NATURA MONTENEGRINA, Podgorica, 2013, 12(2): 531-537

ORIGINAL RESEARCH PAPER

QUANTITATIVE CHANGES IN PROTEIN CONTENT OF SYMBIOTIC SYSTEMS OF SOYBEAN UNDER INOCULATION BY RHIZOBIA WITH DIFFERENT EFFECTIVENESS AND VARIED NITROGEN PROVIDING

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Key words:
nitrogen fixation,
rhizobia,
soybean,
symbiotic systems.

SYNOPSIS

The aim of our study was to investigate the possible changes in protein content of soybean symbiotic systems of varying effectiveness. We have shown the quantitative changes in content of total protein in the roots and nodules of soybean inoculated by active and inactive strains of *Bradyrhizobium japonicum*. In our opinion these changes are associated with the processes of formation and functioning of the symbiotic apparatus formed between plants and rhizobia.

INTRODUCTION

The food crisis is one of the most serious problems of humanity today. Due to rapidly growing of world population and respectively to its rising need in food the crop production will need to increase by a further 50% by 2025 (Khush, 2003). Soybean and other oilseeds provide significant sources of fatty acids and proteins for human and animal nutrition; they also have non-food uses, for example in producing industrial feedstocks and combustible fuels (Komatsu & Ahsan, 2009). Soybean is fourth in the world in terms of grain production, after maize, wheat, rice and the first in terms of biological nitrogen fixation (Bahmat, 2009). Well-developed soybean crops is fixing 155-198 kg/ha of nitrogen. Due to this soybean provides its need for nitrogen at 65-80 %, and significant portion of its reserves in the soil, so that soybean is one of the best crop rotation culture (Babych, & Babych-Poberejna, 2012; Mallarino et al., 2004). In addition, soybean can be a source of environmentally friendly products: biological feature to fix substantial quantities of atmospheric nitrogen in association with nodule-forming bacteria (*Bradyrhizobium*) make possible to grow it without mineral fertilizers, which pollute the environment (Singh, 2010).

High energy costs for production of mineral nitrogen fertilizers inhibit their widespread use in crop production. The current global financial crisis motivates

researchers to find alternative ways to provide the important agricultural crops in necessary compounds of this element. This way is biological fixation from the air by microorganisms which able to bind molecular nitrogen from the atmosphere and convert it to a compound suitable for assimilation by plants (Kots, 2011).

It is known that nitrates can accumulate in the soil and pollute the environment. In addition, increasing the concentration of nitrates in the productive parts of plants has toxic effect on humans and animals: in organisms where they are converted into nitrites (more harmful toxic substances) that cause poisoning, cancer and other diseases (Santamaria, 2006).

That is why the development of new biotechnological methods for increasing the efficiency of nitrogen fixation is very promising to obtain the great crop of this important agricultural plant, soil conservation, saving the environment and peoples' health. Despite significant advances in the study of problems of symbiotic nitrogen fixation, the intensity of this process in large-scale production is much lower than the level obtained in the experiments carried out under controlled conditions. This means that the biological potential of nitrogen-fixing microorganisms is far from full implemented today.

For this reason the aim of our study was to investigate the possible changes in protein content of soybean symbiotic systems of varying effectiveness under different nitrogen amount.

SUBJECT AND METHOD OF RESEARCH

Plant material and growth conditions

The experiments were conducted under natural conditions. Soybean (*Glycine max* (L.) Merr. cv. Mariana) were grown in plastic pots containing 13,5 kg of river sand. The seeds were sterilized at 70 % etanol and inoculated with active (646) and inactive (604k) *Bradyrhizobium japonicum* strains when sowing seeds. Soybean without inoculation were control plants. The Gelrigel medium (Grodzinsky and Grodzinsky,1973) with a complete set of microelements was used as the nutrient solution. Nitrogen was applied to the medium at the rate of 1 and 0,25 of its full volume in the medium (1 full volume is 708 mg $Ca(NO_3)_2 \cdot 4H_2O$ per 1 kg of sand). Plant samples were collected at stage of budding, beginning flowering and full flowering of plants. Roots and nodules were separated, immediately frozen in liquid nitrogen and kept until use.

Protein extraction

Proteins were extracted from roots and nodules as described (Kondratiuk et al., 2013). Pulverized samples (100 mg) were resuspended in 0,175 M Tris-HCl, 5 % SDS, 15 % glycerol, 0,3 M DTT. Proteins were extracted for 1 h at +4 °C and precipitated for 1 h at – 20 °C with four volumes of pre-cooled acetone with 10 % v/v

trichloroacetic acid. The pellet was washed twice with pre-cooled 80 % v/v acetone. The acetone-precipitated proteins were dissolved in 30 mM Tris-HCl (pH 8,5).

PROTEIN QUANTIFICATION

The solubilized proteins were quantified using the Bradford protein assay (Bradford, 1976) with a bovine serum albumin standard curve.

RESULTS AND DISCUSSION

Application of model nitrogen-fixing systems based on contrasting symbiotic characteristics of bacterial strains is an important condition to study the various stages of formation and functioning of plant-microbe symbiosis, particularly in the study of proteins participating in these processes. Previously it was shown that used rhizobia strains differed at nitrogen-fixing activity of root nodules and their effect on growth and development of the host plant (Drozdenko et al., 2009).

In our study we carried out the analysis of quantitative content of proteins in roots and nodules of soybean and revealed the changes of it depending on rhizobia effectiveness under different nitrogen amount.

It was shown quantitative differences in content of total protein in extracts of both the roots (Fig. 1) and nodules (Fig. 2). Moreover it should be noted that some of the trends was observed in root samples and some of it was looked in the nodules. However comparing roots and nodules to each other was observed a significant difference.

In roots' extracts of soybean inoculated by rhizobia strains 646 and 604k and under 0,25 of full volume of nitrogen in the budding phase of growth the total protein content was four and five times higher than in nodules infected with active and inactive strains respectively. At the stage of beginning flowering in the roots these parameters significantly decreased, whereas in the nodules observed a significant increase in the amount of protein. At the phase full flowering content of plant polypeptides in roots increased and in nodules it declined. So, quite reverse quantitative distribution in content of total protein in the roots and nodules may be evidence of various kinds of biological processes occurring in these organs.

In turn, as shown in Figure 1, in the study of quantitative changes of protein extracts of soybean roots was found several trends. So in the control variant was observed an increasing of total amount of protein throughout the period of plant growth both under 1 and 0,25 of full volume of nitrogen in sand. Moreover, at the budding stage of growth the plants were characterized by a lower protein increasing (by 78,7 % at 1 of full volume of nitrogen, by 26,2 % at 0,25 of full volume of nitrogen), at the phase of full flowering was observed a significant increase in these parameters (by 209,6 % at 1 of full volume of nitrogen, by 111,2 % at 0,25 of full volume of nitrogen).

In general, the total amount of protein in the control variant of soybean roots was higher in the first two stages under 0,25 of full volume of nitrogen in sand and the total amount of protein in roots taken in the third phase of plant growth dominated at cultivation of plants in one full volume of nitrogen.

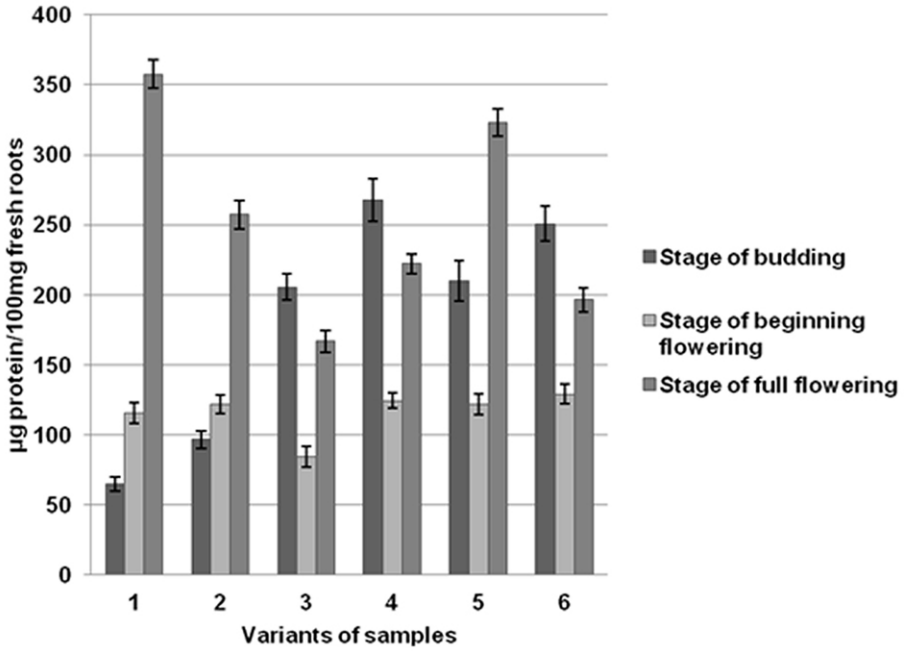

Figure 1. Dynamics of total protein extracts of soybean roots. The soybean seeds were inoculated by strains of *Bradyrhizobium japonicum* with different effectiveness (646 – active strain, 604k – inactive strain). Soybean without inoculation were control plants. The roots were collected at stage of budding, beginning flowering and full flowering of plants. Proteins were extracted from 100 mg plant sample with extraction buffer and precipitated with acetone with trichloroacetic acid. The solubilized proteins were quantified using the Bradford protein assay. The arrow indicates the quantitative changes in protein content of roots of soybean under inoculation by rhizobia varying effectiveness and under different nitrogen amount. Variants of samples: 1 – control; 1 of full volume of nitrogen; 2 – control; 0,25 of full volume of nitrogen; 3 – inoculation by active strain 646; 1 of full volume of nitrogen; 4 – inoculation by active strain 646; 0,25 of full volume of nitrogen; 5 – inoculation by inactive strain 604k; 1 of full volume of nitrogen; 6 – inoculation by inactive strain 604k; 0,25 of full volume of nitrogen.

In soybean plants, inoculated by different strains of *B.japonicum* was observed another trends than in control ones. The content of total protein in all inoculated variants of roots at budding phase was 3-5 times higher than in the control roots. At the same time the amount of protein in the roots of plants decreased rapidly in beginning of flowering and increased during full flowering stage under inoculation the plant by strains of rhizobia, whereas in controls it increased throughout the period of plant growth.

At the same time protein extracts of nodules of soybean inoculated by nitrogen-fixing microorganisms had their own peculiarities (Fig. 2).

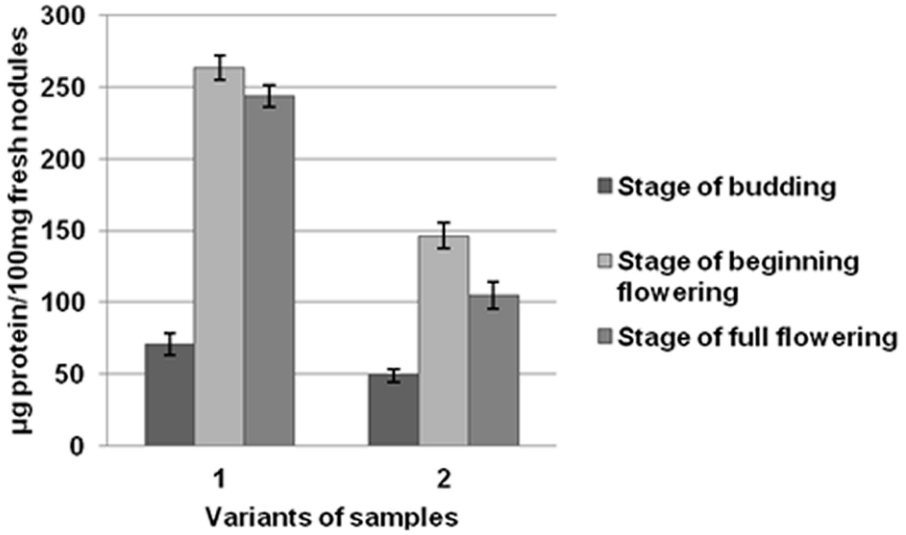

Figure 2. Dynamics of total protein extracts of soybean nodules. The soybean seeds were inoculated by strains of *Bradyrhizobium japonicum* with different effectiveness (646 – active strain, 604k – inactive strain). Soybean without inoculation were control plants. The nodules were collected at stage of budding, beginning flowering and full flowering of plants. Proteins were extracted from 100 mg plant sample with extraction buffer and precipitated with acetone with trichloroacetic acid. The solubilized proteins were quantified using the Bradford protein assay. The arrow indicates the quantitative changes in protein content of nodules of soybean under inoculation by rhizobia varying effectiveness and under different nitrogen amount. Variants of samples: 1 – inoculation by active strain 646; 0,25 of full volume of nitrogen; 2 – inoculation by inactive strain 604k; 0,25 of full volume of nitrogen.

First of all it should be noted that the amount of total protein in nodules of soybean inoculated by inactive strain 604k was lower on average twice than in the variants inoculated by active strain 646 at all stages of plant growth. In previous study (Mamenko, 2008) was shown similar results: the decreasing of total protein content in soybean nodules under inoculated by inactive strains of *B.japonicum* in half, compared with variants of plant inoculated by active strains. Due to the low acetylene reduction activity of these symbiotic systems the authors suggest that this difference may be connected with quantitative and qualitative changes of the protein providing functioning of nitrogenase complex.

However, the overall pattern of protein quantitative distribution was similar to active and inactive strains during the period of plant growth. Quantity of total protein increased at the stage of beginning flowering compared with the phase of budding (almost four times to active strain inoculation and three to inactive) and decreased during full flowering of soybean (by 7,7 % under inoculation by strain 646 and by 28

% under inoculation by strain 604k). In our opinion, these changes can be explained by increased biosynthetic processes during the formation of nitrogen-fixing system and decrease their intensity during active fixation of molecular nitrogen in the nodules. These data are confirmed by other researchers (Karr & Emerich, 1996).They assume that the process of nitrogen fixation is a power-consuming and takes away much of the energy resources of the plant. Under these conditions bacteroids have to exclude all energy-intensive processes including the protein biosynthesis. Thus there is a redistribution of energy coming in nodules to nitrogen fixation. So the authors suggest that this phenomenon explains unevenness process of nitrogen fixation during plant ontogenesis.

CONCLUSION

Hence we have fixed differences in quantitative content of total protein in roots and nodules of soybean inoculated by active and inactive strains of *B.japonicum* under different nitrogen amount. In our opinion these changes are related to the formation and functioning of symbiotic system formed between plants and rhyzobial microorganisms. Therefore, further detailed study of the protein composition of soybean symbiotic systems with contrasting characteristics under different nitrogen amount will allow to detect the influence of biotic and abiotic factors, predict the functions of the identified proteins and their role to symbiosis. It will give the opportunity to influence to effectiveness of plant-microbe symbiosis, improve its productivity and reduce the negative effects of nitrogen pollution to the environment.

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Received: 19 July 2013.