THE COMPOSITION OF FREE AMINO ACIDS IN PLANT ORGANS OF SOYBEAN (*GLYCINE MAX* (L.) Merr.) INOCULATED WITH *BRADYRHIZOBIUM JAPONICUM* RELATED TO THE ACTION OF WATER STRESS

COMPOZIŢIA AMINOACIZILOR LIBERI DIN ORGANELE PLANTELOR DE SOIA (*GLYCINE MAX* (L.) Merr.) INOCULATE CU *BRADYRHIZOBIUM JAPONICUM*, ÎN FUNCŢIE DE ACŢIUNEA STRESULUI HIDRIC

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Abstract. *The composition of free amino acids in the plant organs of soybean (Glycine max* (L.) Merr*) inoculated with Bradyrhizobium japonicum 646 depending on the action of water stress has been studied in this work. The results showed that water stress of 35% soil humidity induced reduction in the free amino acids content of nodules and shouts and increased their content in roots and leaves. The water stress conditions have also led to changes in the qualitative composition of free amino acids. Water stress was observed to decrease the level of aspartic acid (Asp) of the nodules by approximately 12% and to increase its content in roots and shoots by about 4% and 2% respectively. However, the content of γ – amino butyric acid (GABA) increased in all soybean organs: nodules, roots, shoots and leaves of the stressed plants, comparing with the GABA values of the organ plants growing under a soil humidity level of 70%. The water stress also influenced the concentration of essential amino acids, the content and quality of which is determined by the type of vegetative organs.*

Key words: soybean, water stress, free amino acid composition, nodules, roots, shoots, leaves

Rezumat. *În lucrarea de faţă s-a studiat compoziţia aminoacizilor liberi din organele plantelor de soia (Glycine max* (L.) Merr.*) inoculate cu Bradyrhizobium japonicum 646***,** *în funcţie de acţiunea stresului hidric. Rezultatele obţinute au demonstrat că stresul hidric de 35% din capacitatea totală de reţinere a apei (CTRA) din sol a condus la diminuarea conţinutului aminoacizilor liberi din nodozităţi şi tulpini şi la majorarea conţinutului acestora in rădăcini şi frunze. Condiţiile stresului hidric au provocat, de asemenea, şi modificări în compoziţia calitativă a aminoacizilor liberi. S-a observat că stresul hidric a diminuat conţinutul de acid aspartic (Asp) în nodozităţi cu aproximativ 12% şi a majorat conţinutul acestuia în rădăcini şi tulpini cu aproximativ 4% şi respectiv 2%. Conţinutul de acid γ-aminobutiric (GABA) s-a majorat în toate organele: nodozităţile, rădăcinile, tulpinile şi frunzele plantelor, expuse acţiunii stresului hidric, în comparaţie cu valorile GABA din organele plantelor cu nivelul umidităţii solului de 70 % CTRA. Stresul hidric a influenţat de asemenea şi concentraţia aminoacizilor esenţiali, conţinutul calitativ al cărora este determinat şi de tipul organului vegetativ*

Cuvinte cheie: soia, stres hidric, aminoacizi liberi, nodozităţi, rădăcini, tulpini, frunze.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.*)* belongs to the *Fabaceace* family, which comprises more than 18,000 species. Legume plants have a unique feature – that of establishing mutualistic associations with some bacterial groups of the *Rhizobiaceae* family, including the *Bradyrhizobium* genera. Soil *Rhizobia* bacteria colonizing legume roots induce the formation of nodules. Inside nodules, bacteria, being transformed into bacteroids (B), are enclosed within peribacteroid space (PBS) with plant-derived peribacteroid membrane (PBM), forming new structures - simbiosomes, which carry out the process of nitrogen fixation.

Ammonia represents the first stable product of N_2 reduction and it is released from bacteroids to the plant tissues, where is assimilated into amino acids in exchange for plant–derived organic compounds (Mergaert P. et al., 2006; Prell J, Poole P., 2006). So, the metabolism of amino acids and other N compounds in symbiotic legumes depends on the intensity of nodule nitrogen fixation, which, in its turn, is determined by its providing with photosynthetic products as carbon and energy sources, on the one hand, and on the other hand, on the action of environmental factors, including that of water stress (Zahran H. H., 1999)

This study was focused on the distribution of free amino acids in the plant organs of soybean (*Glycine max* (L.) Merr.), inoculated with *Bradyrhizobium japonicum* 646 in relation to the action of edaphic drought.

MATERIAL AND METHOD

Growth conditions. A pot experiment in control conditions was carried out to investigate drought induced changes in amino acid composition of soybean organs.Soybean seeds, cv *"Bucuria"* were inoculated with effective nitrogen fixing bacteria *Bradyrhizobium japonicum* 646 and then sown into pots containing 6 kg of soil. After sowing, soil humidity in all the pots was maintained at 70% of the soil capacity level. The experiment was performed when the plants were at the budding stage of soybean development. At that time, the pots with plants were divided into two sets - control and water stress plants. The soil humidity level of the control plants was continuously maintained at 70%, whereas that of the stress ones was maintained by withholding watering until the soil water content in pots had reached 35 %. Both water regimes at 35 % and 70 % soil humidity were kept daily by replacing the amount of water lost, estimated by weighing each pot. The duration of water stress was 10 days. After the onset of drought period, the plant samples were collected and immediately fixed and kept in liquid nitrogen until their analysis.

Free amino acid determination. Plant samples preparations for amino acid analysis were effectuated according to the Krisshenco method (7). The quantitative and qualitative content of free amino acids in soybean organs was determined, using the amino acid analyzer – AAA 339 (Czechoslovakia).

RESULTS AND DISCUSSIONS

The results of the water stress action on the content of free amino acids in soybean organs are given in table 1. It can be observed that the plants exposed to the action of edaphic drought – 35% soil humidity level are characterized by a reduced content of free amino acids in nodules and shouts and by an increased one in roots and leaves.

Table 1

Influence of water stress conditions on content of total free amino acids in soybean organs

Soil humidity	Total free amino acids, mg/ 100 mg FW			
	Nodules	Roots	Shoots	Leaves
70%	419,67 ± 0,018	82,575 ± 0,003	179,71 ± 0,02	110,07 ± 0,006
35 %	340,375 ± 0,02	100,69 ± 0,009	103,37 ± 0,007	119,04 ± 0,01

It has been widely demonstrated that drought seriously affects biological nitrogen fixation (Zahran H. H., 1999) and that three factors such as: oxygen limitation (Del Castillo L.D. et al., 1994), carbon shortage (Arrese-Igor C. et al., 1999), and regulation by nitrogen metabolism (Purcell L.C. et al., 2000, Vadez V. et al., 2000, King C.A., Purcell L., 2005), can be involved in this inhibition. Particularly, such nitrogen compounds as nodule ureide, nodule aspartate and several amino acids in leaves are considered as potential candidates for feedback inhibition of biological nitrogen fixation (King C.A., Purcell L., 2005).

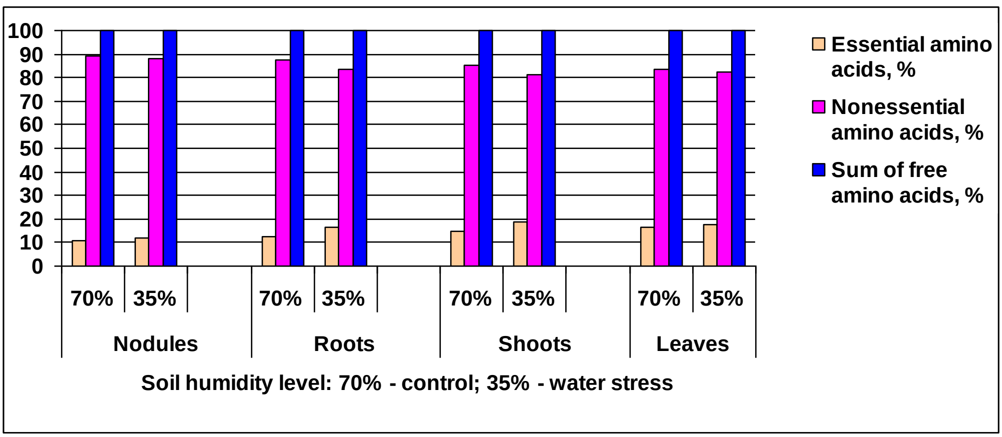

Fig.1. The content of essential and nonessential free amino acids in soybean organs in response to soil water deficit

Soil water deficit also influences the content of essential and nonessential amino acids (fig.1). The ratio between these two amino acid groups is determined by the type of plant organ. In the nodules of the plants grown under both soil water regimes (70% and 35%), the concentration of essential and nonessential amino acids makes about 10% and 90%, respectively, while in roots and shoots, the ratio between essential and nonessential amino acids is changed by the soil water regimes. Soil water deficit led to a decrease in total nonessential free amino acids and an increase of free essential amino acids in roots and shoots, but in-well watered plants, a reverse situation takes place – declining of essential amino acids and increasing of nonessential ones (fig. 1).

Table 2

Impact of water stress on distribution of nonessential amino acids nitrogen compounds in soybean organs. Soil humidity level: 70% - control; 35% - water stress

Nonessential amino acids	Abbreviations	Nodules		Roots		Shoots		Leaves	
		70%	35%	70%	35%	70%	35%	70%	35%
Cysteic acid	**Cyc.ac.**	0	0	2,5	4,1	0,8	0,7	5,6	7,0
Aspartate	**Asp**	34,1	22,2	7,2	11,3	5,0	7,1	1,5	0,7
Serine	**Ser**	4,6	5,4	5,9	7,1	5,0	6,1	1,9	1,9
Asparagine	**Asn**	0	0	39,8	24,9	53,1	40,1	1,8	1,5
Glutamate	**Glu**	10,7	12,5	11,9	9,5	0	0	6,5	4,3
Glutamine	**Gln**	0	0	0	0	3,6	4,0	1,3	1,1
Proline	**Pro**	0,4	0,4	0	0	1,2	1,6	1,7	1,8
Glycine	**Gly**	2,6	3,1	1,4	1,0	0,5	0,7	2,2	2,1
Alanine	**Ala**	16,9	24,0	10,3	8,7	5,7	7,4	15,2	13,6
Cysteine	**Cys**	6,2	7,0	0	0	0	0	0	0
Tyrosine	**Tyr**	1,5	2,0	0	2,7	1,2	2,6	1,9	1,5
β-Alanine	**β-Ala**	1,2	1,3	0	0	0,5	0,9	0,7	0,6
γ- aminobutyric acid	**GABA**	7,6	8,5	8,2	9,5	15,3	16,2	50,8	54,2
Ornithine	**Orn**	0	0	0	0	0,7	1,3	1,0	1,1
Ethanolamine	**EA**	2,5	3,0	3,3	7,1	2,5	1,9	3,9	3,8
Ammonia	**NH_3**	7,0	6,6	7,4	7,5	1,9	4,5	2,9	3,5
Histidine	**His**	2,2	2,1	2,1	3,8	1,7	2,9	0,3	0,3
Arginine	**Arg**	2,5	1,9	0	2,8	1,3	1,8	0,8	1,0
Sum, %		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

The action of soil water deficit is also reflected on the distribution of individual nonessential free amino acids by plant organs (table 2). It can be observed that both at soil humidity levels-70% and 35%, Asp contains the highest concentrations -34.0% and 22.2% - of the total nonessential free amino acids in nodules, respectively. These results show that soil water deficit reduces the content of Asp in nodules, while the content of other nonessential amino acids followed Asp by their concentrations such as Ala, Glu, GABA, Cys and Ser is enhanced under water stress conditions. In roots and shoots, the Asp concentration is much lower than that in the nodules, though soil water deficit led to the increasing of Asp content in these organs. At the same time, a sharp increase in Asn concentration is observed in roots and shoots for both soil humidity levels (70%, 35%), though the water stress conditions declined the content of Asn. So, our results show that after 10 days of water stress, the Asp level in nodules is declined in comparison with that of control – well-watered plants. The recent publication (King C.A., Purcell L., 2005) illustrated that soil water deficit during 2 days led to the accumulation of Asp in soybean nodules. The discordance in the results on Asp concentration in nodules under water stress, perhaps, can be due to the duration of the stress, soybean cultivar or other factors.

The content of Ala in roots and shoots is less than in nodules and leaves. The edaphic drought conditions decreased Ala concentration in roots and leaves (Table 2). The conditions of soil water deficit resulted in the increase of GABA concentration in all the soybean organs tested: nodules, roots, shoots, and leaves. Remarkably, the content of GABA in nodules and roots makes about 7% - 9%, in shoots – about 15% - 16%, but in leaves GABA reached the concentration of about 50% - 54% of the total nonessential free amino acids. GABA is considered to accumulate in plant tissues as a response to diverse stress factors (Bown A.W., Shelp B.J., 1997, Cholewa E. et al., 1997**).** It is suggested that GABA may also serve as a source of carbon and nitrogen in Glu biosynthesis **(**Turano F.J, Fang T, 1998).

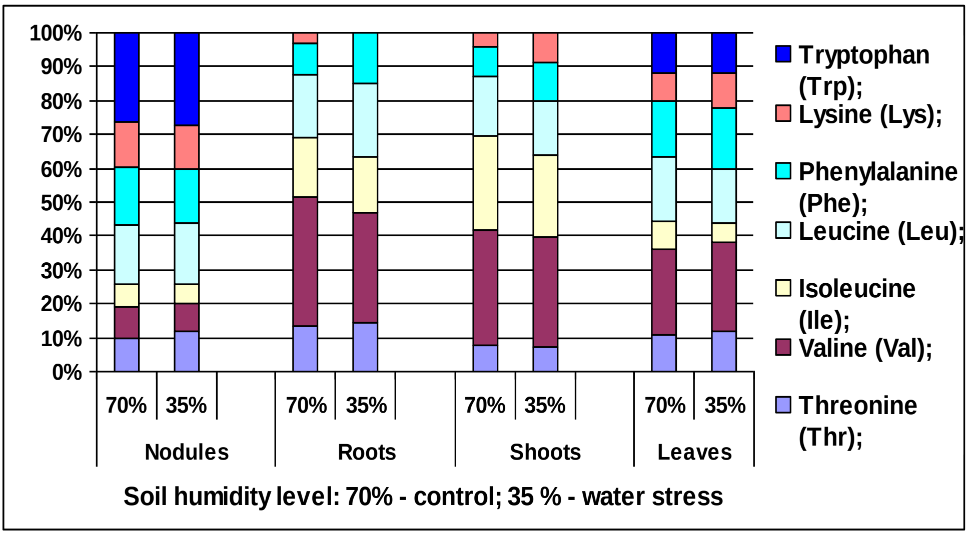

Fig.2. Impact of water stress on essential amino acid composition in soybean organs.

The composition and distribution of essential free amino acids in soybean plants presented in Fig.2 show that this group of free amino acids mostly depends on plant organs, with the exception of some amino acids which are influenced by soil water deficit. The nodules, containing all 7 measured essential amino acids, are characterized by a low level of Val and high level of Trp, comparing to roots, shoots and leaves, where an increasing concentration of Val is observed. The Trp concentration in leaves is less than in nodules and it is absent in roots and shoots.

CONCLUSIONS

1. Water stress conditions led to lower total content of free amino acids in nodules and shoots and increased their content in roots and leaves.

2. The action of water stress was accompanied by significant changes in the quantitative and qualitative distribution of nonessential amino acids, in particular, the content of Asp, Asn and Ala. However, the GABA content increased in all the organs: nodules, roots, shoots, and leaves of plants, exposed to the water stress action, compared with the amino acid values in control plant organs.

3. Water stress also influenced the concentration of essential amino acids, the qualitative content of which is determined by the vegetative organ type.

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