the stage of the three true leaves, there was no significant suppression of the processes of the nodulation and total NFA, and even a slight increase in the specific NFA of root nodules in soybean plants inoculated with active strain 646 and Tn-5 mutant B1-20. Despite the fact that in effective symbiotic systems, the total NFA was suppressed with stress, that is, the sensitivity of fixation of molecular nitrogen at the plant level was elevated, their specific NFA root nodules did not suffer much, indicating the preservation of the work of the symbiotic apparatus. In addition, the rapid restoration of the processes of nodulation and nitrogen fixation to the optimal level in the post-stress period in effective symbiotic systems is an obvious consequence of the inclusion of protective systems and adaptation in conditions of drought. In ineffective symbiotic systems, in the initial of dehydration, the inhibition of the nodulation ability of *B. japonicum* (strain 604k and Tn-5 mutant 107) and even inhibition of NFA of the root nodules (Tn-5 mutant 107) was observed. These symbiotic systems were not able to mobilize their own defense systems and realize their symbiotic potential for drought effects after the restoration of watering.

It has been proved that H_2O_2 is induced in response of plants to the environment, in particular the action of abiotic stressors. It can act as a signaling molecule and induce expression of "protective" genes (apparently by activating protein kinases and phosphorylation of proteins), which is essential for adaptation of plants to stress [5]. Methodological approaches using DNA have allowed to evaluate the expression of genes in response to the ROS. In particular, the exposure of *Arabidopsis* cells in the presence of H_2O_2 resulted in a change of about 175 genes, of which 113 were encoded for proteins with antioxidant functions or associated with protective responses to stress, and the remaining genes were encoded for proteins with signaling functions [24].

The regulation of the nodulation process in the interaction of macro- and microsymbionts is associated with the formation of NFA and the activity of antioxidant enzymes [8, 9, 10, 11, 12]. One of the key antioxidant enzymes is CAT. It protects the aerobic cell from the toxic action of H_2O_2, which is formed in biochemical reactions with ROS [19].

It was shown that the studied symbiotic systems realized their protective mechanisms in different ways and adapted to the conditions of drought. In particular, in the symbiotic system formed with the participation of soybeans and the active strain of rhizobium 646, an increase in H_2O_2 content was observed and a slight decrease in the activity of CAT in the root nodules during drought. It was recorded that in the symbiotic system formed with the participation of the active Tn-5 mutant B1-20 there is the inclusion in the work of protective

systems for the long-term effects of stress, drought. This indicates an increase in CAT activity and an insignificant increase in H_2O_2 content. At the same time there was a rapid restoration of their level to optimal after the restoration of watering the plants. This underlines the ability of effective symbiosis to quickly mobilize their own defense systems and adapt to growing conditions.

Investigated that the symbiotic system, formed with the participation of soybean plants and the low-activity Tn-5 mutant 107, was characterized by high content of H_2O_2 and intensification of CAT activity in the root nodules during the period of drought. At the same time, in the symbiosis of plants with an inactive strain of rhizobium 604k, an increase in H_2O_2 content and a decrease in the activity of CAT in the root nodules for prolonged dehydration was observed. Both symbiotic systems had a low capability to restore the level of prooxidant and enzyme to control in the post-stress period. This indicates a significant dislocation of the prooxidant-antioxidant balance due to the development of oxidative processes in plants in these symbiosis, as well as the inability to realize their symbiotic properties in conditions of drought.

We noted that in all the studied symbiotic systems there was tendency to gradually increase the content of H_2O_2 in the roots with increasing drought and its partial restoration to the level of control in the post-stress period. It should be noted that under optimal conditions of plant growth, the total level of hydrogen peroxide in soybean roots, inoculated with rhizobia, was lower compared to plants that were not inoculated. This indicates possible changes in the prooxidant system in the roots of plants by inoculation of rhizobia.

It was proved that, regardless of the effectiveness of the symbiotic system, CAT activity in soybean roots, inoculated rhizobia increased with moderate drought. With the increase in stress, the level of CAT declined, especially in the roots of soybean, inoculated with an inactive strain of rhizobia 604k. Obviously, this could be due to the depleted enzyme pool due to the active involvement of the H_2O_2 in the work, as well as reduced synthesis and (or) increased degradation of its molecules (in the case of an ineffective symbiotic system). In the post-stress period, the restoration of the CAT activity in the roots to the optimal level is noted only in effective symbiotic systems.

Thus, the efficiency of the functioning of the symbiotic systems *Glycine max – B. japonicum* under drought conditions is marked by adaptive changes in CAT activity in root nodules and roots, which induce regulation of the H_2O_2 content and are accompanied by the preservation of the work of the symbiotic apparatus.

ВМІСТ ПЕРОКСИДУ ВОДНЮ ТА АКТИВНІСТЬ КАТАЛАЗИ У РІЗНИХ ЗА ЕФЕКТИВНІСТЮ СИМБІОТИЧНИХ СИСТЕМАХ *GLYCINE MAX – BRADYRHIZOBIUM JAPONICUM* В УМОВАХ ПОСУХИ

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Мета. Дослідити вплив тривалої посухи на зміни вмісту пероксиду водню та активність каталази у коренях і кореневих бульбочках сої за інокуляції штамами і Tn5-мутантами *Bradyrhizobium japonicum* із контрастними симбіотичними властивостями. **Методи.** Мікробіологічні, фізіологічні, біохімічні, газова хроматографія, спектрофотометрія. **Результати.** Встановлено, що ефективні симбіотичні системи, утворені за участю сої та активного штаму ризобій 646 і Tn5-мутанта B1-20, відрізнялись за проявом активності каталази та вмістом пероксиду водню у коренях і кореневих бульбочках впродовж дії посухи. Однак ці зміни супроводжувались розвитком стрес-захисних реакцій і мали адаптивний характер, що обумовило збереження ефективної роботи їх симбіотичного апарату в умовах посухи. У симбіотичних системах, утворених за участю сої та малоактивного Tn5-мутанта 107 і неактивного штаму 604к, виявлено підвищення вмісту пероксиду та нестабільну роботу активності ензиму за дії стресу, а також слабке відновлення їх рівня до оптимального у післястресовий період. **Висновки.** Ефективність функціонування симбіотичних систем *Glycine max – B. japonicum* за тривалої дії посухи відзначається адаптаційними змінами активності каталази у коренях і кореневих бульбочках, що індукують регуляцію вмісту пероксиду водню та супроводжуються збереженням роботи симбіотичного апарату.

Ключові слова: Glycine max (L.) Merr., *Bradyrhizobium japonicum*, пероксид водню, каталаза, активні форми кисню, посуха.

СОДЕРЖАНИЕ ПЕРЕКИСИ ВОДОРОДА И АКТИВНОСТЬ КАТАЛАЗЫ В РАЗЛИЧНЫХ ПО ЭФФЕКТИВНОСТИ СИМБИОТИЧЕСКИХ СИСТЕМАХ *GLYCINE MAX – BRADYRHIZOBIUM JAPONICUM* В УСЛОВИЯХ ЗАСУХИ

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Цель. Исследовать влияние длительной засухи на изменения содержания перекиси водорода и активность каталазы в корнях и корневых клубеньках сои при инокуляции штаммами и Tn5-мутантами *Bradyrhizobium japonicum* с контрастными симбиотическими свойствами. **Методы.** Микробиологические, физиологические, биохимические, газовая хроматография, спектрофотометрия. **Результаты.** Установлено, что эффективные симбиотические системы, образованные с участием сои и активного штамма ризобий 646 и Tn5-мутанта B1-20, отличались проявлением

активности каталазы и содержанием перекиси водорода в корнях и корневых клубеньках на протяжении действия засухи. Однако эти изменения сопровождались развитием стресс-защитных реакций и имели адаптивный характер, что обусловило сохранение эффективной работы их симбиотического аппарата в условиях засухи. В симбиотических системах, образованных с участием сои и малоактивного Tn5-мутанта 107 и неактивного штамма 604k, выявлено повышение содержания перекиси и нестабильная работа активности энзима при воздействии стресса, а также слабое восстановление их уровня до оптимального в послестрессовый период.

Выводы. Эффективность функционирования симбиотических систем *Glycine max – B. japonicum* при длительном воздействии засухи отмечается адаптационными изменениями активности каталазы в корнях и корневых клубеньках, которые индуцируют регуляцию содержания пероксида водорода и сопровождаются сохранением работы симбиотического аппарата.

Ключевые слова: Glycine max (L.) Merr., Bradyrhizobium japonicum, перекись водорода, каталаза, активные формы кислорода, засуха.

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