



RESEARCH ARTICLE

THE ACTION OF SOME CYTOKININ-TYPE COMPOUNDS ON THE ANTIOXIDANT PROTECTION CAPACITY OF PLANTS IN CONDITIONS OF UNFAVORABLE VARIATION OF HUMIDITY AND REPEATED WATER STRESS

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Abstract

The effect of cytokinin-type compound on the capacity of the enzymatic and non-enzymatic system of antioxidant protection by plants in conditions of humidity fluctuation and repeated drought were studied. As subjects served the plants *Glycine max* (Merr) L. cultivar Nadejda, grown in the Mitcherli vegetation pots with volume of 40 kg soil and exposed to the drought stress at the phases "first trifoliate leaf" and "flowering – pods formation". The antioxidant protection capacity of soybeans pre-treated with cytokinin, thiourea and with the composition Polyel + phytohormones (AIA, Gb, CK) is ensured both by the intensification of the activity of antioxidant enzymes and by the increase of content of non-enzymatic substances with low molecular weight. After restoring the optimal moisture content, the plants pre-treated with thiourea and Polyel + F more fully restore their functional processes.

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Introduction:-

The statement that drought-induced dehydration is one of the most common external stresses suffered by plants has become an axiom. To cope with the stress caused by adverse conditions, plants often activate several biochemical, physiological and morphological mechanisms, which then facilitate the induction of a faster and more appropriate response to the occurrence of adverse conditions. From the literature information [Bhattacharjee S., 2005, 2012; Mittler R., Blumwald E., 2010; Munne-Bosch S., 2013; Walter J. et al., 2013] and from the results of their own investigations [Ștefîrță A. et al., 2017] it follows that the key mechanisms, correlated with the adequate response to the unfavorable fluctuation of humidity and repeated installation of drought conditions are mechanisms coupled with the self-regulation of the formation and neutralization of reactive oxygen species (ROS) by activating the antioxidant potential, with the intensification / stabilization of the synthesis of compounds with regulation and protection functions. Traditionally, ROS are considered toxic products, which being in high concentrations cause oxidative destruction of cells. Recently, however, it has been shown that plants produce ROS as signaling molecules, which, in moderate concentrations, control the responses to abiotic stress [Mittler R., 2002] by accessing a number of protective mechanisms: activating the synthesis of certain phytohormones (AS, ABA, AJ, CK), antioxidant enzymes, non-enzymatic antioxidant compounds with low molecular weight, protective substances, etc. The destructive effect of ROS is controlled by activating the enzymatic and non-enzymatic antioxidant protection systems. At the same time, in plants the activity of antioxidant enzymes is regulated by various factors, including

phytohormones [Mytinova Z. et al., 2011]. Phytohormones are one of the main endogenous systems for regulating and coordinating the physiological processes of the plant organism. They are involved in the complex of signal transmission systems due to which the coordination of growth and development processes with the response reactions of plants to the conditions of the external environment takes place. In this aspect, more and more data demonstrate the interactive relationships of drought tolerance, changes in hormonal status and self-regulation of water homeostasis. [Dobra J., Motyka V., Dobrev P. et al., 2010]. Currently, the mechanisms involved in increasing plant tolerance to drought are being studied insistently by the exogenous application of phytohormones. The possibility of regulating adaptive reactions through the exogenous use of phytohormones has been demonstrated in multiple investigations [rev. Ștefărită A. et al., 2015, 2017]. At the same time, another important factor that determines the tolerance of cells and tissues from the adverse conditions is the content of native antioxidant protection substances [Munne-Bosch S. et al., 2013; Bartolía C.G., Casalongué C.A., Simontacchia M., Marquez-García B., Foyerc C.H., 2013; Ștefărită A. et al., 2012; 2017]. The high level of substances with hydrogen sulphidic groups, the presence of antioxidants, free radical acceptors, inhibitors of peroxide reactions in the chain, reduces the sensitivity to drought due to the capture of free radicals, inhibition of lipid peroxidation in membranes, etc. In general, tolerant plants have a higher level of anti-stress metabolites under normal growth conditions and / or accumulate in unfavorable conditions several metabolites with protective function such as amino acids, soluble carbohydrates, etc. The former is pre-adapted to possible unfavorable conditions due to the constitutive presence of metabolites with protective functions.

The *objective* of the research expected for this paper was to elucidate the effect of exogenous inducers of non-specific mechanisms, correlated with the potential for stress-memory formation to the repeated drought of *Glycine max* (Merr.) L. plants. Follow elucidate the effect of cytokine-type compounds on the ability of plants antioxidant protection under conditions of drought fluctuation of humidity and episodic repeated water stress.

Research Materials And Methods: -

As study objects served plants of *Glycine max*(Merr)L. Nadejda variety, selected in IGFPP (author, PhD Budac Alexandru). Testing of the effect of tolerance inducers in conditions of fluctuating humidity and repeated water stress was performed in vegetation experiments with plants grown in Mitcherli containers with a capacity of 40 kg absolutely dry soil. A new complex compound, conventionally called Polyel, which contains macro- and microelements in the form of salts and coordination complexes, as well as vitamins – components necessary for normal plant development was obtained and tested. It is a solid beige mixture of coordination complexes of iron(III), cobalt(III), micro- and macroelements, vitamins, NO₃⁻ ions, substances that optimize the chemical composition of the compound and make it effective for plant growth and development.

Scheme of experience at the phase "I trifoliate leaf"

1. Control – plants on a background of permanent humidity 70% TWC (total water capacity of the soil);
2. Plants on a background of humidity 40% TWC (moderate drought);
3. Plants pre-treated with cytokinin solution and exposed to moderate drought (40% TWC);
4. Plants pre-treated with thiourea solution and exposed to moderate drought (40% TWC);
5. Plants pre-treated with the solution of the composition Polyel + phytohormones (AIA, Gb, CK) and exposed to moderate drought (40% TWC).

Scheme of experience in the phase "flowering - formation of pods"

1. Control, humidity 70% TWC;
2. Plants exposed for the first time to moderate drought, 70-40% CTA (first cycle of drought);
3. Plants repeatedly exposed to drought, 70-40-70-40% CTA (2 drought cycles);
4. Plants pre-treated with CK and exposed to humidity 70-40-70-40% CTA (2 cycles of drought);
5. Plants treated with thiourea and exposed to humidity 70-40-70-40% CTA (2 cycles of drought);
6. Plants pre-treated with the composition Polyel + phytohormones (AIA, Gb, CK) and exposed to humidity 70-40-70-40% (2 cycles of drought).

In the experiments, the plants were exposed to consecutive cycles of water deficit, each cycle followed by a recovery period, and in each cycle new, fully developed leaves were analyzed, formed under the corresponding stress conditions. Analyses of the parameters of the components of the enzymatic and non-enzymatic antioxidant protection systems were performed after each drought cycle (on the seventh day of water stress) and after 7 days of recovery in the post-stress period. Testing of the intensity of peroxide oxidation of lipids was performed by

determining the final product – the content of malonic di-aldehyde [Curganova L.H., Veselov A.P., Goncharova T.A., Sinitsina V., 1997]. The activity of key antioxidant protection enzymes was investigated by the spectrophotometric method: SOD – by the method [Cevari S., Ceaba I., Sekei I., 1985]; CAT – according to the method of Chance B. and Machly A. (1955) by spectrophotometric determination at λ 240 nm of H_2O_2 decomposition; GwPX – after the intensity of oxidation of guaiacol (2-methoxy-phenol) as a hydrogen donor in the presence of H_2O_2 , at λ 470 nm; APX – by monitoring the oxidation rate of ascorbate at λ 290 nm [Nacano Y., Asada K., 1981]. Homogenization of plant material and extraction – as described [Keshavkant S., Naithani S.C., 2001]. The proline content was determined according to [Bates L., Waldren R.P., Teare I.D., 1973]; the ascorbic acid content was determined by the spectrophotometric method; the content of assimilating pigments was determined spectrophotometrically in 80% acetone extracts. The results were statistically analyzed, using the "Statistics 7" software package for computers. The parameters MDA content, proline, ascorbic acid and pigments content were used as indicators to quantify the intensity of stress and tolerance to moisture fluctuation and moderate drought repeated over time.

Results And Discussions:-

Plants react to unfavorable environmental conditions by forming reactive oxygen species, which, depending on the concentration, can serve as signaling molecules for the expression of protective mechanisms or can lead to the appearance of oxidative stress in cells. Much of the damage caused by drought is associated with oxidative damage at the cellular level due to excessive ROS formation caused by cell dehydration. It is logically correct to assume that the property of plants to control and maintain at a certain level the content of ROS is directly correlated with their resistance to the action of destructive factors.

According to the data obtained the process of ROS formation, assessed by the content of malonic di-aldehyde, in control plants (untreated with physiological active substances, PAS) and exposed to the phase of "I trifoliolate leaves" in conditions of moderate moisture insufficiency increased by $17.5 \mu M \cdot g^{-1} \text{ fr.w.}$ compared with the MDA content in the leaves of plants on the optimal background of moisture (Table 1). Oxidative stress, conditioned by drought at this stage of development in plants pre-treated with CK, thiourea and Polyel+F, is actually weaker compared to untreated plants. In the leaves of these plants the MDA content, formed after dehydration, is respectively with 14.85; 14.26 and $8.5 \mu M \cdot g^{-1} \text{ fr.w.}$ smaller compared to control plants. The data demonstrate an attenuation of the oxidative stress caused by drought plants pre-treated with cytokinin by 7.14% , in plants pre-treated with thiourea - by 8.73% and in plants pre-treated with Polyel + F - by 24.21% comparative with control plants.

The differences in DAM content and oxidative stress intensity in these plants can be explained both by the different degree of dehydration [Ştefărița A. et al., 2017] and by the differences in the degree of neutralization of ROS provided by enzymatic and non-enzymatic protection systems. Adapted plants have the property of retaining ROS production coupled with effective antioxidant protection, provided by enzymatic and non-enzymatic ROS neutralization systems. The antioxidant protection system includes a wide range of enzymes – superoxid dismutase, catalase, ascorbate peroxidase, glutathione reductase, glutathione peroxidase, guaiacol peroxidase, etc., the activity of which changes due to unfavorable factors.

The antioxidant enzyme protection capacity, manifested by the activity of SOD, CAT, APX and GPX, of plants pre-treated with thiourea and exposed to moderate water stress at the stage of "I trifoliolate leaves" increased for 62.0; 25.7; 61.7 and 55.97 percent compared to the activity of these enzymes in the leaves of control plants on the optimal background of moisture (Table 1). In the leaves of plants pre-treated with Polyel + F the activity of these enzymes increased by 76.50; 35.95; 118.30 and 71.06% compared to the activity of these enzymes in the leaves of control plants (70% CTA). In the untreated plants, the insufficiency of humidity at the "I trifoliolate leaf" stage conditioned a weaker activation of these enzymes - by 21.4; 18.1; 13.5 and 24.8% respectively. Superoxid dismutase is the key enzyme, which plays a decisive role in the transformation of $O_2 \cdot^-$ - into H_2O_2 and the protection of cells from oxidative destruction. This enzyme is present in all plants in mitochondria, chloroplasts and cytoplasm. The increase of the enzyme activity to the action of stress factors can be conditioned by the post-translational metabolic regulation, by mobilizing the latent forms and / or the *de novo* synthesis. Based on these data in the literature, the hypothesis emerged that the high activity of SOD is a necessary premise for the resistance of plants to oxidative stress, conditioned by the action of various adverse factors [Baranenco V.V., 2006]. As follows from the data presented in Table 1, the MDA content in plants pre-treated with the compounds used is a result of a greater intensification of SOD activity as well as APX. Thus, the SOD / MDA ratio in control plants (70% CTA) was 1.89,

Table 1:- Influence of cytokine-type compounds and moderate drought in the early stages of ontogenesis on malonic di-aldehyde content and the activity of antioxidant enzymes in the leaves of soybeans, the variety Nadejda.

variants, humidity % TWC	MDA, $\mu\text{M} \cdot \text{g}^{-1}\text{fr. w.}$		SOD, conv. un. \cdot $\text{g}^{-1}\text{fr. w.}$		CAT, $\text{mM} \cdot \text{g}^{-1}\text{fr. w.}$		APX, $\text{mM} \cdot \text{g}^{-1}\text{fr. w.}$		GPX, $\text{mM} \cdot \text{g}^{-1}\text{fr. w.}$		GR, $\text{mM} \cdot \text{g}^{-1}\text{fr. w.}$		GwPX, $\text{mM} \cdot \text{g}^{-1}\text{fr. w.}$	
	M \pm m	Δ , % *	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %
Control, 70	19.59 \pm 0.5		37.15 \pm 0.3		7.40 \pm .2		4.59 \pm 0.1		53.54 \pm 0.9		183.51 \pm 2.3		209.95 \pm 3.8	
Drought, 70-40	37.09 \pm 0.9		45.09 \pm 0.5		8.74 \pm .2		5.21 \pm 0.1		81.79 \pm 1.1		200.74 \pm 3.7		262.01 \pm 4.5	
CK, 70-40	34.40 \pm 1.0	- 7.1	52.90 \pm 1.1	17 .3	8.85 \pm .2	1.3	7.13 \pm 0.1	36 .9	83.03 \pm 2.1	1.51	224.85 \pm 5.4	12 .0	286.23 \pm 3.6	9.2
Tiourée, 70-40	33.85 \pm 0.8	- 8.7	60.20 \pm 1.6	33 .5	9.30 \pm .1	6.4	7.42 \pm 0.1	42 .4	93.28 \pm 1.6	14 .1	243.71 \pm 0.4	21 .4	317.0 \pm 1.2	21 .0
Polyel +F, 70-40	28.11 \pm 0.5	- 24.2	65.57 \pm 1.7	45 .4	10.06 \pm 0.2	15 .1	9.54 \pm 0.1	83 .1	94.14 \pm 0.7	15 .1	246.12 \pm 4.2	22 .6	335.5 \pm 5.8	28 .1

* Compared with untreated plants exposed to the same drought conditions.

Table 2:- Malonic di-aldehyde content and antioxidant enzymes activity of in soybean leaves after improving humidity conditions.

variants, humidity % TWC	MDA, $\mu\text{M} \cdot \text{g}^{-1}\text{fr. w.}$		SOD, conv. un. \cdot $\text{g}^{-1}\text{fr. w.}$		CAT, $\text{mM} \cdot \text{g}^{-1}\text{fr. w.}$		APX, $\text{mM} \cdot \text{g}^{-1}\text{fr. w.}$		GPX, $\text{mM} \cdot \text{g}^{-1}\text{fr. w.}$		GR, $\text{mM} \cdot \text{g}^{-1}\text{fr. w.}$		GwPX, $\text{mM} \cdot \text{g}^{-1}\text{fr. w.}$	
	M \pm m	Δ , % *	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %
Control, 70	18.89 \pm 0.4		34.87 \pm 0.9		7.66 \pm 0.1		4.68 \pm 0.1		65.62 \pm 0.4		174.64 \pm 0.8		236.09 \pm 4.7	
Drought, 70-40-70	22.34 \pm 0.6		38.12 \pm 0.8		7.91 \pm 0.1		4.93 \pm 0.1		73.76 \pm 1.1		175.94 \pm 3.8		249.47 \pm 5.5	
CK, 70-40-70	20.69 \pm 0.5	- 7.4	38.43 \pm 0.8	0.8	7.88 \pm 0.1	- 0.4	5.11 \pm 0.1	3.7	73.34 \pm 1.3	- 0.6	175.33 \pm 2.4	- 0.3	253.70 \pm 4.3	1.7
Tiourée, 70-40-70	19.09 \pm 0.5	- 14.5	39.83 \pm 0.5	4.5	8.36 \pm 0.2	5.7	5.10 \pm 0.1	3.5	74.68 \pm 1.1	1.8	169.91 \pm 1.3	- 3.4	247.15 \pm 5.7	- 0.9
Polyel +F, 70-40-70	17.90 \pm 0.2	- 19.9	38.69 \pm 0.6	1.5	8.46 \pm 0.1	7.0	5.63 \pm 0.1	14.2	74.42 \pm 1.5	0.9	178.52 \pm 1.2	1.5	257.83 \pm 3.9	3.4

* Compared with untreated plants exposed to the same drought conditions.

in control plants, but exposed to the action of moderate drought - 1.21, in plants pre-treated with CK - 1.53, pre-treated with thiourea - 1.78 and for plants pre-treated with Polyel + F - 2.33. These data demonstrate that in plant cells pre-treated with SRO cytokinin-type compounds formed under the influence of dehydration are to a greater extent captured by SOD and ascorbate peroxidase compared to untreated plants. The increase in the activity of these enzymes in drought conditions in pre-treated plants is significantly higher. Improvement of humidity conditions led to a significant decrease in the content of malonic di-aldehyde and the activity of ROS neutralizing enzymes (Table 2), compared to the values of these indices in plants on the 7th day of water stress (Table 1). The MDA content in the leaves of plants pre-treated with thiourea and, in particular, in those pre-treated with Polyel + F, returned to the MDA content of control plants (70% CTA), and the activity of antioxidant protection enzymes continues to be maintained at a higher level compared to the control. The SOD / MDA ratio after 7 days of improving the humidity conditions was 1.84 in the control plants; in plants preventively exposed to drought - 1.70, in plants pre-treated with CK - 1.86 and in plants pre-treated with thiourea and Polyel + F - 2.08 and 2.16 accordingly, which shows that the decrease malonic di-aldehyde content may be not only a consequence of reduced ROS production due to optimizing cell hydration, but also maintaining the activity of the key enzyme to capture them at a higher level. In conditions of unfavorable humidity fluctuation and the installation of periods of repeated drought the degree of increase in ROS formation, induced by insufficient moisture during flowering and pod formation, is significantly lower in plants, which endured moderate water stress at the first leaf stage. trifoliolate, compared to plants exposed to the first cycle of drought in the critical period (Table 3). At the same time, it is remarkable that the drought of the same intensity and duration conditions the appearance of a weaker oxidative stress weakens the plants preventively treated with cytokinin-type compounds. The degree of increase of MDA content in plants first exposed to drought in the "flowering-formation of pods" phase is higher compared to plants, which were first exposed to moderate water stress at the stage "I trifoliolate leaf". The same character of the changes of the MDA content under the influence of repeated water stress was registered in the plants pre-treated with CK, T and Polyel + F, the only difference being the lower true content of the ROS formation indicator (Table 3). As reported above, the maintenance of high antioxidant capacity is closely correlated with the activity of the enzyme antioxidant protection system. The activity of antioxidant protection enzymes is more pronounced in plants that have suffered moderate water stress in the early stages of development and, in particular, in those pre-treated with cytokinin-type compounds. Maximum values of antioxidant enzymes were recorded in conditions of repeated moderate drought in plants pre-treated with Polyel + F.

The activity of the enzymatic system of antioxidant protection, induced by repeated drought, is maintained at a higher true level even after the improvement of the soil moisture (Table 4). The decrease in malonic di-aldehyde content after optimizing soil moisture is significantly more pronounced in plants that have endured a repeated moderate drought, especially in plants pre-treated with thiourea and Polyel + F. In them the activity of antioxidant protection enzymes is maintained at a level truly higher.

Summarizing the information provided by the results of the analyzes performed in the current paper, it can be concluded that ROS, along with the components of water status in cells are involved in perceiving external environmental factors and triggering the molecular mechanisms of plant resistance. as shown above, many authors consider that ROS are the central component in the plant's response to stress and the level of ROS or red-ox status cells determines the type of response, as in low concentrations ROS conditions protective expressions and initiates adaptive processes, and at high concentrations cell death is initiated. Antioxidant systems are considered universal mechanisms of plant protection in various adverse environmental conditions.

It is demonstrated that ROS conditions the expression of protective genes and the initiation of adaptive processes through the synthesis and accumulation of various substances with a protective role: specific proteins, amino acids, carbohydrates, etc. [Bruce T.J.A., Matthes M.C., Napier J.A., Pickett J.A.T., 2007; Bhattacharjee S., 2012]. For effective antioxidant protection, it is not enough to activate only one of the annihilation systems of reactive oxygen species, requiring both antioxidant enzymes and low molecular weight antioxidant metabolites. The functioning of a certain defense biochemical reaction is not enough for the survival of the plant, even in the short-term action of the stress factor. Some changes inevitably lead to the initiation of other protective reactions. It is considered that the involvement of non-enzymatic compounds in the process of neutralizing ROS is a compensation mechanism, which works between low molecular weight antioxidants and antioxidant protective enzymes and the reliability of the entire antioxidant protection system in case of possible disturbances in the activity of certain components.

Table 3:- Malonic di-aldehyde content and activity of antioxidant enzymes in soybean leaves in conditions of repeated drought at the stage of "flowering-formation of pods"

variant s, humidi ty, % TWC	MDA, $\mu\text{M} \cdot \text{g}^{-1}\text{fr. w.}$		SOD, conv. un. $\cdot \text{g}^{-1}\text{fr.w.}$		CAT, $\text{mM} \cdot \text{g}^{-1}\text{fr.w.}$		APX, $\text{mM} \cdot \text{g}^{-1}\text{fr.w.}$		GPX, $\text{mM} \cdot \text{g}^{-1}\text{fr.w.}$		GR, $\text{mM} \cdot \text{g}^{-1}\text{fr.w.}$		GwPX, $\text{mM} \cdot \text{g}^{-1}\text{fr.w.}$	
	M \pm m	Δ , % *	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %
Martor, 70	23.8 \pm 0.5		47.4 \pm 1.3		7.3 \pm 0.2		7.6 \pm 0.2		55.2 \pm 0.7		115.2 \pm 2.7		81.9 \pm 1.7	
Drought, I cycle 70 - 40	36.0 \pm 0.8		54.1 \pm 1.2		8.1 \pm 0.1		11.3 \pm 0.1		73.0 \pm 1.7		127.1 \pm 1.9		100.9 \pm 2.3	
Drought, II cycle, 70-40-70-40	33.9 \pm 0.6	- 5.8	57.3 \pm 1.6	5.9	8.4 \pm 0.2	3.7	14.7 \pm 0.2	30.1	86.0 \pm 1.4	17.8	139.3 \pm 2.1	9.6	120.9 \pm 2.9	19.8
CK, drought, II cycle 70-40-70-40	32.5 \pm 0.4	- 9.7	57.0 \pm 1.0	5.4	8.8 \pm 0.1	8.6	20.5 \pm 0.2	81.4	84.2 \pm 0.6	15.3	164.1 \pm 0.2	29.1	131.5 \pm 2.5	30.3
Thiourea, drought, II cycle, 70-40-70-40	32.1 \pm 0.7	- 10.8	66.1 \pm 1.2	22.2	8.9 \pm 0.1	9.8	21.8 \pm 0.1	92.9	89.6 \pm 2.4	22.7	176.8 \pm 3.3	39.1	146.9 \pm 4.1	45.5
Polyel + F, drought, II cycle, 70-40-70-40	30.1 \pm 0.4	- 16.4	76.3 \pm 1.5	41.0	9.9 \pm 0.1	22.2	23.4 \pm 0.2	107.1	109.6 \pm 3.1	50.1	171.9 \pm 1.6	35.2	158.5 \pm 4.5	57.1

* Compared with untreated plants exposed to the same drought conditions.

Table 4:- The content of malonic di-aldehyde and the activity of antioxidant enzymes in the leaves of soybean plants after the improvement of humidity conditions

variants , humidit y, % TWC	MDA, $\mu\text{M} \cdot \text{g}^{-1}\text{fr. w.}$		SOD, conv. un. $\cdot \text{g}^{-1}\text{fr.w.}$		CAT, $\text{mM} \cdot \text{g}^{-1}\text{fr.w.}$		APX, $\text{mM} \cdot \text{g}^{-1}\text{fr.w.}$		GPX, $\text{mM} \cdot \text{g}^{-1}\text{fr.w.}$		GR, $\text{mM} \cdot \text{g}^{-1}\text{fr.w.}$		GwPX, $\text{mM} \cdot \text{g}^{-1}\text{fr.w.}$	
	M \pm m	Δ , % *	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %	M \pm m	Δ , %
Martor, 70	27.2 \pm 0.3		37.4 \pm 0.6		6.3 \pm 1.0		8.6 \pm 0.2		69.8 \pm 1.2		90.9 \pm 1.2		162.4 \pm 2.9	
Drought, I cycle, 70 - 40	28.1 \pm 0.4		39.4 \pm 1.1		6.5 \pm 0.1		8.9 \pm 0.2		72.2 \pm 1.1		92.5 \pm 2.7		165.6 \pm 3.1	
Drought, II cycle,	28.5 \pm 0.3	1.4	40.1 \pm 0.5	1.8	6.6 \pm 0.1	1.5	9.3 \pm 0.2	4.5	74.6 \pm 0.9	3.3	94.7 \pm 0.8	2.4	167.2 \pm 2.5	1.0

70-40-70-40-70														
CK, drought, II cycle, 70-40-70-40-70	29.2±0.3	3.9	40.5±1.0	2.8	6.8±0.1	4.6	9.4±0.3	5.6	75.2±1.5	4.2	95.8±1.7	3.6	170.7±1.8	3.1
Thiourea, drought II cycle, 70-40-70-40-70	29.5±0.4	4.9	42.5±1.0	7.9	6.8±0.1	4.6	8.8±0.2	-1.1	83.2±1.3	15.2	96.2±2.0	4.0	170.9±2.3	3.2
Polyel + F, drought, II cycle, 70-40-70-40-70	29.1±0.6	3.6	42.6±0.7	8.1	6.7±0.2	3.1	9.5±0.2	6.7	82.1±0.6	13.7	97.8±1.1	5.7	171.2±2.9	3.4

* Compared with untreated plants exposed to the same drought conditions.

From the obtained data (Table 5) it is following that the antioxidant protection capacity of Nadejda soybean plants is ensured both by the increased intensification of the activity of antioxidant enzymes and by the increase in conditions of moderate moisture insufficiency of non-enzymatic antioxidants with mass low molecular weight. It was established that even a moderate and short-term drought in the "I trifoliate leaf" phase conditioned the increase of the content of non-enzymatic antioxidants, such as carotenoids, ascorbic acid and proline (Table 5).

Table 5:- Modification of the content of non-enzymatic antioxidants in the leaves of soybeans at stage "I trifoliate leaf" under the influence of cytokinin-type compounds in conditions of moderate moisture insufficiency.

variants, humidity % TWC	carotenoids, mg · 100 g fr.w.		ascorbic acid μg · g ⁻¹ fr.w.		proline, μg · g ⁻¹ fr.w.	
	M ± m	Δ, %*	M ± m	Δ, %*	M ± m	Δ, %*
Control, 70	50.730 ± 0.4		47.18 ± 1.5		0.76 ± 3.9	
Drought, 70-40	48.535 ± 0.7		54.67 ± 0.9		2.92 ± 1.1	
CK, 70-40	56.098 ± 1.0	15.6	56.66 ± 0.9	3.6	3.13 ± 0.2	7.2
Tiourea, 70-40	59.178 ± 0.9	21.9	57.29 ± 1.9	4.8	3.38 ± 0.4	15.7
Polyel + F, 70-40	60.106 ± 0.3	23.8	60.51 ± 1.2	10.7	4.18 ± 0.1	43.1

* Compared with untreated plants exposed to the same drought conditions.

It is known that dehydration and the formation of ROS in drought conditions largely affect chloroplasts, because in the leaves of plants exposed to drought occurs the photochemical reorganization of chloroplasts as a consequence of excess photon dissipation. More intensive ROS formation takes place on the membranes of chloroplasts and mitochondria [Ivanov B., 1998]. It is considered that chloroplasts are important sensors of changes in the environment, producing red-ox signals and other signals, which can be transferred directly or indirectly to the nucleus to influence gene expression, which leads to acclimatization to predominant environmental conditions. Naturally, chloroplasts are equipped with an abundance of antioxidants with protective functions from ROS. In the elimination of singlet oxygen, compounds that can extinguish the excited states (mainly triplet states) of pigments and singlet oxygen are of particular importance. Of these, according to the authors, carotenoids are the most effective natural mechanism of protection of chloroplasts from oxidative destruction.

Maintaining a more stable level of carotenoids, as it is known, has an important role in protecting chloroplasts and chlorophyll from the destruction of singlet oxygen molecules. In conditions of moderate humidity insufficiency, there was a tendency to increase the carotenoid content in the fruits of the plants (Table 5, Fig.1).

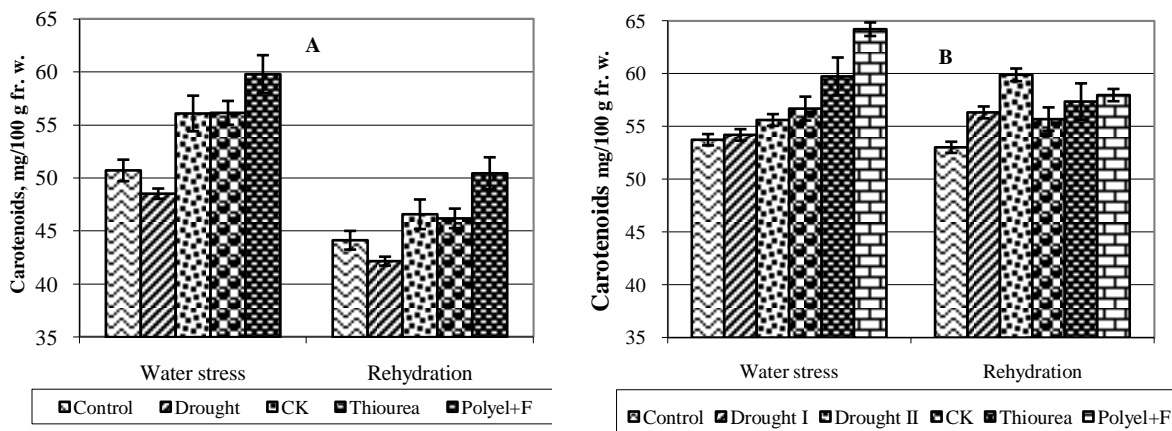


Fig. 1:- Modification of carotenoid content in the leaves of *Glycine max* (Merr.) L. plants, Nadejda variety under conditions of humidity fluctuation and repeated water stress. A - at the "First trifoliolate leaf" phase; B - at the phase "flowering - formation of pods". The effect of cytokinin-type compounds.

After the first cycle "drought / recovery" a significant increase took place in plants pre-treated with Polyel + F. After the improvement of humidity conditions, the carotenoid content decreases significantly, but in plants preventively exposed to drought their content is kept at a high compared to untreated plants and even plants not preventively exposed to drought. It is worth mentioning that the same character of the change of the carotenoid content was registered in the "flowering-formation of the pods" phase: the double stressed plants are distinguished by a higher carotenoid content. The compounds used to treat the seeds and foliar apparatus of plants help to maintain the background of non-enzymatic antioxidants in conditions of moisture insufficiency at a higher level.

It is considered that the central role in the operative change of antioxidant status to oxidative stress caused by excessive formation of reactive oxygen species belongs to ascorbate. Ascorbate and glutathione are the central components in regulating the red-ox balance of plant cells [Noctor G., Foyer CH, 1998]. Ascorbic acid possesses a wide range of antioxidant properties is the most effective inhibitor of singlet oxygen in chloroplasts. H_2O_2 can be reduced both directly by ascorbate and by ascorbate peroxidase. In this study there was a significant influence of plant pre-treatment with solutions of cytokinin-type compounds, in particular, with the preparation Polyel + F on the accumulation of ascorbic acid (Table 5; 6; Fig.2).

Table 6:- The content of non-enzymatic antioxidants in the leaves of soybean plants at the phase "flowering - formation of pods" in conditions of repeated drought.

variants, humidity, % TWC	Carotenoids, mg · 100 mg fr.w.		ascorbic acid $\mu\text{g} \cdot \text{g}^{-1}\text{fr.w.}$		proline, $\mu\text{g} \cdot \text{g}^{-1}\text{fr. w.}$	
	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %
Martor,70	57.80 ± 0.3		73.18± 0.7		0.85 ±0.1	
Drought, I cycle 70 - 40	53.50 ± 1.8		80.46± 1.8		1.91 ± 0.1	
Drought, II cycle, 70 – 40 – 70-40	56.24 ±0.4	5.1	84.11± 1.0	4.5	2.59 ± 0.2	35.3
CK, drought, II cycle, 70 – 40 – 70-40	58.11 ± 0.4	8.7	88.07± 1.1	9.5	3.00 ± 0.1	56.9
Thiourea, drought, II cycle, 70-40-70-40	60.16 ± 0.5	12.5	94.12± 0.9	17.0	3.88 ±0.1	102.7
Polyel + F, 70 – 40 – 70-40	64.22 ± 0.5	20.0	9.06± 0.7	19.4	4.28 ±0.3	123.6

In the "I trifoliolate leaf" phase, the drought caused an increase in ascorbate content by about 16 percent, and in plants pre-treated with CK, T and Polyel + F in the same drought conditions the ascorbate content exceeded its level in the leaves of untreated plants with 3.64, 4.80 and 10.7% compared to the control (70% CTA). After improving the soil moisture, the content of ascorbate decreases, but in preventively stressed plants it is kept at a higher level (Fig. 2A).

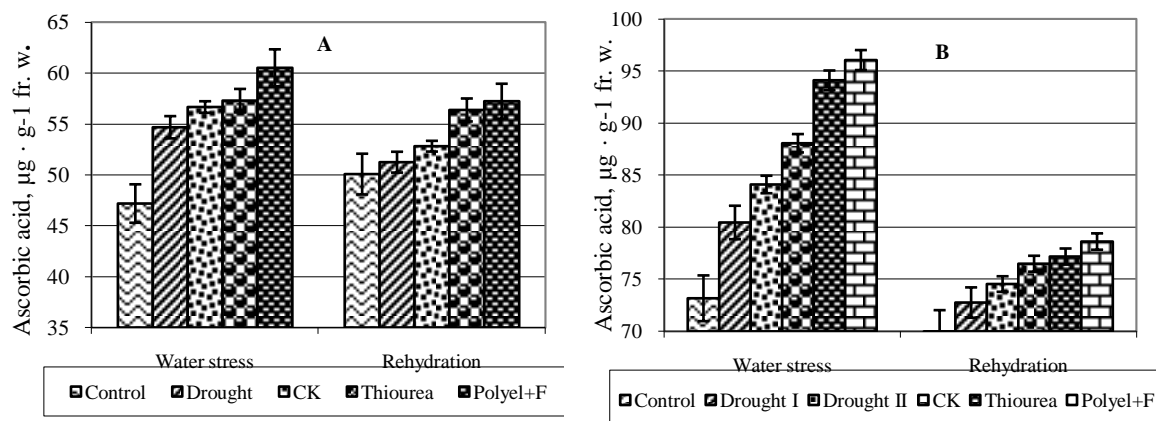
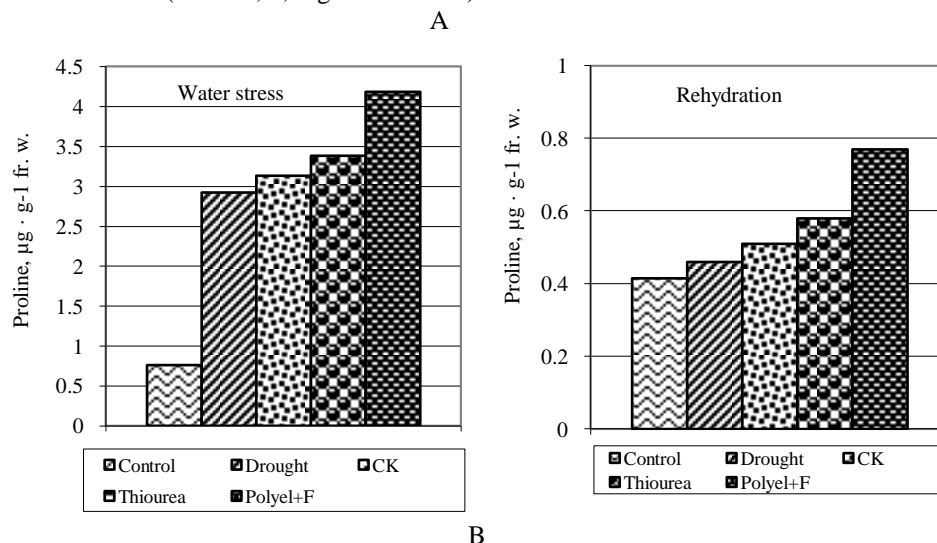


Fig. 2:- Modification of the ascorbic acid content in the leaves of *Glycine max* (Merr.) L. plants, in conditions of humidity fluctuation and repeated water stress. A - at the "First trifoliate leaf" phase; B - at the phase "flowering - formation of pods". The effect of cytokinin-type compounds.

Another important component of the antioxidant defense system, a regulator of cellular red-ox potential, a stabilizer of subcellular structures and a component of the signal transduction pathways that regulate stress-receptive genes is considered proline [Noctor G., Foyer CH, 1998]. Proline is a low molecular weight antioxidant, a regulator of cellular red-ox potential, a stabilizer of subcellular structures and a component of signal transduction pathways, which regulates stress-receptive genes. Proline has a universal mechanism for generating tolerance to various abiotic stress factors by stabilizing structural components, enzyme structure and osmotic regulation. Proline is one of the main organic osmolytes that accumulates in many plant species in response to environmental stress such as drought, salinity, extreme temperatures, ultraviolet radiation and heavy metals. In fact, there is evidence that Pro has both positive and negative effects on stress tolerance. Much of the conflicting evidence came from physiological investigations examining Pro levels in different plant genotypes and in different drought conditions by intensity and duration. Recent Ding Y., Fromm M., Avramova Z. (2012) reported that a Repeated pre-treatment by dehydration / rehydration induces the expression of specific genes that control the enzymes proline dehydrogenase and proline oxidase in drought conditions.

The data obtained in the current investigations confirmed the information on changes in proline content in the leaves of plants exposed to soil moisture at the initial stages of ontogenesis and on the nature of these changes in plants exposed to repeated water stress (Table 5; 6; Fig. 3 A and 3B).



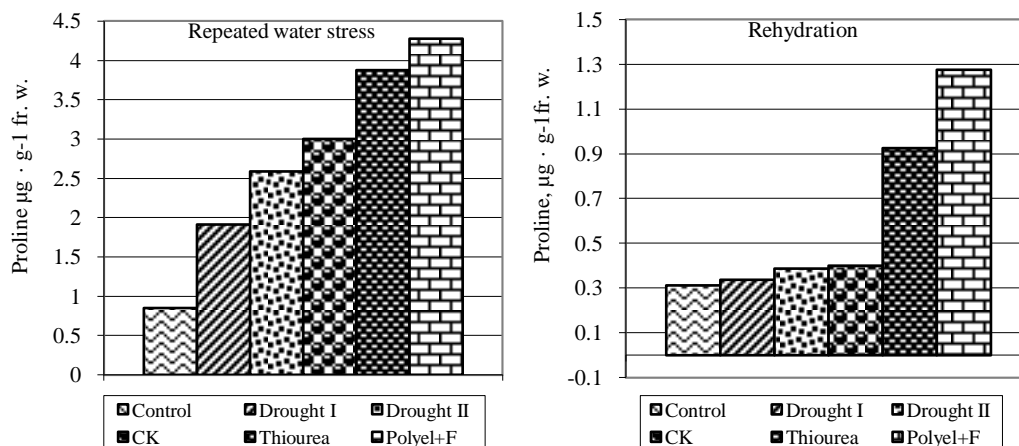


Fig. 3:- Changing the proline content in leaves of plants of *Glycine max* (Merr.) L. cultivar trust in terms of fluctuation of humidity and water stress repeatedly: A - at the "First trifoliate leaf" phase; B - at the phase "flowering - formation of pods". The effect of cytokinin-type compounds.

The insufficiency of moderate humidity at the "I trifoliate leaf" phase conditioned a significant increase of the proline content in the leaves. In the leaves of plants pre-treated with CK, T and, in particular, with Polyel + F in drought conditions, the Pro content was significantly higher compared to untreated plants: by 7.2; 15.75 and 43.2%. The insufficiency of moderate humidity at the "I trifoliate leaf" phase conditioned a significant increase of the proline content in the leaves. In the leaves of plants pre-treated with CK, T and, in particular, with Polyel + F in drought conditions, the Pro content was significantly higher compared to untreated plants: by 7.2; 15.75 and 43.2%. After the improvement of the humidity conditions during the recovery period, the Pro content in the leaves decreased, but in the plants pre-treated with the compounds used the amino acid level remained higher both compared to the control and to the plants preventively exposed to drought (Fig. 3A). In the post-stress period, the Pro content in the leaves of plants not treated with cytokinin-type compounds exceed the value of this amino acid in the leaves of control plants by 1.2%, while in plants pre-treated with CK, thiourea and Polyel + F after moisture improvement the Pro content exceeded its comparative level with untreated plants with 3.61; 36.14 and 68.7%.

So, the obtained data argue the idea that the moderate insufficiency of humidity at the initial stages of plant development induces the stimulation of the processes of synthesis / accumulation of proline, a memorable process, which speaks of preserving the amino acid content and improving moisture.

Due to the increased potential for protection of antioxidant systems provided by thiourea and Polyel+F, the repeated occurrence of drought in the phase "flowering - formation of pods" in plants more pronounced tolerance is manifested by activating the accumulation of non-enzymatic antioxidants. It was found that plants, which have withstood stress in the early stages of development, at the onset of new stress keep the proline content at a true higher level by 35.3% compared to unadapted plants, exposed for the first time to drought. In plants pre-treated with cytokinin-type compounds, there was a significant increase in non-enzymatic antioxidants. Under the conditions of the second drought cycle, the proline content in plants pre-treated with CK prevailed with: 56.90; in plants treated with thiourea - with 102.67 and in plants treated with Polyel + F - with 123.6% compared to the value of these compounds in the leaves of untreated plants and exposed to a single cycle of water stress in the phase "flowering - formation of pods". Improvement of humidity conditions after the second cycle of drought-rehydration proline background decreases truthfully, but in plants pre-treated with cytokinin-type compounds, especially in plants pre-treated with thiourea and those pre-treated with Polyel + F it is kept at a significantly higher level (Fig. 3B).

Therefore, the exogenous application of physiologically active cytokinin-type substances has the effect of increasing the tolerance of plants to the occurrence of repeated stress by amplifying the antioxidant potential. The antioxidant protection capacity of soybean plants is ensured both by the increased intensification of the activity of antioxidant enzymes and by the increase in conditions of moderate moisture insufficiency of non-enzymatic antioxidants with low molecular weight.

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