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RESEARCH ARTICLE

WATER STATUS AND STRESS-MEMORY FORMATION IN *PHASEOLUS* PLANTS WITH DIFFERENT DROUGHT RESISTANCE POTENTIAL.

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Abstract

The results of the investigations about the impact of repeated stress on the plant water status have been analysed by taking the literature information about some mechanisms, associated with the stress-memory formation and resistance of plants to drought. The concept of stress-memory existence as an ability of quick and adequate plant response to recurring stress exposure (it is stated that reaction of plants affected by repeated stress differs from the reaction of plants, exposed to stress factors for the first time) was experimentally demonstrated. It was established that beans plants of the species *Phaseolus lunatus*, L., variety 'Fetanisa', *Phaseolus acutifolius*, Gray, variety 'Acutifolius 5' and *Phaseolus vulgaris* L., variety 'Nina', which have been undergone a mild drought condition at phase "first trifoliate leaf" more easily supports repeated stress to the stage of "flowering- pods formation" as a result of the formation of stress memory - the ability to react appropriately to the unfavorable factor. The dependence of stress-memory formation on homeostatic water capacity in tissues and on the constitutive resistance of the genotype have been demonstrated. The self-regulation of water status by increasing water retention capacity and preserving turgescence are nonspecific mechanisms important for stress-memory formation and plant adaptation to fluctuating humidity conditions and water stress recurring over time.

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Introduction:-

Plants, both spontaneous and especially cultured, are frequently exposed to unfavorable growth conditions. Climate factors, such as extreme temperatures, droughts, increased of concentrations soil solution, are major impediments that limit growth and development, and, therefore, crop yield. Increasing the climate change and the frequency of extreme conditions lead to plant exposure not just to one single drought event but also to multiple stress situations repeated in different periods of development [Jentsch A., Kreyling J., Elmer M. et al., 2011; Krasensky J., Jonak C., 2012]. Short-term moderate droughts (3-5 days), which are repeated periodically, negatively affect productivity, but contribute to increasing plant resistance. The withering of plants that have endured the several moderate droughts takes place at lower soil moisture as compared to plants grown permanent under optimal conditions. Stimulation of tolerance to repeated stress through pre-exposure to various stress factors is well known. This phenomenon of hardened plants to drought was observed in 1926 by Tumanov I. (1926), who showed that certain mesophyte plants in conditions of humidity deficiency form resistance, which facilitates their passage through drought conditions.

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Newer researches [Acatrinei Gh., 1991] on wheat, barley, corn, soybean, etc. demonstrated that this process involves modifications on every level of organization of plant organism. Plants tempered to drought have a well-developed root system, high absorption activity of root hairs, absorbent cells show reduced osmotic potential due to increased level of osmotic active substances' concentration etc. This phenomenon is associated with changes in gene expression pattern, enzymatic activity modification, synthesis of isozymes and some other proteins, activation of new regulation systems that contribute to increasing of cytoplasmic tolerance to dehydration. Ding Y., Fromm M. and Avramova Z. (2012), Tetsu Kinoshita and Motoaki Seki (2014) have demonstrated that multiple exposure to drought stress condition cause a quicker and adequate plant response to a new stress situation through differential gene expression changes compared to plants that weren't previously exposed to drought stress. These researches contributed to elaboration of stress-memory concept, which implies that plant exposure to subsequent stress induces a different reaction than that which accessed in plants exposed for the first time under stress [Bruce T.J., Matthes M.C., Napler J.A., Pickett J. A., 2007]. It is assumed that plants that have sustained moderate stress at the beginning of vegetation may form the so-called "stress memory" - the ability to adequately react to repeated stress over time [Walter J. et al., 2011; 2013]. Stress memory is defined as the capacity of plant to memorize information about previous stress incident and which is used to optimize subsequent protection against repeated stress case [Trewavas A., 2003]. According to [Fleta-Soriano E., Munne-Bosch S., 2016], stress printing or memory can be defined as structural, genetic and biochemical changes that occurred as a result of preventive exposure to stress and which makes the plant more resistant to subsequent exposure to the same stress factor. Expression of particular genes, induced by environmental stimuli, triggers activation of certain hormonal, metabolic, structural and other defense mechanisms that provide tolerance and organism's survival under moderate unfavorable environmental conditions. Genes that ensure changes of plant response to subsequent stress were designated as a category of "memory genes" [Ding Y., Ning Liu, Virlouvert L. et al., 2013]. On the other hand, if the stress is too severe, the plants may be adversely affected. Walter J., Nagy L., Hein R. et al. (2010) have shown that severe drought has not only resulted in the loss of biomass in herbaceous plants but also in reducing photosynthesis and inhibiting the photosynthetic apparatus when the plants were exposed to a second drought. As a result, extreme stress may cause serious adverse effects in twice-stressed plants.

What are the mechanisms by which the plant stores information about changes associated with stress tolerance and how they provide adequate response to successive stress so far is not known. One of general primary responses to different stressors, both biotic and abiotic, is change of plant water status [Bartoli C.G. et al. 1999; Ștefiriță A. et al., 2012, 2013, 2016] and activation of antioxidant protection system. ROS are involved in perceiving of the external signals. Their moderate increase causes the expression of protective genes and the initiation of adaptive processes [Bhattacharjee S., 2012] by synthesizing and accumulating various protective substances: specific proteins, amino acids, carbohydrates, etc. [Davies W.J., Zhang J., 1991; Bruce et al., 2007]. According to [Bacon M.A., Wilkinson S., Davies W.J. 1998; Munns R., M. Tester, 2008; Wilkinson S., Davies W.J. 2002], modification of hydraulic pressure in plant organs caused by changes of ABA levels under drought conditions, jointly with both chemical (pH, ROS, etc.) and hormonal mediators trigger anti-stress subroutines and execution of adaptive and defense reactions. It is mentioned [Bacon M.A., Wilkinson S., Davies W.J. 1998; Wilkinson S., Davies W.J. 2002], that in some species the hydraulic signals are more significant for inducing the plant's response to the unfavourable factor action. Another possibility to preserve an ecological stress memory is the morphological changes of the plant, which remain stable for a longer time [Kalapos T. et al., 1996; Bartoli C.G. et al., 2013]. Under these conditions, biological traits of a crop, formed during species' evolution, manifest themselves at organismic level as an integral adaptive system through formation of corresponding evaporation surface area, number of stomata, cationization, leaf pubescence, root vigor and rates of radicular system growth, vegetative and generative organs' mass rate, etc. [Aroca R., 2012].

Studies of the mechanisms related with stress - memory of plant are still rare. A few researches regarding this topic are in most cases conducted on species of spontaneous flora and a certain data on mesophytes crops is absent. Through elucidating of plant resistance mechanisms and regulation pathways, it may have a potential significance for agronomy as a theoretical basis for elaboration of effective field management strategies regarding crop productivity retention in the context of future climate changes.

In this study, on example of *Phaseolus* plants exposed to repeatedly drought, we argue that the features of water status could play an important role in the formation of stress-memory and adaptation to humidity fluctuation conditions.

Material and research methods:-

Bean plants of *Phaseolus vulgaris*, L. variety 'Nina', *Phaseolus lunatus* L. variety 'Fetanisa' and *Phaseolus acutifolius* Gray variety 'Acutifolius 5' have been used as study objects. The representatives of these species are distinguished by the potential of resistance to drought. Plants of the *Ph. vulgaris*, L. after ecological characters is attributed to mesophytes, develops a vigorous root system, horizontally oriented in the arable layer, and develops well in areas with sufficient humidity. Plants of the *Ph. vulgaris*, L. according to ecological characters is attributed to mesophytes, develops a vigorous root system, horizontally oriented in the arable layer, grows well and develops in areas with sufficient humidity. It is considered that the root system formed by the plant at the first stages of ontogenesis can predetermine the organism's resistance or receptivity to water supply at later stages of development. Genotypes of *Ph. lunatus* L. and *Ph. acutifolius*, Gray, are distinguished by morphogenetic properties pre-adaptation to drought. According to ecological characters, the plants of the Fetanisa cultivar are attributed to xerophytes, and the plants of Acutifolius 5 are typically xerophytes. Adaptive morphological characters that contribute to drought tolerance of lime and tepary bean are attributed to: small leaves, pubescent, cutinisation, wax stratum, the properties of leaf leaves change the position of the sun over the day, deep penetrating root system, with well-developed absorbent surface. The critical period in the individual development of bean plants is the phase from flowering to baking when they are most affected by drought. During this period the drought cause the downfall of flowers and ovaries in many species of the *Phaseolus* tribe.

In experiments with different humidity conditions, realised in the Greene house of the Institute of Genetics, Physiology and Plant Protection, plants were grown in Mitcherli pots with a capacity by 30 kg absolutely dry soil. The moisture regime is maintained by watering the plants according to the weight of the pots with the soil and plant respectively. Drought-induced stress was modelled by reducing the watering level from 70% of total soil water capacity (TWC) to 30% TWC humidity. We mention that such a stressful condition is a drought, when the soil humidity reaches the value of 1.2 - 1.3 wilting coefficient for *Ph. vulgaris* L.

Water stress conditions have been created: I cycle of drought – at the phase "first trifoliate leaves"; II cycle of drought - at the phase "flowering – pods formation".

Scheme of experiment:

At the phase "first trifoliate leaf":

I variant - control, humidity 70% TWC (total water capacity of the soil);

II variant - humidity 70 - 35% TWC;

At the phase "flowering - pods formation":

I variant - control, humidity 70% TWC;

II variant - 70-35% TWC (first cycle of drought);

III variant - 70-35-70-35% TWC (plant under repeated drought).

Water stress conditions lasted for 7 days. After 7 days of drought moisture has been restored to the optimal level. The study of morpho-functional characters was performed after 7 days of water stress and after the 1st and 7th days after restoring optimal humidity conditions.

For water status assessment in roots, leaves and pods several indices were taken in account: the total water content - by drying the sample to constant weight at a temperature of 105° C; saturation deficit - expressed as a percentage of full saturation; water retention capacity in tissues was characterized by water retained after 2 hours of induced abscission, expressed as a percentage of its initial content. Relative water content was determined according to Vasseu T.L., Sharkey T.D. (1989). Water parameters in roots were determined considering the entire root system. Same procedure was performed with inflorescences and pods.

Plant's resistance to water stress was judged upon the degree of modification / stabilization of its physiological processes. The differences between the variants were documented by statistical analysis of the data, using the software set "Statistics 7" - ANOVA for PC.

Results and Discussions:-

By the virtue of the fact that plant organism as an open biological is closely connected to environment, and the water in the plant represents a continuous water from the soil, the lack of moisture primarily affects the status of the water

in the plane and the dehydration of the tissues is a direct consequence of the external factor action [Levitt J., 1980; Kramer P., Boyer P., 1995]. At the same time, due to the presence of mechanisms of self-regulation, plants can ensure certain level of autonomy of the metabolic processes independently of the environmental conditions.

Comparative analysis of self-regulation of water homeostasis has shown that *Phaseolus* plants of the investigated species are distinguished by their reactivity to the variation in humidity regime and the degree of change in the parameters of the water status in the leaves depends on the genotypic peculiarities and the potential of their resistance (Table 1).

Table 1:-Influence of soil moisture on the water content ($\text{g} \cdot 100\text{g}^{-1}$ f.w.) in the leaves of bean plants at phase "First trifoliate leaf"

Species / variety	Soil humidity, % TWC	WC, $\text{g} \cdot 100\text{g}^{-1}$ f.w.*		SD, % of full saturation		WRC, % water retained from the initial content	
		M \pm m	Δ , % control	M \pm m	Δ , % control	M \pm m	Δ , % control
<i>Ph. lunatus</i> , L.	Control, 70	83.74 \pm 0.81		6.64 \pm 0.43		76,86 \pm 0.35	
	Drought, 35	82.26 \pm 0.37	-1,77	9.37 \pm 0.11	41,11	79,03 \pm 0.31	2,82
<i>Ph. acutifolius</i> , Gray	Control, 70	85.01 \pm 1.39		6.49 \pm 0.22		74,31 \pm 0.44	
	Drought, 35	83.15 \pm 1.55	-2,19	9.28 \pm 0.28	42,99	77,02 \pm 0.18	3,64
<i>Ph. vulgaris</i> , L.	Control, 70	86.48 \pm 0.95		8.70 \pm 0.33		73,06 \pm 0.36	
	Drought, 35	83.34 \pm 0.63	-3,64	12.82 \pm 0.19	47,37	75,03 \pm 0.22	1,90

*WC - water content; f.w.- fresh weight; SD-saturation deficit; WRC - water retention capacity

Under favourable humidity conditions, the water potential and the leaf tissues degree of hydration of *Ph. vulgaris*, L. plants are characterized by slightly higher values compared to *Ph. acutifolius*, Gray and *Ph. lunatus*, L. (Table 1). The degree of hydration of the tissues of *Ph. vulgaris*, L. is higher by 1.4 - 2.4% compared to that of the *Ph. acutifolius*, Gray and *Ph. lunatus*, L. respectively. The differences are not significant but where true to the I truthfulness level of veracity I ($t_d = 1.24$; $t_d = 2.98$). Under these conditions (70% TWC), the saturation deficit in lima bean leaves, variety 'Fetanisa' arrives 6.7-7.1% from full saturation, to tepary bean variety 'Acutifolius 5' is 6.5%, and to vulgaris bean variety 'Nina' - 8.7% from full saturation.

It is considered that the character of changes in the water status parameters and relative water content, water use efficiency, water retention capacity of tissues can be used to obtain information about the tolerance or sensitivity of plants to drought. In fact, the parameters of the water status in plant are used as quantitative criteria of plant resistance to stressful climatic factors everywhere and for a very long time [Ștefăruț A. et al., 2017]. One index of drought resistance is high water retention capacity and low saturation deficit of the tissue. The humidity deficiency at the phase "First trifoliate leaf" naturally causes a decrease in the degree of hydration of plant tissues (Table 1). The level of maintaining of the relative water content in leaves differs significantly for every studied variety. After 7 days of water stress (35% TWC, the water content in leaves was reduced respectively: 'Fetanisa' lima bean - by 1.8%, 'Acutifolius 5' tepary beans - by 2.2%, and in 'Nina' common beans reduction of tissue hydration constituted 3.70% compared to the water content in control plants (70% TWC). The increment of saturation deficiency level was considerably lower in the leaves of varieties with higher resistance potential. Minimal values of saturation depletion were recorded in *Ph. lunatus*, L. and *Ph. acutifolius*, Gray. Similar changes were detected regarding plant turgidity. Lack of moisture induced loss of turgescence by 2, 3 - 2, 9% compared to control plants (70% TWC) in *Ph. acutifolius*, Gray and *Ph. lunatus*, L. plants and by 4, 5 - 5, 6% in *Ph. vulgaris*, L. plants. The difference in

saturation deficit and turgescence changes in tolerant plants (lima and tepary beans) was significantly lower compared to *Ph. vulgaris*, L. plants, sensitive to drought.

Water retention capacity is one of the internal factors regulating the water homeostasis of plants. It has been established that the lima bean leaves from the optimum moisture background after 2 hours of experimental wilting retain 76.86% of the initial water content in the tissues; tepary beans - 74.31% and common beans - 73.06% of the water reserve in tissues. The leaves of plants of the species *Ph. lunatus*, L., and *Ph. acutifolius*, Gray are characterized by maximum water retention. Thus, it could be expected that the representatives of these species, having a high capacity of water retention in tissues, will be less affected in drought conditions. The amount of water retained after experimental wilting (2 h) of plant leaves variety 'Fetanisa' from 35% TWC humidity background increases with 2.82%; 'Acutifolius 5' - 3.64%, and variety 'Nina' - with 1.9% compared with control plants (70% TWC). According by the amount of water retained in leaves under conditions of moisture insufficiency in the soil, the plants taken in the investigations, can be distributed in descending order as follows: 'Fetanisa' > 'Acutifolius 5' > 'Nina'. Plants with high resistance potential are characterized by increased capacity to retain water in leaf tissues. Plants of the species *Ph. vulgaris*, L., susceptible to drought, distinguished with high water loss velocity.

Therefore, the results of this study lead to the conclusion that under conditions of soil moisture deficiency the intensity of water exchange processes in plants is diminished: hydration and tissue turgidity degree decreases, saturation deficit increases. The character of changes in the water status of leaves under moisture fluctuations and short-term action of stress factor are determined by genotype particularities. *Ph. lunatus*, L. and *Ph. acutifolius*, Gray are characterized by more stable water status and a higher level of preservation of water content under moderate drought conditions. Studied cultivars show different constitutive resistance. Plants considered to have a high potential of resistance (*Ph. lunatus*, L. and *Ph. acutifolius*, Gray) under the same conditions of suboptimal humidity level (35% CTA) are characterized by the property to maintain a relatively stable tissue hydration, while *Ph. vulgaris*, L. is distinguished by increased sensitivity to stress.

Current investigations show that plants form stress memory upon moderate water stress at juvenile stage (Table 2).

Table 2:-The water content ($\text{g} \cdot 100\text{g}^{-1}\text{f.w.}$) in organs of bean plants exposed to repeat drought

Table 2. The water content (g 100g DW) in organs of bean plants exposed to repeat drought							
Species	Variety	Organ	70 % TWC, control	The first cycle of drought		2 cycles of drought	
			M ± m	M ± m	Δ, % control	M ± m	Δ, % control
<i>P. lunatus</i> L.	Fetanisa	pods	87,07 ± 0,46	85,52 ± 0,19	-1,78	85,76 ± ,24	-1,50
		leaves	82,60 ± 0,12	79,67 ± 0,49	-3,54	81,32 ± 0,44	-1,55
		stems	79,48 ± 0,45	77,52 ± 0,27	-2,47	78,71 ± 0,28	-0,97
		roots	85,45± 0,39	74,98 ± 1,77	-12,25	79,91 ± 0,54	-6,48
Degree of parameter change, % / plant					Σ -20,04		Σ-10,50
<i>P. acutifolius</i> Gray.	Acutifolius 5	pods	87,52 ± 0,50	84,97 ± 0,29	-2,99	85,23 ± 0,31	-2,62
		leaves	84,56 ± 0,41	81,72 ± 0,47	-3,36	82,76 ± 0,33	-2,13

		stems	80,12 ± 0,19	77,79 ± 1,56	-2,91	78,59 ± 0,69	-1,91
		roots	85,06 ± 0,42	74,77 ± 1,05	-12,10	78,68 ± 0,92	-7,50
Degree of parameter change, % / plant					Σ-21,36		Σ-14,16
P. vulgaris L.	Nina	Pods	90,00 ± 0,66	86,11 ± 0,70	-4,32	86,76 ± 0,60	-3,60
		leaves	84,79± 0,23	80,29 ± 0,55	-5,31	81,13 ± 0,42	-4,32
		stems	82,77 ± 0,62	80,11 ± 0,45	-3,21	80,51 ± 0,85	-2,73
		roots	86,56± 0,38	73,02 ± 0,68	-15,64	77,74 ± 0,82	-10,19
Degree of parameter change, % / plant					Σ-28,48		Σ-20,84

A slight dehydration caused by a moderate drought (35% CTA, 7 days) at the early stages of development results in increased plant tolerance to a repeated drought over time. This allows assuming that changes that occur at the cellular level and conferring resistance are initiated to lower water content and can be enhanced when water loss is not too rapid. Both *Ph. lunatus* L and *Ph. acutifolius* Gray, and those the *Ph. vulgaris* L, which have suffered moderate short-term water stress at the first trifoliate leaf phase, during the further development there are no significant quantitative differences of the water status components. Towards the flowering and the formation of the pods, the degree of hydration of the vegetative organs is approximately the same in the control plants and in the plants, which underwent a cycle of drought in early phase of development. On the occurrence of repeated drought conditions at the phase "flowering - forming pods" has been found a more adequately response of the plants, which have undergone a mild water stress at early stage compared with the plants exposed the first time to drought.

Ecological classification of plants is mainly based on overall characteristic of the functional state of the leaves. This comes from the fact that any changes in environmental conditions are reflected to a certain extent on the development and activity of assimilation apparatus, which ultimately determines the productivity of the plant. The experimental data obtained in the current study demonstrates the reaction and the different degree of modification of plant water status parameters not only by species, variety tolerance, but also by organ (Table 2). It draws attention to the fact that pods and stems are distinguished by a significantly higher internal water homeostatic capacity compared to leaves and roots.

Therefore, the degree of hydration of the pods in the plants exposed to the first drought cycle was 1.78 - 2.99 - 4.32% lower compared to the water content in the control plant pods. Thus, the degree of hydration of the pods to the representatives of the *Ph. lunatus*, L., *Ph. acutifolius*, Gray and *Ph. vulgaris*, L., exposed to the first drought cycle, was 1.78 - 2.99 - 4.32% lower compared to the water content in the control plants. In plants exposed to repeated water stress there was a less significant decrease in the degree of hydration of the pods: by 1.50 - 2.62 - 3.60% from the control plant parameter value. In 'Fetanisa', 'Acutifolius 5' and 'Nina' stems after the first drought cycle a decrease of water content by 2.47 - 2.91 - 3.21% correspondingly was recorded. Repeated drought conditioned the decrease of the water content in the studied plant stems by 0.97 -1.91 and 2.73% respectively. Plants that have sustained a moderate drought at the early of ontogenesis stages become more tolerant to moderate water stress during flooring and pods formation (Figure 1).

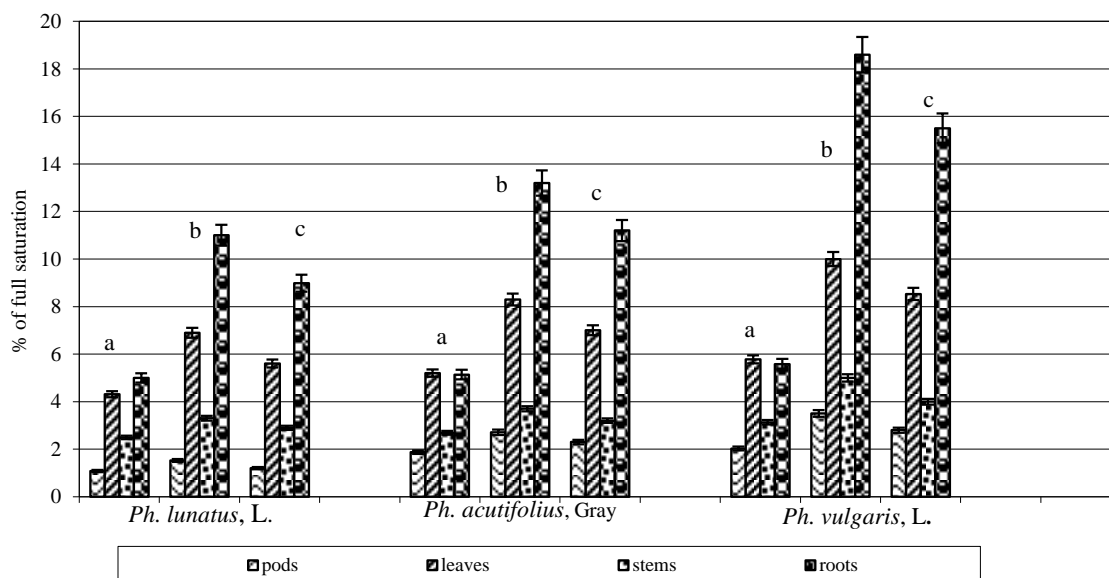


Fig. 1:-Modification of the saturation deficit in organs of bean plants exposed to repeated drought action: a - control, unstressed plants; b - plants exposed to the first drought cycle at the "flowering – formation of pods" "; c – twice-stressed plants.

The representatives of sp. *Ph. lunatus*, L. and *Ph. acutifolius*, Gray, demonstrated a more pronounced adaptive response compared to *Ph. vulgaris*, L., the adaptation effect is better pronounced as a result of self-regulation property of water status. Thus, the degree of hydration of 'Fetanisa' and 'Acutifolius 5' leaves under influence of the second drought cycle decreased insignificantly - by 1.55 and 2.13% respectively compared to control plants, and in 'Nina' plants dehydration accounted for 4.32%. In tissues of plants exposed for the first time to drought stress, saturation deficit was by 59.4% higher compared to control plants. In plants exposed repeatedly under drought conditions, the saturation deficit in leaf tissues increased by 34.3 - 41.9% at varieties 'Fetanisa' and 'Acutifolius 5'. Maximum saturation deficiency values were registered for *Ph. vulgaris*: an increase by 65.4% - for the plants primarily exposed to drought during the "flowering - formation of pods" stage, and by 47.6% for the plants that were exposed to the second cycle of drought. Saturation deficiency and turgidity values of leaves in plants, previously stressed at the juvenile stage, at the phase "flowering - formation of pods" are not different from the control plants.

It is known [Kramer P.J., Boyer J.S., 1995] that the survival of plants under drought conditions depends on the activity of the root system and the water reserves in the roots and stems. It is to be noted that the insufficiency of moisture causes a significantly greater saturation deficiency in the plant roots. Deficiency of saturation in plant roots, prior to the juvenile stage stressed, at the phase of "flowering - pods forming" has increased to *Ph. lunatus*, L., and to *Ph. acutifolius* Gray of 1.8 and 2.2 times more compared to plants on the optimum moisture background (Figure 1). In plants exposed for the first time to water stress at the flowering stage, the saturation deficit in the roots increased respectively 2.2 and 2.6 times compared to control plants. For *Ph. vulgaris* L. 'Nina' plants maximum deficit was recorded in the roots of plants exposed for the first time to drought conditions and reached a 3.6-fold increase, compared to control plants. Repeated water stress in these plants conditioned by 3.0 times increase of the saturation deficit against control, which was significantly higher in *Ph. lunatus* L. and *Ph. acutifolius* Gray but less significantly compared to plants exposed to a single drought cycle. Impact of drought on water content and saturation deficit of the root system is the fact that with the reduction of soil moisture water absorption by roots becoming more difficult. Besides, during the drought in increasing the tissue saturation deficit, the leaves drain water from the roots, which leads to a greater inhibition of their functional activity. From the data obtained, the symptoms of disturbance of the functional activity of the root system are manifested significantly weaker at the repeated occurrence of the drought conditions compared to the non-adapted plants.

The value of saturation deficit to some extent also depends on the capacity of the cytoplasm biopolymers to retain water. Changing the water retention capacity in cells and tissues is an integral endogenous factor in regulating the response of the plant to adverse environmental influences, as intracellular water provides cooperative enzyme

restructuring, concentration and compartmental effects, pH, etc. [Ștefiriță A. et al., 2017]. There is no doubt that the normal functioning of the organs and therefore the integrated organism requires a certain level of hydration and activity of the water molecules, which is ensured by the conditions of water supply. At the same time, for plants under unfavorable conditions, it is important to reduce water losses for the prevent excessive dehydration of tissues. The more strained the conditions of the external environment, the more necessary is their metabolic-controlled and genotypically regulated defense function. Tolerant plants are characterized by increased capacity to retain water in leaf tissues under reduced humidity conditions. Under conditions of moderate soil humidity deficiency, they show a lower degree of water loss, manifested differently in plant organs. It has been established that when the drought occurs repeatedly, the water retaining capacity in leaf tissues is higher compared to plants exposed for the first time to stress, whereas the property of pods, stems and especially roots decreases significantly (Figure 2). The roots firstly lose the ability to retain water, which negatively affects the degree of water saturation and tissue turgescence.

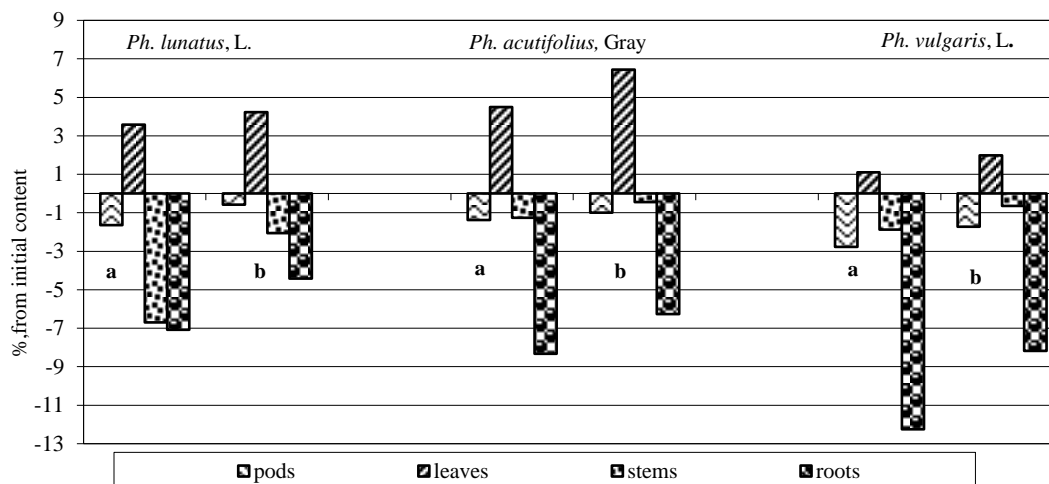


Fig. 2:-Degree of change in water retention capacity in bean plant organs exposed to repeated drought at the "flowering - pods formation" phase: a - a drought cycle b - two drought cycles.

The values of water retention capacity in the leaves for the adapted plants are higher, compared to plants exposed for the first time to drought. 'Fetanisa' plants exposed to recurring drought retains by 4.25% more water in the tissues, 'Acutifolius 5' - by 6.43 and 'Nina' - 2.0% compared to control plants. Water retention capacity of the first leaves in plants exposed to water stress increased respectively by 3.5%, 4.5% and by 1.1% compared to the water retention capacity of the leaves of the control plants. The root tissues of the plants exposed to drought for some period (7 days) lost the property to retain water. Significant adverse impact on water retention capacity was recorded in drought-sensitive plants of *Ph. vulgaris*, L. The water retention capacity of the roots of these plants decreases significantly. The plants previously exposed to drought stress maintained a higher level of water retention in tissues and after optimization of soil moisture (Figure 3).

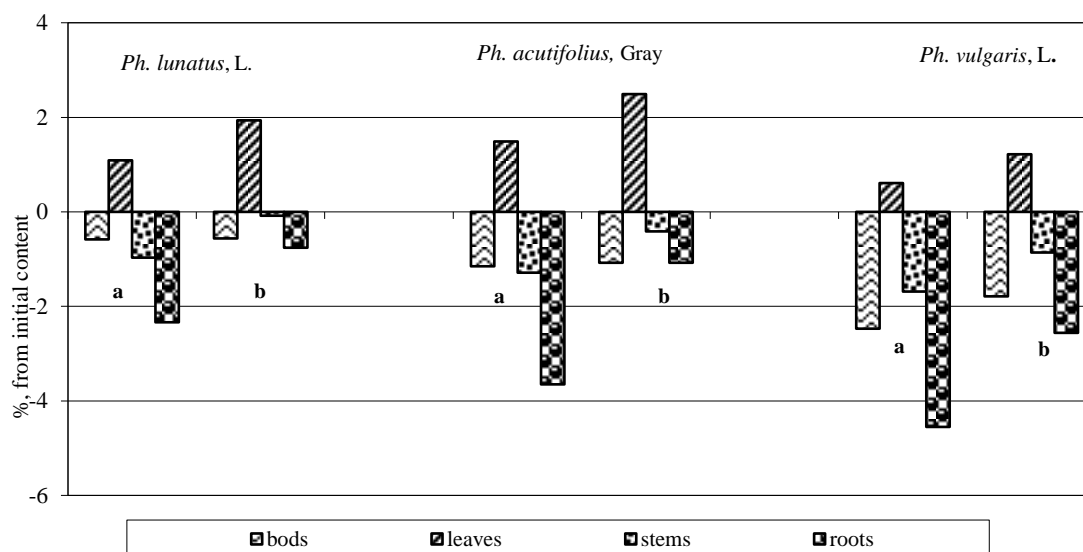


Fig. 3:-Water retention capacity in bean plant organs, after improvement of humidity conditions: control, 70% TWC; b - 70-35-70% TWC; c - 70-35-70-35-70% TWC

The investigations have confirmed that plants are able to keep memory of stress caused by previous actions of unfavorable factors and that the response of bean plants pre-exposed to repeated stress is more adequate. In the formation of stress-memory an important role belongs to the potential of self-regulation of the water status by activating the mechanisms of stabilizing / increasing the water retention capacity, maintaining the turgidity and the degree of hydration at a necessary level for the normal course of vital processes.

It is necessary to mention that after the restoration of the optimal humidity fund, the water content increases and the saturation deficit decreases to a different extent depending on the genotype and organ (Table 3). Pods and stems more fully recover their degree of moisture, more poorly - the root system. Restoring of the degree of hydration in plants exposed to the first drought cycle and in plants that withstood two cycles of drought runs differently. So, if the I drought cycle at the "flowering – formation of pods" phase conditioned a decrease in the degree of hydration in *P. lunatus* by 20.04%, already after 24 hours of soil moisture optimization, the water content difference was 12.92% against control (non-stressed plants).

Table 3:-Water content (g·100g⁻¹f.w.) in bean plants after optimization of humidity conditions.

Table 4. Water content (g 100 g TWC) in bean plants after optimization of humidity conditions.							
Specie	Variety	Organ	70 % TWC, control	The first cycle of drought		2 cycles of drought	
			M ± m	M ± m	Δ, % control	M ± m	Δ, % control
Ph. lunatus L.	Fetanisa	pods	87,01 ±0,25	85,90 ± 0,23	-1,28	86,63±0,16	-0,43
		leaves	82,60 ± 0,23	80,35 ±0,31	-2,72	81,64 ± 0,37	-1,16
		stems	79,49 ±0,24	78,24 ±0,46	-1,57	79,59± 0,61	0,12
		roots	87,65±0,39	81,24 ±0,32	-7,35	82,53±0,71	-5,84
Degree of parameter change, % / plant					Σ -12,92	Σ -7,31	
Ph. acutifolius L.	Acutifolius 5	pods	87,70 ±0,30	85,64 ±0,34	-2,35	86,34± 0,28	-1,55

		leaves	84,51 ± 0,17	82,63± 0,52	-2,22	83,61 ±0,39	-1,06
		stems	80,05 ± 0,48	78,28± 0,58	-2,21	79,33± 0,52	-0,90
		roots	87,16± 0,51	81,01± 0,38	-6,95	82,84± 0,52	-4,96
Degree of parameter change, % / plant					Σ -13,73		Σ -8,47
Phaseolus vulgaris L.	Nina	Pods	88,50 ±0,24	85,45 ±0,58	-3,44	86,52± 0,47	-2,23
		leaves	84,79±0,32	81,61 ±0,35	-3,75	82,33 ±0,23	-2,90
		stems	81,96± 0,39	80,04 ±0,44	-2,34	81,23±0,65	-0,89
		roots	87,76±0,63	78,97 ± 0,48	-10,01	81,83 ± 0,73	-6,76
Degree of parameter change, % / plant					Σ -19,54		Σ -12,78

The hydration rate of plants, that suffered a repeated drought, was 10.50% from control during stress period, and after optimal soil moisture recovery - 7, 31%. In *Ph. acutifolius* plants after the first drought cycle, the decrease of tissue moisture level was 21.36%, and after increasing soil moisture level – 13.73%; in plants exposed to repeated water stress these changes were within the limit: 14.16% and 8.47%. For *Ph. vulgaris* 'Nina', degree of dehydration of the tissues in plants primary exposed to stress constituted 28.48%, and in plants that suffered a repeated drought - 20.84% against control plants. At soil moisture optimization, the gap was 19.54 and 12.78%, respectively. Therefore, the rate of restoration of the hydration degree in plants that sustained 2 cycles was significantly higher. Already after 24 hours past restoration of moisture level, in *Ph. lunatus* the degree of hydration remained uncompensated at 7.12 and 3.19; *Ph. acutifolius* - 7.63 and 5.69 and *Ph. vulgaris* - at 8.94 and 8.06% of tissues' hydration level in control plants. Tolerant cultivars have the property of preserving water homeostasis at a more stable level, and are quicker to restore their water status in response to soil moisture optimization. The rapid restoration of organ hydration level is a consequence of the fact that plants, especially those that have been preventively subjected to two drought cycles, continue to maintain at a higher level the property of retaining water in the tissues and consequently, water consumption in the sweat process is low (Figure 3). Corresponding decreases saturation deficit and increase the hydration and turgescence of organs.

Thus, pre-exposure to moderate stress at early stages of ontogenesis forms in plants a memory of the past stress, which represents an ability of plants exposed to stress conditions to respond adequately and to withstand a new stress more easily. The memory about response to previous impact of the environmental factors is possible due to activation of certain defense mechanisms via signals about water status change, hydrostatic pressure and water retention capacity being in particular. As a result, moderate short-term drought (5 - 7 days), which periodically repeat, help to increase plant resistance. Such plants more easily endure repeated stress compared to plants cultivated under optimum conditions. A mild water deficiency at juvenile stages provides adaptation to survive in water-limiting conditions through forming a xeromorphic phenotype with better water-preserving mechanisms, increased water retention capacity and a balance between components of plant water status. Processes that provide plant resistance to the unfavorable factors are associated with self-regulation of metabolic functions and maintaining a dynamic balance of water with the participation of water. So, at the molecular, cellular and organ level, stress memory and drought tolerance is ensured by increasing the water retention capacity of macromolecules and maintaining the degree of hydration and tissue turgidity. The formation of stress memory depends on the constitutive resistance of plants: plants with a higher resistance potential, in particular with a higher constitutive tolerance, easier adapt to repeated water stress.

The beans plants of the species *Phaseolus lunatus*, L., variety 'Fetanisa', *Phaseolus acutifolius*, Gray, variety 'Acutifolius 5' and *Phaseolus vulgaris* L., variety 'Nina', which have undergone a mild drought to step "first trifoliate leaf" more easily supports repeated stress to the stage of "flowering - pods formation" from the formation of stress-memory. The dependence of stress-memory formation on homeostatic water capacity in tissues and the constitutive resistance of the genotype have been demonstrated. The self-regulation of water status by increasing water retention capacity and preserving turgescence are nonspecific mechanisms important for stress-memory formation and plant adaptation to fluctuating humidity conditions and water stress recurring over time.

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