

LIPID PEROXIDATION INTENSITY OF *GLYCINE MAX - BRADYRHIZOBIUM JAPONICUM* SYMBIOTIC SYSTEMS WITH DIFFERENT EFFECTIVENESS UNDER DROUGHT CONDITIONS

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*One of the mechanisms of reactive oxygen species action on plant tissues is the activation of lipid peroxidation, which can cause damage to cell membranes. It is known about the signaling functions of both reactive oxygen species and lipoperoxidation products, as well as the dependence of the intensity of oxidative processes on the ability of plants to realize their adaptive potential under stress. **Aim.** To study the intensity of lipid peroxidation processes in nodules, roots and leaves of soybean inoculated with strains and Tn5-mutants of *Bradyrhizobium japonicum* with different symbiotic properties under drought conditions. **Methods.** Microbiological, physiological, biochemical methods, gas chromatography and spectrophotometry. **Results.** It was found that effective symbiotic systems formed from the active strain *Bradyrhizobium japonicum* 646 and Tn5-mutant B1-20 are characterized by a slight increase in the content of malondialdehyde in nodules, roots and leaves during prolonged dehydration and quick restoration of its level to optimal after exposure to stress. For ineffective symbiotic systems formed with the participation of soybean and inactive *Bradyrhizobium japonicum* 604k strain, as well as inactive Tn5-mutant 107, significant development of lipoperoxidation processes under drought conditions was observed, which indicates a violation of cell membrane integrity. **Conclusions.** Activation of the lipid peroxidation process is a universal reaction of different symbiotic systems to the effect of drought, the intensity of which depends on their ability to realize their adaptive potential under unfavorable growing conditions.*

*Keywords: *Bradyrhizobium japonicum*, *Glycine max* (L.) Merr., malondialdehyde, lipid peroxidation, symbiotic systems, drought.*

Early nonspecific response of plants to different naturally occurring stress factors is an increase in the level of reactive oxygen species (ROS) that initiate the processes of oxidative destruction of the cell membrane structures [1]. The major initiator of free radical oxidation of lipid membranes is superoxide, which is easily generated in many spontaneous and enzymatic oxidation reactions. The products of its secondary conversion may be singlet oxygen, hydroxyl radical, hydrogen peroxide, organic peroxides and their radicals [2, 3]. With the participation of ROS, the processes of lipoperoxidation lead to the destruction of polyunsaturated fatty acids and decrease the content of polar lipids and unsaturated fatty acids, the appearance of hydroperoxide groups in the hydrophobic zone of membranes [4, 5]. Such rearrangements change the fluidity and ability to lateral diffusion of membranes, which leads to a deviation in the functioning of membrane-bound enzymes, an increase in the permeability of membranes for many substances and ions [6].

Under drought an increase in the process of lipoperoxidation is accompanied by the accumulation of unsaturated fatty acids, hydroperoxides, diene conjugates, malonic dialdehyde (MDA). This leads to an increase in the fluidity of the lipid matrix of biomembranes, which increases the mobility of membrane proteins and changes the kinetic properties of membrane-dependent enzymes [7].

Lipid peroxidation (LPO) is the primary reaction in the chain of physico-chemical transformations that leads to the destruction of lipoprotein membrane complex and disrupts their transport functions, as well as inhibits the processes of energy generation, which ultimately reduce cell life [4, 7]. At the same time, these processes are the most significant in the adaptive updating and repair of functioning structures, lipoprotein membranes, capacity growth and buffering capacity of the redox system. In addition, they are important in increasing the efficiency of enzymatic and non-enzymatic antioxidant defense and in the fine regulation of

LPO reactions in membrane structures due to the functioning of mechanisms for controlling the content of ROS, lipid peroxides, and peroxidase reaction catalysts [8].

The participation of LPO activation in the mechanism of the general stress adaptation syndrome as a primary and secondary stress mediator demonstrates the importance of quantitative determination of LPO activation parameters for the timely and effective use of anti-stress measures [9]. Therefore, it is believed that the shift of equilibrium toward prooxidants is the most informative indicator for assessing the degree of influence of various factors on plant organism [10]. At the same time, LPO activation is one of the first non-specific links in the general stress response of the plant organism and can initiate the inclusion of other defense mechanisms and LPO products are considered as “indicators” and “primary mediators” of stress [4].

Despite a significant number of studies on the role of POL in the regulation of support systems for cell homeostasis in plants for the effects of stress factors, there are issues that require further study. The understanding of LPO processes involvement in the mechanisms of systems regulation that maintain cells homeostasis in microbial-plant interactions under stress influences remains poorly understood. Particularly relevant is the study of this issue in terms of the efficiency of symbiotic systems under stress, which depends on the ability of macro- and micro-symbionts in symbiosis to induce defense mechanisms and realize its symbiotic potential [11]. It was proved that LPO in soybean nodules is intensified at the initial stages of inoculation of *Bradyrhizobium japonicum*, but decreases after an increase in the content of leghemoglobin and the rate of nitrogen fixation. In addition, it was shown that MDA levels in ineffective nodules were higher than in effective [12]. Such data indicate the participation of LPO process in the ensuring of optimal functioning of plant metabolism with symbiotic interaction with nodule bacteria and the prospects for further study of its role in cell activity from mutualistic relationships. Knowledge of homeostasis regulation mechanisms with the participation of POL processes will allow developing new approaches to the search for effective symbiotic systems that are able to realize and maintain their adaptive potential due to adverse factors.

The aim of the work was to study the intensity of LPO processes in nodules, roots and soybean leaves by inoculation with strains and Tn5-mutants of *B. japonicum* with different symbiotic properties under drought conditions.

Materials and methods. The objects of the study were selected symbiotic systems formed with the participation of soybean plants (*Glycine max* (L.) Merr.) of Diamond cultivar and strains of nodule bacteria (*B. japonicum*) – 646 (active, virulent), 604k (inactive, highly-virulent) and Tn5-mutants *B. japonicum* B1-20 (B-7538) (active, virulent), 107 (low-active, virulent). We used *B. japonicum* strains and Tn5-mutants from the collection of the Symbiotic Nitrogen Fixation Department of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine.

Before sowing, soybean seeds were 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 inoculation load was 200–300 thousand rhizobia cells per seed. The cultures of rhizobia were grown on solid mannitol – yeast medium for 7 days at 26–28° C.

Plants were grown in sand pots with the application of Gerligel nutritional mixture with 0.25 nitrogen rates with natural light and optimal water supply (60 % of full moisture capacity). The combined drought model was created by decreasing the watering of plants at the stage of three true leaves to 40 % of full moisture capacity (duration – 7 days) and at the budding stage – to 30 % of full moisture capacity (duration – 5 days). After the termination of the drought, the moisture content of the substrate was increased to 60 % of full capacity at the stage of mass flowering – the watering restoration (duration – 5 days). The control was provided by non-inoculated and inoculated rhizobium plants, which grew with optimal water supply (60 % of full moisture capacity). The soybeans root nodules, roots and leaves were selected for research at the stages of three true leaves, budding and mass flowering.

The intensity of LPO was evaluated by the content of its final product – MDA as a result of color reaction with thiobarbituric acid on a spectrophotometer “Smart Spec Plus” (USA). To obtain a plant extract, a sample of the test material (0.5 g) was homogenized with 3 ml of distilled water. 3 ml of trichloroacetic acid (TCA) were added to the homogenate and suspension was

homogenized for a second time. Two samples of 2 ml were taken from the obtained homogenate into volumetric tubes with ground stoppers. To one of the samples the same volume (2 ml) of TCA was added, and then this sample was used as a control for spectrophotometry. 2 ml of TBA solution were added to the second sample. Samples were incubated for 30 min in a boiling water bath, then cooled and centrifuged for 10 min at 3000 rpm. The supernatant was carefully selected by syringe into tubes and measured on a spectrophotometer at $\lambda = 532$ nm [13]. The results were presented in nmol per 1 g of dry weight. The dry mass was determined by drying the samples of the aboveground and underground parts of the plants to a constant value at +105°C.

The results were statistically analyzed in the Statistica 6.0 (Statsoft Inc., USA) program pack. The tables and figures show the arithmetic mean values and their standard errors ($x \pm SE$). The reliability of the differences between the samples was evaluated using the single-factor dispersion analysis (ANOVA), using the Mann-Whitney U-criterion. Differences were considered to be significant at $P < 0.05$.

Results. It was found that in the symbiotic system formed with the participation of soybean plants and the active Tn5-mutant of *B. japonicum*

B1-20, no significant changes were observed in the LPO processes in root nodules at the initial stages of plant dehydration. An increase in the moisture deficit resulted in an increase in the content of MDA by 94.8 %, compared with the control – inoculated rhizobium (Tn5-mutant B1-20) plants with optimal water supply (Fig. 1). An effective symbiotic system formed with the participation of soybeans and the active rhizobia strain 646 recorded an increase in MDA content in root nodules by 42.4 and 95.1 %, respectively, with moderate (40 %) and severe (30 %) moisture deficiency. In the post-stress period, restoration of LPO processes to the optimum level in these symbiotic systems was observed, as evidenced by a slight increase in MDA content in soybean nodules by 29.6 and 38.4 %, respectively, by inoculation with rhizobia of strain 646 and Tn5-mutant B1-20.

It was shown that drought causes intensification of LPO processes in soybean inoculated with inactive *B. japonicum* 604k strain. This is evidenced by an increase in the MDA content of root nodules by 50.6 and 188.8 %, respectively, under moderate and severe dehydration conditions. After the restoration of plant irrigation, the content of MDA in nodules remained 124.6 % higher than the control level (inoculated rhizobium (604k strain) plants with optimal water supply).

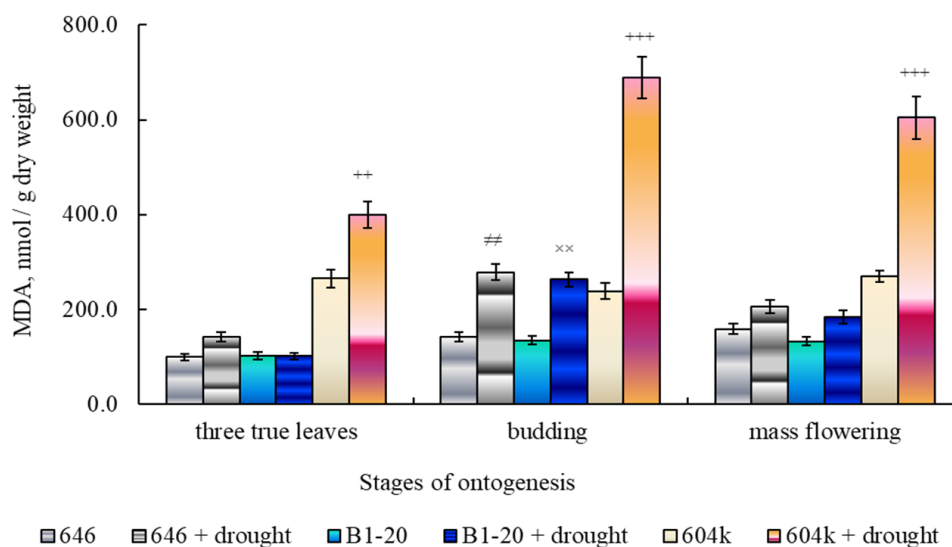


Fig. 1. Influence of drought on the content of MDA in soybean nodules inoculated with *B. japonicum* strains and Tn5-mutants ($x \pm SE$, $n = 5$). Data compared to the control (inoculated rhizobium plants with optimal water supply) are significant at $P < 0.05$ ($\#$ – relative to the variants inoculated with strains 646 and 604k; \times – relative to the variant inoculated with Tn5-mutant B1-20). Here and in Fig. 2–3 the significance of the differences between the samples was evaluated using the single-factor dispersion analysis (ANOVA), using the Mann-Whitney U-criterion.

Analysis of the effect of drought on changes in the MDA content in soybean root nodules upon inoculation with inactive Tn5-mutant 107 was not performed, since there was a significant inhibition of the nodulation processes and there was not a sufficient amount of the studied material. A similar tendency of significant suppression of the processes of nodulation and nitrogen fixation in this symbiotic system was observed in previous studies during studying the effect of drought on the peculiarities of formation and functioning of the symbiotic apparatus of soybeans [14, 15].

In the effective symbiotic systems formed with the participation of the active strains of *B. japonicum* 646 and Tn5-mutant B1-20, there is no significant increase in the processes of LPO in the roots under drought conditions (Fig. 2). In particular, the MDA content of soybean roots inoculated with strain 646 increased by 68.1 % for moderate dehydration and by 53.7 % for enhanced stressor exposure. In soybeans inoculated with Tn5-mutant B1-20, there was an increase in MDA in the roots by 49.9 % under conditions of prolonged dehydration. In the post-stress period, the processes of LPO in the roots of these symbiotic systems were restored to the level of control (inoculated rhizobium (604k strain) plants with optimal water supply).

At the initial stages of dehydration, an increase in MDA content by 81.7 % was noted in soybean roots inoculated with the inactive Tn5-mutant 107. Its level remained unchanged upon further influence of moisture deficiency and did not recover to optimal after exposure to stress.

An ineffective symbiotic system formed with the participation of soybean plants and an inactive *B. japonicum* 604k strain was characterized by an increased content of MDA in the roots by 67.7 and 106.3 % during the drought period. After the restoration of plants watering, its content in the roots was higher by 168.0 % in comparison with control level (inoculated rhizobium (604k strain) plants with optimal water supply). It indicates the significant development of LPO processes.

There has been a sharp increase in the processes of LPO in the roots of non-inoculated soybean rhizobia under moderate drought, as MDA content increased by 292.3 % compared to control (non-inoculated plants with optimal water supply). Its content increased by 326.8 % with increasing exposure to water stress while in the post-stress period its level did not recover to the optimum.

In the leaves of non-inoculated soybeans, as well as in the roots under drought conditions, a similar tendency for the development of LPO processes was recorded (Fig. 3). Thus, for moderate

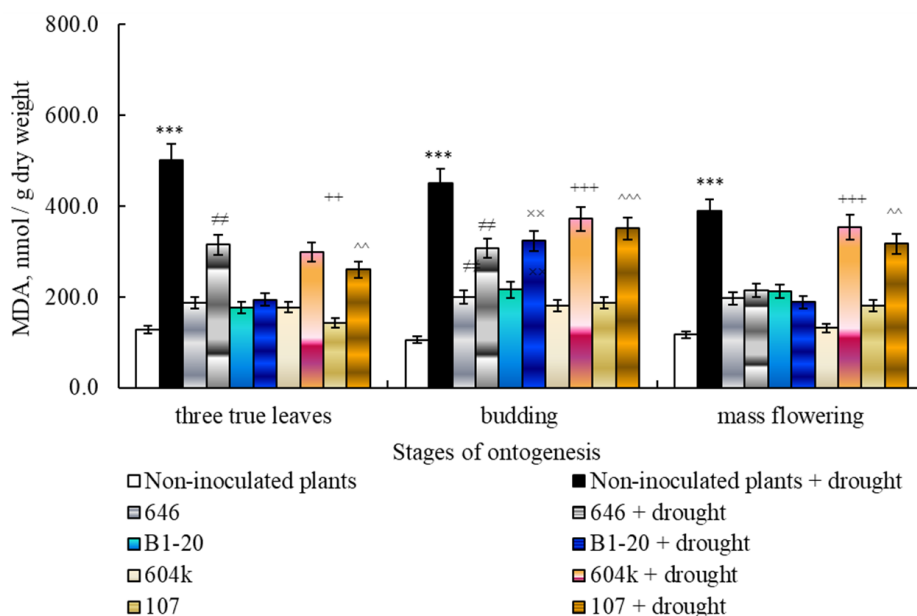


Fig. 2. Influence of drought on the content of MDA in soybean roots inoculated with *B. japonicum* strains and Tn5-mutants ($x \pm SE$, $n = 5$). Here and in Fig. 3: data compared to the control are significant at $P < 0.05$ (* – relative to the variant with non-inoculated plants; #+ – relative to the variants inoculated with strains 646 and 604k; ^ – relative to the variants inoculated with Tn5-mutants B1-20 and 107) (See Fig. 1).

moisture deficiency, the MDA content in the leaves was higher than the control by 42.6 %, and for prolonged water stress – by 86.4 %, in the recovery period – by 93.65 %. The control was provided by non-inoculated plants, which grew with optimal water supply. After exposure to stress, the MDA content in leaves increased and did not recover to the control level in non-inoculated plants with optimal water supply.

According to soybean inoculation with the active strain *B. japonicum* 646 and Tn5-mutant B1-20, there was a slight increase in LPO processes in the leaves under the influence of drought. In

particular, in the effective symbiotic system formed with the participation of strain *B. japonicum* 646, the MDA content in leaves increased by 60.9 % for moderate dehydration and by 50.5 % for long-term exposure to water stress (Fig. 3). In an effective symbiotic system, formed with the participation of Tn5-mutant *B. japonicum* B1-20 strain, intensification of LPO processes in leaves by 43.9 % was observed for long-term moisture deficiency. In the post-stress period, restoration of LPO processes in these symbiotic systems was recorded.

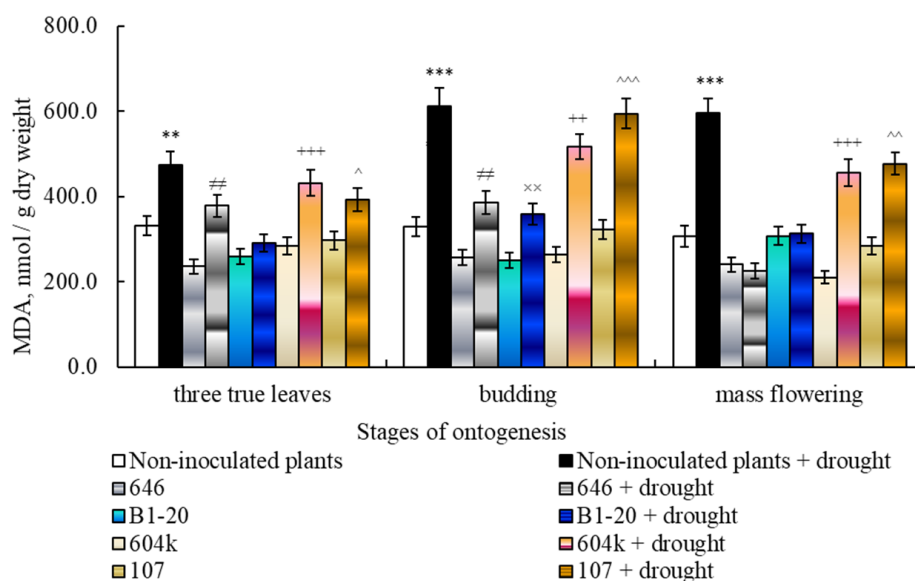


Fig. 3. Influence of drought on the content of MDA in soybean leaves inoculated with *B. japonicum* strains and Tn5-mutants ($x \pm SE$, $n = 5$) (See Fig. 2).

Moderate drought induced a slight intensification of LPO processes in the leaves of soybean inoculated with the inactive Tn5-mutant 107 by 32.1 %, where inoculated with the inactive 604k strain – by 51.9 %. Long-term exposure to the stress factor revealed a significant increase in LPO processes in ineffective symbiotic systems, since the content of MDA increased by 83.8 and 96.3 % in plants inoculated with *B. japonicum* Tn5-mutant 107 and strain 604k respectively. After the restoration of plants watering, the MDA content in the leaves remained at a rather high level compared with control (inoculated rhizobium plants with optimal water supply) – by 68.3 % (Tn5-mutant 107) and 117.2 % (strain 604k).

Discussion. Insufficient water supply leads to changes in water exchange of plant cells and, as a consequence, to impaired metabolic functions of the plant organism [16, 17, 18]. A plant organism under stress is characterized by the change in genome expression, increase in the activity of antioxidant enzymes, accumulation of low molecular weight organic compounds, and the synthesis and release of ethylene [19, 20, 21, 22]. The signal for triggering this complex of reactions is a stereotyped and biologically important change in the internal environment of the cell, which occurs under the influence of stress factors. Such a signal is a change in the level of LPO in the state of prooxidant-antioxidant balance in biological membranes, which is due to the increased production of ROS [1, 3].

The initial stage of LPO is the formation of diene conjugates. The following steps of peroxidation are associated with an increase in lipid hydroperoxides. One of the end products of this process is MDA, a highly toxic compound that disrupts cell metabolism [4]. Intermediate and terminal products of LPO, which accumulate upon activation of this process by the action of stressors, have some modifying effect on membranes and some enzymes [23]. MDA interacts with free amino groups of proteins, components of phospholipids, causes the appearance of ethylene in membranes, which leads to changes in the properties of both individual components and membranes in general [24].

The analysis of the obtained results showed that the effective symbiotic system formed with the participation of soybean and the active strain 646 and Tn5-mutant B1-20 differ with no insignificant increase in MDA content in root nodules during drought and rapid restoration of its level to the optimum after its action. Such changes in LPO processes were accompanied by the preservation of the efficiency of the symbiotic apparatus, which in our opinion may indicate the adaptation of these legume-rhizobial systems in drought conditions. The data obtained by us are consistent with previous studies, which revealed no significant disturbances of the processes of nitrogen fixation and nodulation in these symbiotic systems during droughts and after watering restoration of plants [14, 15].

An ineffective symbiotic system formed with the participation of soybeans and an inactive *B. japonicum* 604k strain recorded an increase in MDA content in the nodules during the drought and a slight restoration of its level to the control in the post-stress period. This indicates the intensification of LPO processes and the violation of the integrity of cell membranes in soybean nodules. This occurred along with the suppression of nodulation processes in this symbiotic system [14, 15], which we have observed earlier in our studies on the peculiarities of the symbiotic apparatus formation of soybean under the action of prolonged drought.

In effective symbiotic systems, drought induced a slight intensification of LPO processes in leaves and roots and a rapid restoration of their level to optimal after stress. For ineffective symbiotic systems, a significant intensification of LPO processes in leaves and roots was revealed with an increase in the effect of drought and a weak restoration of their level to the control level after restoration of plant irrigation.

Under optimal water supply conditions in soybeans inoculated with inactive Tn5-mutant 107 and inactive 604k strain, the content of MDA in root nodules was higher compared to the effective symbiotic systems formed with active *B. japonicum* strain 646 and Tn5-mutant B1-20. Such a pattern is not fixed in the leaves and roots of soybeans.

Conclusions. Therefore, on the basis of the obtained results, it can be concluded that activation of the process of lipid peroxidation is a universal reaction of different symbiotic systems to the effect of drought, the intensity of which depends on their ability to realize their adaptive potential under unfavorable growing conditions.

ІНТЕНСИВНІСТЬ ПЕРОКСИДНОГО ОКИСНЕННЯ ЛІПІДІВ У РІЗНИХ ЗА ЕФЕКТИВНІСТЮ СИМБІОТИЧНИХ СИСТЕМАХ СОЯ–*BRADYRHIZOBIUM* *JAPONICUM* ЗА ДІЇ ПОСУХИ

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Резюме

Одним із механізмів дії активних форм кисню на рослинні тканини є активація пероксидного окиснення ліпідів, що може спричинити пошкодження клітинних мембран. Відомо про сигнальні функції як активних форм кисню, так і продуктів ліпопероксидації, а також про залежність інтенсивності розвитку окиснювальних процесів від здатності рослин реалізувати свій адаптаційний потенціал за дії стресів. **Мета.** Дослідити інтенсивність процесів ліпопероксидації у бульбочках, коренях і листках сої за інокуляції різними за симбіотичними властивостями штамми та Tn5-мутантами *Bradyrhizobium japonicum* за умов посухи. **Методи.** Мікробіологічні, фізіологічні, біохімічні методи, газова хроматографія та спектрофотометрія. **Результати.** Встановлено, що ефективні симбіотичні системи, утворені за участі активних штамів *Bradyrhizobium japonicum* 646 і Tn5-мутанту B1-20, відрізняються незначним зростанням вмісту малонового діальдегіду у бульбочках, коренях і листках за тривалого зневоднення та швидким відновленням його рівня до оптимального після дії стресу. Для неефективних

симбіотичних систем, утворених за участі сої та неактивного штаму *B. japonicum* 604к, а також малоактивного Tn5-мутанту 107, зафіксовано посилений розвиток процесів ліпопероксидації в умовах водного стресу, що свідчить про порушення цілісності клітинних мембран. **Висновки.** Активізація процесу пероксидного окиснення ліпідів є універсальною реакцією різних за ефективністю

симбіотичних систем на дію посухи, інтенсивність розвитку якої залежить від їх здатності реалізувати свій адаптаційний потенціал за несприятливих умов вирощування.

Ключові слова: *Bradyrhizobium japonicum*, соя (*Glycine max* (L.) Merr.), малоновий діальдегід, пероксидне окиснення ліпідів, симбіотичні системи, посуха.

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