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THE STUDY OF THE MUTAGENIC EFFECT OF CARBON DIOXIDE ON SOYBEAN LINE T-219 (*GLYCINE MAX* (L.) MERRILL)

The mutagenic activity of carbon dioxide (CO₂) on the mutant soybean line T-219 (*Glycine max* (L.) Merrill), in which the synthesis of chlorophyll depends on the allelic state of the gene, was studied. The dominant allele Y11 causes a dark green plant, and the recessive allele y11 causes a golden yellow plant. We examined the level of somatic mutations by the presence of light green spots on dark green leaves and yellow spots on light green leaves (direct mutation Y11 → y11), light green spots on golden yellow leaves (reverse mutation y11 → Y11). Methyl methanesulfonate (MMS), a direct-acting mutagen, was used as a positive control. Soybean seeds, treated with MMS for 24 hours, were germinated under standard laboratory conditions and conditions of high CO₂ concentration (5000 ppm) in a specialized box with adjustable CO₂ content. In plants grown under high CO₂ concentration, the frequency of leaf somatic mutations was at the control level. However, in plants whose seeds were treated in MMS and grown under standard laboratory conditions, the total number of spots per leaf statistically significantly increased 4.3 times ($p < 0.05$). When treating seeds with a mutagen followed by cultivation under high CO₂ concentration, the total number of spots per leaf increased 6.3 times ($p < 0.001$) compared with the control and 14.1 times ($p < 0.001$) compared with the cultivation variant high CO₂ concentration. Revealed co-mutagenic effect of CO₂ when combined with the mutagen. The impact of CO₂ on the morphophysiological parameters of plants, the height and rate of photosynthesis, has been studied. On the 10th day of the experiment, the highest plant growth was under high CO₂ concentration and was 7.60 ± 0.92 cm. In plants grown under standard conditions (control), the height of plants was 1.4 times lower ($p < 0.05$). When treating seeds with MMS, followed by germination both under normal conditions and with high CO₂ concentration, plant height was at the control level. However, the accelerated growth of plants under high CO₂ concentration was observed only during the first two weeks, then they died. The rate of photosynthesis in conditions of high CO₂ level increased by 2.0 times, but the difference is not statistically significant. When growing plants at high CO₂ concentrations, photosynthesis intensity increased 2.0 times compared with the control, but the difference was not statistically significant. In plants grown from seeds treated with MMS, the rate of photosynthesis was 1.7 times lower ($p < 0.05$) compared with the control, but when grown under high CO₂ concentration, it increased 1.6 times. At the same time, the rate of photosynthesis decreased 2.2 times ($p < 0.05$) in comparison with the control. A correlation analysis was made between the values of the studied morphophysiological parameters of experimental soybean plants. The correlation coefficient was $r = 0.91$, which indicates a high positive correlation between the rate of photosynthesis and the plant height. Thus, CO₂ did not show mutagenic activity on the mutant soybean line T-219. However, the combined action of CO₂ and the classic mutagen MMS a statistically significant increased the frequency of mutations of the gene responsible for the chlorophyll synthesis. The results obtained indicate the ability of carbon dioxide to co-mutagenic action.

Key words: soybean, carbon dioxide, mutation, comutagen, chlorophyll, mutant line.

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Сояның T-219 линиясына (*Glycine max* (L.) Merrill) диоксидті көміртегінің мутагендік әсерін зерттеу

Сояның (*Glycine max* (L.) Merrill) T-219 линиясындағы диоксид көміртегінің (CO₂) мутагенді белсенділігі зерттелді. Осы мутантты линияда хлорофиллдің түзілуі геннің аллельді жағдайына байланысты. Y11 доминантты аллель жапырақтардың қою-жасыл түстің, ал рецессивті аллель y11 – сары түстің пайда болуын қамтамасыз етеді. Соматикалық мутациялардың деңгейі қою-жасыл жапырақтарда ақшыл-жасыл дақтардың, ақшыл-жасыл жапырақтарда сары дақтардың (Y11→y11 тура мутация) және ақшыл-жасыл жапырақтарда сары дақтардың пайда болуымен (y11→Y11 кері мутация) зерттелді. Оң бақылау ретінде тура әсер ететін метилметансульфонат (ММС, 5 мг/л) мутагеннің ерітіндісі қолданылды. ММС-пен 24 сағат бойы өңделген соя дәндері, қалыпты лабораториялық жағдайда және CO₂ деңгейі реттелінетін мамандандырылған бокста, CO₂ концентрациясының жоғарғы жағдайларында (5000 ppm) өсірілді. CO₂ концентрациясының жоғарғы жағдайында өсірілген өсімдіктерде жапырақтардың соматикалық мутацияларының жиілігі бақылаумен бірдей болды. Алайда, дәндері ММС ерітіндісімен өңделген және қалыпты лабораториялық жағдайларда өсірілген өсімдіктерде, бір жапырақтағы дақтардың жалпы саны статистикалық жағынан маңызды 4,3 есе өсті ($p < 0,05$). Дәндерді мутагенмен өңдеп CO₂ жоғары концентрациясында өсірген жағдайда, бір жапырақтағы дақтардың жалпы саны бақылаумен салыстырғанда 6,3 есе ($p < 0,001$) және CO₂ жоғары концентрациясы жағдайларында өсірумен салыстырғанда 14,1 есе ($p < 0,001$) өсті. CO₂ мутагенмен қоса әсер еткенде комутагенді нәтижесі анықталды. CO₂-нің өсімдіктердің морфофизиологиялық параметрлері – биіктігі және фотосинтез интенсивтілігіне әсері зерттелді. Эксперименттің 10 тәулігінде, CO₂ жоғары концентрация жағдайларында өсірілген өсімдіктердің биіктігінің ең жоғары көрсеткіші $7,60 \pm 0,92$ см. құрады. Қалыпты жағдайда өсірілген өсімдіктерде (бақылау), биіктігі 1,4 есе ($p < 0,05$) аз болды. Дәндерді ММС-пен өңдеп, қалыпты және CO₂ жоғары концентрация жағдайында өсірген кезде өсімдіктердің биіктігі бақылаумен бір деңгейде болды. Алайда, CO₂ концентрациясының жоғары жағдайларындағы өсімдіктердің тез өсуі, тек, алғашқы екі аптада ғана байқалды, кейінгі уақытта өсімдіктер өлімге ұшырады. CO₂ жоғары концентрациясында фотосинтездің қарқындылығы 2,0 есе артты, бірақ бұл айырмашылық статистикалық жағынан мәнді болмады. Өсімдіктерді CO₂ жоғары концентрациялар жағдайында өсіргенде фотосинтез қарқындылығын бақылаумен салыстырғанда 2,0 есе артуы байқалды, бірақ бұл айырмашылық статистикалық жағынан мәнді болмады. ММС өңделген дәндерден өсірілген өсімдіктерде, бақылаумен салыстырғанда, фотосинтез қарқындылығы 1,7 есе ($p < 0,05$) төмен болды, бірақ CO₂ жоғары концентрациясы жағдайларында өсірген кезде – 1,6 есе артты. Сонымен қатар, бақылаумен салыстырғанда фотосинтез қарқындылығы 2,2 есе ($p < 0,05$) азайды. Сояның тәжірибелік өсімдіктерінде зерттелген морфофизиологиялық параметрлердің шамаларымен фотосинтездің қарқындылығы арасында корреляциялық талдау өткізілді. Корреляция коэффициенті $r = 0,91$ құрады, бұл зерттелетін өсімдіктердің фотосинтез қарқындылығымен өсімдік бойының биіктігі арасындағы жоғары оң корреляцияның бар екендігі анықталды. Сонымен, сояның мутантты T-219 линиясына CO₂ мутагендік белсенділігі анықталған жоқ. Дегенмен, CO₂ классикалық ММС мутагенмен бірлесіп әсер еткенде хлорофиллдің түзілуіне жауап беретін геннің мутациялар жиілігінің статистикалық мәнділігінің өсуі байқалды. Алынған нәтижелер диоксидті көміртегінің комутагендік әсерінің қабілеттілігін көрсетеді.

Түйін сөздер: соя, диоксидті көміртегі, мутация, комутаген, хлорофилл, мутантты линия.

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Исследование мутагенного действия диоксида углерода на сою (*Glycine max (L.) Merrill*) линии Т-219

Изучена мутагенная активность диоксида углерода (CO₂) на мутантной линии сои Т-219 (*Glycine max (L.) Merrill*), у которой синтез хлорофилла зависит от аллельного состояния гена. Доминантный аллель Y11 обуславливает темно-зеленую окраску листьев, а рецессивный аллель y11 – желтую окраску. Изучали уровень соматических мутаций по наличию светло-зеленых пятен на темно-зеленых листьях и желтых пятен на светло-зеленых листьях (прямая мутация Y11→y11), а также светло-зеленых пятен на желтых листьях (обратная мутация y11→Y11). В качестве положительного контроля использовали метилметансульфонат (ММС), мутаген прямого действия. Семена сои, обработанные ММС в течение 24 ч., проращивали в обычных лабораторных условиях и в условиях повышенной концентрации CO₂ (5000 ppm) в специализированном боксе с регулируемым содержанием CO₂. У растений, выращенных в условиях повышенной концентрации CO₂, частота соматических мутаций листьев была на уровне контроля. Однако у растений, семена которых были замочены в ММС и выращены в обычных условиях лаборатории, общее количество пятен на лист статистически значимо возросло в 4,3 раза (p < 0,05). При обработке семян мутагеном с последующим выращиванием в условиях высокой концентрации CO₂ общее количество пятен на лист возросло в 6,3 раза (p < 0,001) по сравнению с контролем и в 14,1 раза (p < 0,001) по сравнению с вариантом выращивания в условиях высокой концентрации CO₂. Выявлен комутагенный эффект, оказываемый CO₂ при совместном действии с мутагеном. Изучено влияние CO₂ на морфофизиологические параметры растений – высоту и интенсивность фотосинтеза. На 10 день эксперимента наибольшая высота была у растений, выращенных в условиях повышенной концентрации CO₂, и составила 7,60 ± 0,92 см. У растений, выращенных в обычных условиях (контроль), высота растений была меньше в 1,4 раза (p < 0,05). При обработке семян ММС с последующим проращиванием как в обычных условиях, так и при повышенной концентрации CO₂ высота растений была на уровне контроля. Однако ускоренный рост растений в условиях повышенной концентрации CO₂ наблюдался только в течение первых двух недель, в дальнейшем они погибали. Интенсивность фотосинтеза в условиях высокой концентрации CO₂ увеличилась в 2,0 раза, но разница статистически не значима. При выращивании растений при повышенных концентрациях CO₂ отмечено увеличение интенсивности фотосинтеза в 2,0 раза по сравнению с контролем, однако разница статистически не значима. У растений, выращенных из обработанных ММС семян, интенсивность фотосинтеза по сравнению с контролем была ниже в 1,7 раза (p < 0,05), но при выращивании в условиях повышенной концентрации CO₂ увеличилась в 1,6 раза. При этом интенсивность фотосинтеза уменьшилась в 2,2 раза (p < 0,05) в сравнении с контролем. Проведен корреляционный анализ между величинами изученных морфофизиологических параметров опытных растений сои. Коэффициент корреляции составил r=0,91, что свидетельствует о высокой положительной корреляции между интенсивностью фотосинтеза и высотой изучаемых растений. Таким образом, CO₂ не проявил мутагенной активности при воздействии на мутантную линию сои Т-219. Однако при совместном действии CO₂ с классическим мутагеном ММС наблюдалось статистически значимое увеличение частоты мутаций гена, ответственного за синтез хлорофилла. Полученные результаты свидетельствуют о способности диоксида углерода к комутагенному действию.

Ключевые слова: соя, диоксид углерода, мутация, комутаген, хлорофилл, мутантная линия.

Introduction

The worsening global environmental crisis is associated with the widespread pollution of the environment by various chemical compounds [1-5]. The use of artificially synthesized chemical compounds in agriculture, industry, households, and medicine, which may be get into environmental objects (air, water, soil), increases annually. Most of them have toxic, carcinogenic, teratogenic and mutagenic effects on living organisms. There is a result of activating the formation of intracellular free radicals, inhibiting the DNA repair activity or direct interaction with DNA molecules [6-11]. According to CAS (Chemical Abstracts Service, USA), 10 million chemical compounds were registered in the period from 1957 to 1990, in 2008 – 40 million, and as of September 2018 – 144 million [12]. Several dozens of compounds can simultaneously enter the body [13, 14]. The number and range of xenobiotics in the environment increases, which can lead to an increase in the genetic load and, consequently, an increased risk of extinction of one or another species. One of the environmental pollutants is carbon dioxide (CO₂). Carbon dioxide as a natural component of the atmosphere is necessary for the normal functioning of the biosphere since it is a source of primary organic matter. However, in recent decades, due to the rapid urbanization of the environment and the intensification of human economic activity, the natural CO₂ level is increasing, making it one of the air pollutants. Carbon dioxide is a colorless, odorless gas with a density of 1.98 kg/m³ under normal conditions, it is 1.5 times heavier than air [15]. At atmospheric pressure, carbon dioxide from a solid state (dry ice) turns into gaseous (sublimation), and its concentration in the Earth's atmosphere is 0.04% [16, 17]. Carbon dioxide easily passes ultraviolet rays and rays of the visible part of the spectrum, but absorbs infrared rays emitted by the Earth, and is one of the greenhouse gases and participates in the process of global warming.

Annual changes in the concentration of carbon dioxide in the atmosphere are determined by the vegetation of mid-latitudes (40-70°C) in the northern hemisphere [18]. Therefore, from March to September due to photosynthesis, the CO₂ content in the atmosphere decreases, and from October to February it increases due to the wood oxidation (heterotrophic plant respiration, decay, decomposition of humus, forest fires) and the burning of fossil fuels (coal, oil, gas). A large amount of carbon dioxide is dissolved in the ocean [19]. A slight increase in con-

centration up to 2-4% in the premises leads to the development of drowsiness and weakness of people. The CO₂ levels from 7 to 10% are considered dangerous, at which suffocation, headache, dizziness, loss of hearing and consciousness develop. When inhaling air with high gas concentrations, death occurs due to asphyxiation [20]. The carbon dioxide level in the atmosphere has steadily increased from approximately 315 ppm in 1959 to 405 ppm at present [16, 21]. Predict an increase in level to 500-1000 ppm in 2100 [22]. The increased carbon dioxide concentration raises the rate of photosynthesis and growth but reduces the content of nitrogen and, possibly, minerals in plant tissue [23-30]. High CO₂ levels in the air lead to the body intoxication, reducing the ability of oxygen in the blood to bind to hemoglobin. The physiological consequences of exposure to CO₂ on the body are well known; however, possible mutagenic or mutagen-modifying effects of high concentrations of carbon dioxide are of interest. Knowledge of the mutagenic potential of anthropogenic environmental pollutants, including carbon dioxide, will make it possible to identify possible genetic risks for humans and develop preventive measures to protect them. Thus, the aim of this paper to explore the mutagenic effect of carbon dioxide on the mutant soybean line T-219.

Materials and methods

The research object was line T-219 of soybean (*Glycine max* (L.) Merrill, fam. *Fabaceae*), kindly provided by the Laboratory of Environmental Genetics of the N.I. Vavilov Institute of General Genetics of the Russian Academy of Sciences (Moscow, Russia). Carbon dioxide (CO₂) was examined for mutagenic activity. Methyl methanesulfonate (MMS, C₂H₆O₃S) at a concentration of 5 mg/L was used as a mutagen (positive control). Air-dried soybean seeds soaked in distilled water for 20 hours, then some of them were placed in the mutagen solution for 24 hours, and part of the seeds were placed in a specialized box with an adjustable CO₂ content for the same period. After treatment, the seeds were washed and germinated for 4-5 weeks until the appearance of two simple and the first complex ternary leaf. The box made plexiglass measuring 20*30*40 cm (length*width*height) and a total volume of 24 liters. A gas cylinder with CO₂ connected to the hermetic lid through a hose. The pressure gauge fixed on the lid. The CO₂ analyzer recorded the gas content inside the box (Fig. 1).



Figure 1 – Box for CO₂ supply

There are 4 experimental groups of plants: I – intact plants; II – plants germinated in a box with CO₂; III – plants treated with MMS and germinated under standard laboratory conditions (SLC); IV – plants treated with MMS and germinated in a box with CO₂. Soybean plants were grown for 4-5 weeks until the appearance of two simple and the first complex ternary leaf (Fig. 2).

In line T-219, chlorophyll synthesis depends on the allelic state of the gene. The dominant allele Y11 causes a dark green leaf, and the recessive allele y11 causes a golden yellow leaf. As a result of the splitting of heterozygotes in the next generation, plants are represented by three categories based on the color of leaves: homozygous dominant Y11Y11 plants, characterized by a dark green leaf; heterozygous plants Y11y11, characterized by a light green leaf; homozygous plants in the recessive allele y11y11, characterized by a golden yellow color.

On the leaves of all three types of plants, various kinds of mosaic spots may appear, which are the result of different types of mutations (Table 1).

These spots have clear boundaries, which makes it quite easy to distinguish them from spots that appear as a result of physiological processes [31, 32].

We looked through only the upper surface of the leaves, on which there were more than 80% of the spots. The data were given as the number of spots

per leaf and analyzed as the total number of spots, and the frequency of individual types of spots.

The assimilation test was used to determine the rate of photosynthesis in experimental and intact plants [33]. This method is based on determining the amount of carbon dioxide absorbed by the leaves during photosynthesis. Two empty flasks with a capacity of 250 ml for 20-30 minutes were kept in the same conditions for filling with air. Then a leaf of the plant was taken, and its area was measured by the formula (1): $S = a \times b \times 0.7$, where a is the leaf length, b is the maximum width; 0.7 is conversion factor. The leaf cut was updated under water, and the leaf stalk was tied to a rubber stopper. Flasks were moved to the sun or under the lamp for 5 minutes. After that, the leaf was removed and a 0.025N solution of Ba(OH)₂ was poured into the flask, then 2-3 drops of phenolphthalein were added as an indicator. The flask walls were carefully moistened with a solution of Ba(OH)₂, then it was periodically shaken for 3 minutes, titrated with a 0.025 N HCl until the pink disappeared. The rate of photosynthesis was calculated by the formula (2):

$$I_{ph} = \frac{(A-B) \times K \times 0.55 \times 60}{S \times t}$$

where A is the amount of HCl used for titration of barite in the test flask, ml; B is the amount of HCl used for titration of barite in the control flask, ml; K

– amendment to the titer of HCl; 0.55 is the amount of CO₂ mg corresponding to 1 ml of 0.025 N HCl; S is the leaf area, dm²; t – exposure, min; 60 – the conversion rate of minutes to hours.

Statistical processing of the results was performed in the Data Analysis add-in Microsoft Ex-

cel and StatPlus5Pro version 6 (Analyst Soft Inc., USA). In all cases, mean values and standard errors were determined. Student's t-test evaluated the significance of differences between averages, the differences were considered significant at a confidence level of 0.95 (p < 0.05).



Figure 2 – Growing plants under standard laboratory conditions (A) and in boxing under high CO₂ concentration (B)

Table 1 – The relationship between possible genetic disorders and types of somatic mosaicism analyzed on *Glycine max* leaves [31]

Types of leaf	Types of spots	Type of genetic disorders
Light green	Yellow	Direct mutation Y11 → y11 chromosome nondisjunction
	Dark green	Reverse mutation y11 → Y11 chromosome nondisjunction
	Double	Somatic cross over
Dark green	Light green	Direct mutation Y11 → y11
	Very dark green	chromosome nondisjunction Y11Y11Y1
Golden yellow	Light green	Reverse mutation y11 → Y11

Results and discussion

The somatic mutation counting test is based on recording and analyzing various types of spots appearing on soybean leaves after seed treatment with mutagens. As noted earlier, in variety T-219 of *Glycine max* (L.) Merrill, the synthesis of chlorophyll depends on the allelic state of the gene. The dominant allele Y11 causes a dark green, and the recessive allele y11 causes a golden yellow plant (Fig. 3).

The results of the study of somatic mutations on leaves of variety T-219 of soybean (*Glycine max* (L.) Merrill) under the high concentration of carbon dioxide (5000 ppm) and the classical mutagen of methyl methanesulfonate (MMS) are presented in Table 2.

As can be seen from the presented results, the level of spontaneous mutagenesis in soybean leaves (variant I) was $0.38 \pm 0.19\%$. In plants from water-treated seeds and grown under high concentrations of carbon dioxide (variant II), the total number of

spots per leaf was 0.17 ± 0.18 . Although the number of spots per leaf in variant II decreased by 2.2 times, this decrease was not statistically significant. In plants from MMS-treated seeds and grown under standard laboratory conditions (MMS + SLC), the total number of spots per leaf statistically increased

4.3 times ($p < 0.05$) and was 1.64 ± 0.63 . When treating seeds with MMS, followed by cultivation under high concentration of CO_2 (MMS + CO_2), the total number of spots per leaf increased 6.3 times ($p < 0.001$) compared to the control and 14.1 times ($p < 0.001$) compared to with variant II ($\text{H}_2\text{O} + \text{CO}_2$).

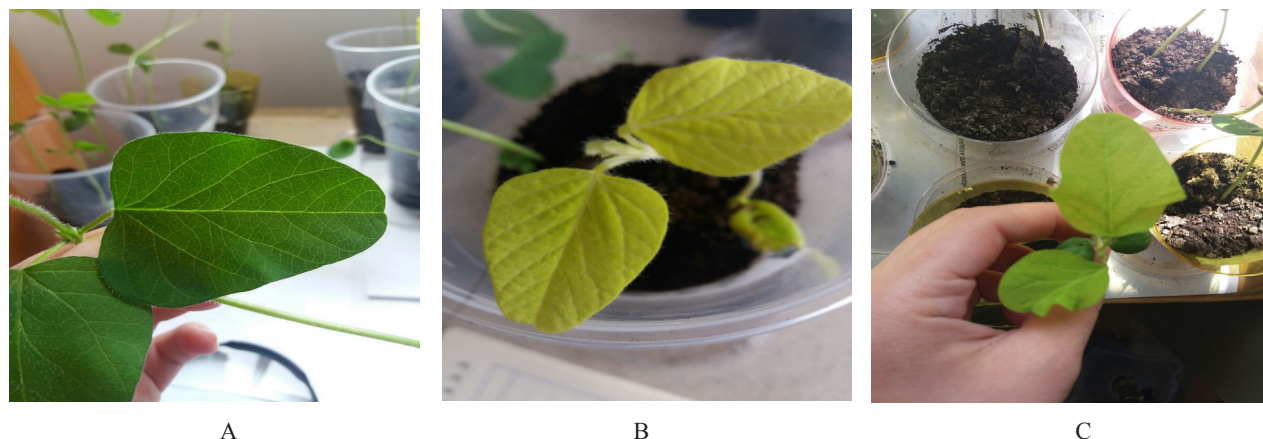


Figure 3 – Dark green (A, the dominant allele of chlorophyll), yellow (B, the recessive allele of chlorophyll) and light green (C, heterozygous plant Y11y11) coloring of leaves of T-219 soybean (*Glycine max*)

Table 2 – The effect of the separate and joint action of CO_2 and MMS on variety T-219 of soybean (*Glycine max* (L.) Merrill) (data by leaf type)

Variants	Dark green leaves (spots per leaf)	Light green leaves (spots per leaf)		Golden yellow leaves (spots per leaf)	Average spots per leaf
	Light green spots (direct mutation Y11 → y11)	Yellow spots (direct mutation Y11 → y11, chromosome nondisjunction)	Dark green spots (reverse mutation y11 → Y11, chromosome nondisjunction)	Light green spots (reverse mutation y11 → Y11)	
I variant, control	$0,38 \pm 0,19$	-	-	-	$0,38 \pm 0,19$
II variant, $\text{H}_2\text{O} + \text{CO}_2$	$0,17 \pm 0,18$	-	-	-	$0,17 \pm 0,18$
III variant, MMS+SLC	$0,95 \pm 0,58$	$0,47 \pm 0,29^*$	$0,23 \pm 0,23$	-	$1,64 \pm 0,63^*$
IV variant, MMS+ CO_2	$1,89 \pm 0,49^{**\bullet}$	-	-	$0,50 \pm 0,53$	$2,39 \pm 0,51^{***\bullet}$

* – $p < 0,05$; ** – $p < 0,01$; *** – $p < 0,001$ compared to control;
 • – $p < 0,001$ compared to variant $\text{H}_2\text{O} + \text{CO}_2$

The appearance of the same type of stains on soybean leaves may be due to different reasons. Therefore, it is necessary to analyze the relative increase of all types of spots on all types of leaves to conclude the specificity and possible mechanisms of the mutagenic action of the studied factor. In the

experiment, soybean revealed four types of spots: light green spots on dark green leaves, yellow and dark green spots on light green leaves, as well as light green spots on golden yellow leaves.

In the control and the variant II ($\text{H}_2\text{O} + \text{CO}_2$), only light green spots were observed on the dark

green leaves, the cause of which is the direct mutation (Y11 → y11). These spots were found in all variants and prevailed in plants.

In plants grown from seeds treated with MMS (variant III), the number of light green spots on dark green leaves (Figure 4, A) increased 2.5 times compared with the control, but this increase was not statistically significant. At the same time, in this variant (III), there were yellow spots and dark green spots on light green leaves, which are absent in all other variants. Yellow spots are formed through a direct gene mutation (Y11 → y11) or loss of a Y11 fragment as a result of the deletion. Dark green spots are formed through a reverse point mutation, nondisjunction of chromosomes, and the acquisition

by the cell of Y11 fragment resulting from a deletion in the nearest cell.

When MMS-treated seeds germinated in a box with a high CO₂ concentration (5000 ppm), we found two types of mutations: direct mutation (light green spots on dark green leaves) and reverse mutation (light green spots on yellow leaves) (Figure 4, B and C). The formation of light green spots on dark green leaves increased statistically significantly by 5.0 (p <0.01) and 11.1 (p <0.001) times compared with the control and variant II (H₂O + CO₂), respectively. The light green spots on the golden yellow leaves, resulting from the reverse mutation y11 → Y11, were found only in variant IV (MMS + CO₂), but there was only one plant with this type of spots.

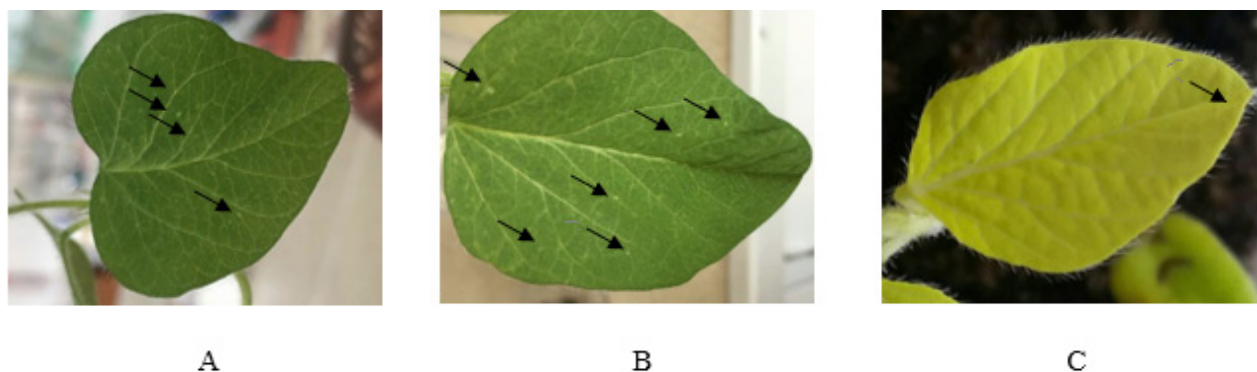


Figure 4 – Types of leaves and spots on leaves of variety T-219 of *Glycine max* (L.) Merrill under MMS-treatment: A – Dark green leaf with light green spots (direct mutation Y11 → y11); B – Dark leaf with light green spots (direct mutation Y11 → y11); C – Golden yellow leaf with dark green spots (reverse mutation y 11 → Y 11)

When growing soybean under high CO₂ concentration, no statistically significant increase in the level of chlorophyll mutations was observed. But when combined with MMS, carbon dioxide modified MMS-induced mutagenesis towards it enhanced. Based on the results obtained, it can be assumed that CO₂ has a comutagenic effect. This assumption is consistent with the research of Zhang Q. et al. The authors found that high CO₂ concentrations can to have a comutagenic impact, modifying the action of hydrazine hydrate [34].

Analysis of morphophysiological parameters helps to obtain information about the viability of the plant organism and its processes. In our studies, morphophysiological parameters such as plant height (Fig. 5) and photosynthesis intensity (Fig. 6) were also studied. On the 10th day of the experiment, the highest plant growth was under high CO₂ concentration and was 7.60±0.92 cm. In plants grown under standard conditions (control),

the height of plants was 1.4 times lower (p <0.05).

When MMS-treated seeds germinated in a box with a high CO₂ concentration, the plant height was not statistically significantly different from the control plants. At the same time, an increase in growth parameters was observed in plants of experimental group IV, treated with MMS and grown in box with high CO₂ concentrations, as compared to only MMS treated. However, this difference was not statistically significant. In plants of the IV experimental variant (MMS + CO₂), the average plant height was 1.2 times lower than plants of variant II (H₂O + CO₂); however, this difference was also not statistically significant.

It should be noted that the accelerated growth of plants grown under high CO₂ concentration (variants II and IV) was observed only during the first two weeks. Plants actively absorbed carbon dioxide and increased biomass. But after the first two weeks, the growth slowed down and was significantly inferior

to the plants of the control group. As a result of prolonged exposure to the high concentration of carbon dioxide, the growth process stopped, which further led to the plant death.

The amount of CO₂ absorbed by a unit of the leaf surface per unit of time is one of the most important physiological parameters that ensure

the viability of the plant. The results of the rate of photosynthesis under different experimental conditions are presented in Fig. 6. When plants were growing under high CO₂ concentrations, the rate of photosynthesis increased by 2.0 times compared with the control; however, the difference is not statistically significant.

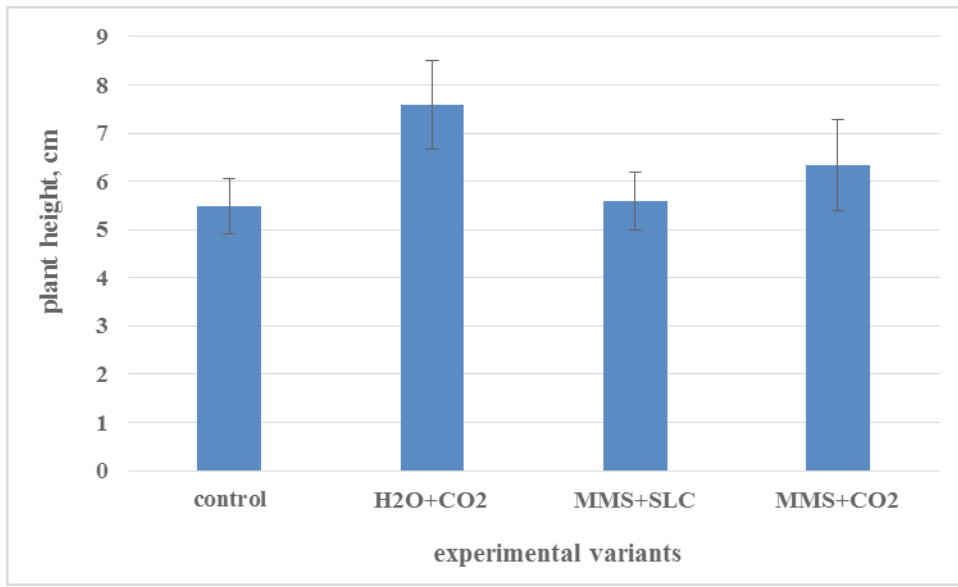


Figure 5 – The height of the soybean plant (*Glycine max*) with the separate and combined action of MMS and high CO₂ concentration

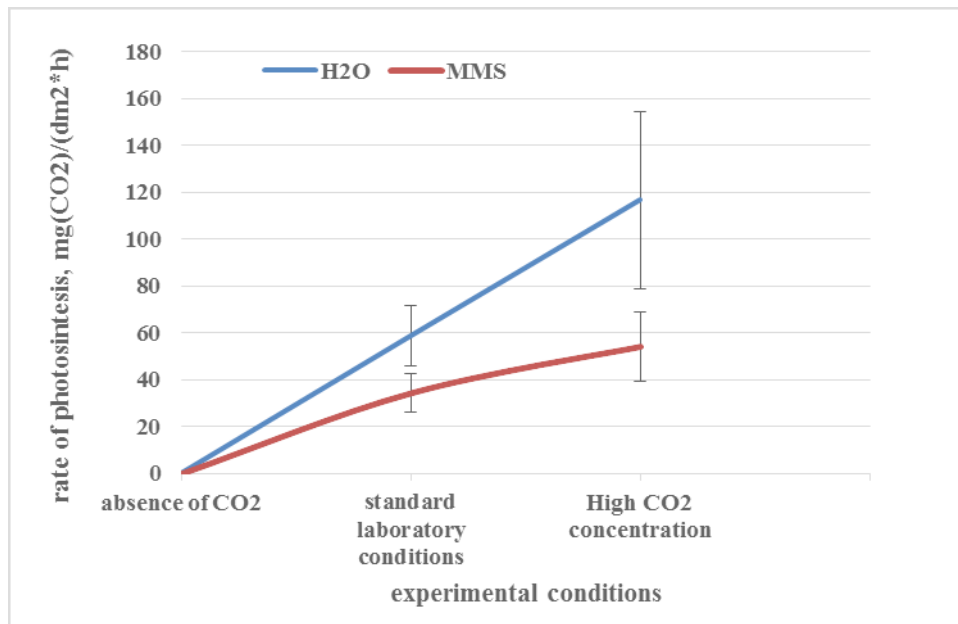


Figure 6 – The rate of photosynthesis with the separate and joint action of MMS and high CO₂ concentration

In plants from MMS-pre-treated seeds, the rate of photosynthesis was statistically significantly different from the control values and was 1.7 times lower ($p < 0.05$). However, if the seeds treated with MMS were grown under high CO_2 concentration, then the rate of photosynthesis increased 1.6 times. At the same time, the rate of photosynthesis decreased 2.2 times ($p < 0.05$) as compared with the control plants. A correlation analysis was performed

to determine the dependence of the intensity of photosynthesis and growth processes (Fig. 7). The correlation coefficient was $r = 0.91$, which indicates a strong positive correlation between the intensity of photosynthesis and the height of the plants.

The results are consistent with the study by Ainsworth et al. The authors found an increase in the intensity of photosynthesis by 40% when growing plants with high CO_2 concentration (600 ppm) [35].

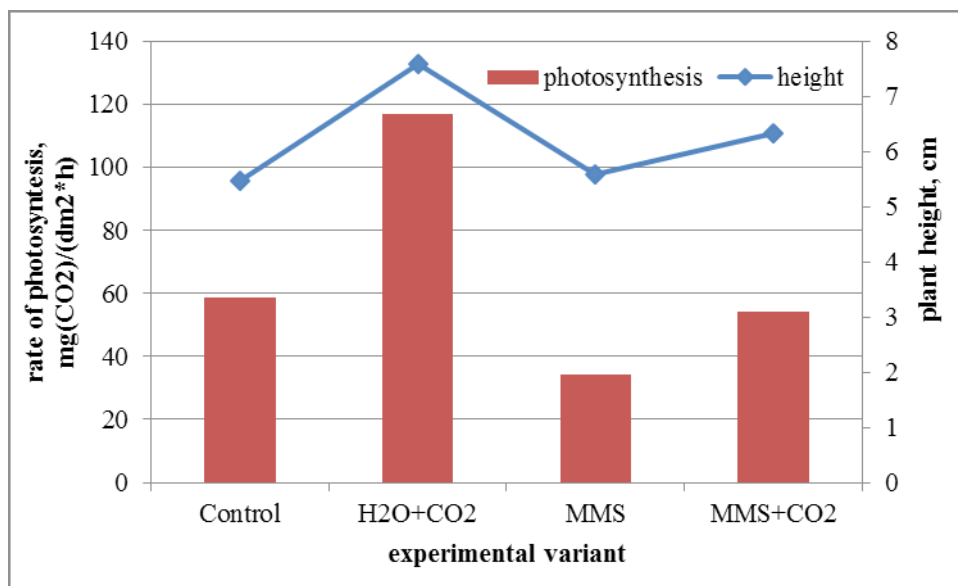


Figure 7 – Plant height and photosynthesis intensity with separate and joint effects of MMS and CO_2 on soybean

Thus, as the research result, it was found that CO_2 at a concentration of 5000 ppm did not have a mutagenic effect on the mutant line T-219 soybean. When combined with MMS, carbon dioxide significantly modified the action of the mutagen, statistically considerably increasing the direct chlorophyll mutations in soybean leaves of variety T-219. The results obtained indicate a co-mutagenic effect of CO_2 , which can enhance the damaging effects. The danger of comutagens is determined not only by their ability to improve the detrimental impact of mutagens present in the human environment, but also by the possibility of influencing the processes of endogenous mutagenesis [36]. It can be assumed that high concentrations of carbon dioxide suppressed the activity of the cellular repair system, resulting in increased levels of MMS induced structural mutations. Besides, it was found that high CO_2 concentrations (5000 ppm) enhanced the growth

processes and the rate of photosynthesis in the initial stages. However, as a result of prolonged exposure to the high concentration of carbon dioxide, the growth process was slowed down, then stopped, leading to the plant death.

Conclusion

The results of this investigation show that CO_2 has no mutagenic effect on the mutant soybean line T-219. However, the combined action of CO_2 and the classic mutagen MMS a statistically significant increased the frequency of mutations of the gene responsible for the chlorophyll synthesis. The results obtained demonstrate the ability of carbon dioxide to co-mutagenic action. These data suggest that due to the rising anthropogenic CO_2 concentration in the atmosphere, there is a risk of increased mutagenic effect of potential genotoxicants in the environment.

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