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# PROTEIN ENRICHMENT OF SOYBEAN AS AFFECTED BY DIFFERENT NITROGEN METABOLISM ENZYMES

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**ABSTRACT**
The nitrogen metabolism and enzymology are related to the selection of high-yielding crops. One of the most important plant products are proteins, and to synthesize them, plants should be well supplied with nitrogen. Considering the prices of energy and the environmental hazards related to excessive use of nitrogen fertilizers, the natural sources of proteins become even more important. Knowing and enhancing the activity of nitrogen assimilation and metabolism enzymes could help in breeding and selection of protein-rich genotypes. Thus, the aim of this study was to study the NG, NR and GDH enzyme activities and determine the content of proteins in different soybean cultivars.

**KEYWORDS**
proteins, nitrogen assimilation and metabolism enzymes, enzyme activities

## 1. INTRODUCTION

Dinitrogen ($N_2$) fixation is responsible for the conversion of inert nitrogen gas into usible ammonia. Whether derived from the atmosphere by simbiotic nitrogen fixation or from the soil, nitrogen is reduced to ammonia to become available for amino acid and protein synthesis [1].

Beside that, nitrogen is incorporated in plant tissues as nucleotides, nucleic acids, coenzymes, vitamins, pigments, alkaloids, amines and other compounds [2]. On average, proteins contain about 15% nitrogen and nucleic acids about 13% nitrogen. The most common sources of inorganic nitrogen in autotrophic organisms, cyanobacteria, algae, land plants, and some fungi, are ammonia ($NH_4^+$) and nitrate ($NO_3^-$), although some cyanobacteria can use nitrogen gas. Heterotrophic organisms, animals, most bacteria, and most fungi, utilize organic nitrogen produced by plants [3].

Several enzymes are involved in nitrogen assimilation and metabolism in plants. Among them, most important are nitrate reductase (NR; NADH, EC 1.6.6.1 and NADPH, EC 1.6.6.2), nitrite reductase (NiR, EC 1.7.7.1), glutamine synthetase (GS, EC 6.3.1.2), glutamate synthase (GOGAT, EC 1.4.7.1), glutamate dehydrogenase (GDH;

NADH, EC 1.4.1.2; NAD(P)H, EC 1.4.1.3 and NADPH, EC 1.4.1.4), aspartate aminotransferase (AspAT, EC 2.6.1.1), asparagine synthase (AS, EC 6.3.5.4) and phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) [4].

In symbiotic nitrogen fixation by root nodules of Fabaceae plants, the key enzyme is nitrogenase (NG, EC 1.18.2.1). NG is the enzyme responsible for biological nitrogen fixation i. e. the reduction of atmospheric nitrogen to $NH_4^+$ in the presence of some microorganisms, such as Bradyrhizobium, developed on the root system of Fabaceae species such as soybean [Glycine max (L.) Merr.] [5].

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Knowing and enhancing the activity of nitrogen assimilation and metabolism enzymes could help in breeding and selection of protein-rich genotypes. Thus, the aim of this study was to study the NG, NR and GDH enzyme activities and determine the content of proteins in different soybean cultivars.

Soybean genotypes Ranka, Panonka, Balkan and Vojvodjanka, obtained from the Institute of Field and Vegetable Crops at Rimski Šančevi, near Novi Sad, were chosen for the experiment. Plants were grown under field conditions on slightly calcareus chernozem soil with neutral reaction (4.65% of $CaCO_3$, 2.41% of humus, 0.16% of nitrogen, 7.05 pH in KCl, 7.93 pH in $H_2O$, $P_2O_5$ 12.80 mg 100 $g^{-1}$ soil and $K_2O$ 19.59 mg 100 $g^{-1}$ soil). The trial was set in a complete randomized block design in four replications. 35-days-old leaves from the second nodus were taken at the beginning of flowering (R1 phase) [6], for the assay of NR and GDH activities.

NG activity in the rhizosphere was determined by the acetylene reduction assay. The roots of plants were taken from the soil and shaken slightly to remove most of the soil from the rhizosphere. The sand adhering to the root surface was preserved.

The roots were incubated with 10% acetylene at 28°C for 4 h. Reduction formed ethylene was separated and determined on a 7600 A gas chromatograph system Hewlett-Packard. The concentration of ethylene in the gas sample was calculated from the peak area via a calibration curve [7].

The activities of in vitro NR and NADH-dependent GDH were determined in a common extract from leaves. The extract was prepared by homogenization of 1.0 g fresh leaves in 10 $cm^3$ extraction buffer (containing 50 mM imidazole, 5 mM 2-mercaptoethanol and 0.5 mM EDTA, pH 7.2), followed by centrifugation at 10.000 g for 15 min.

The NR activity was determined on the basis of the nitrite concentration, calculated from the nitrite-complex absorbancy measured spectrophotometrically at 540 nm [8].

The GDH activity was determined on the basis of the ratio of the oxidized and reduced form of nicotinamide adenine dinucleotide, estimated from the absorbancy measured at 340 nm [9].

The soluble protein content was measured according to the method described by Bradford et al. [10]. Biochemical data were statistically processed using variance analysis and the values were tested with the LSD at 5% and 1%.

## 2. RESULTS AND DISSCUSION

Values of the enzyme activities in investigated soybean cultivars are shown in figures 1-4.

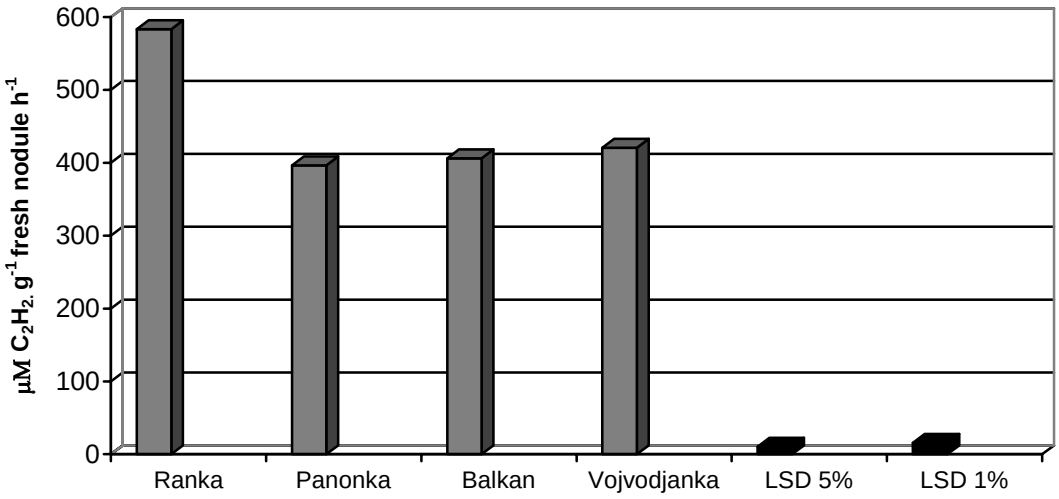


Fig. 1. Nitrogenase (NG) activity
in different soybean cultivars

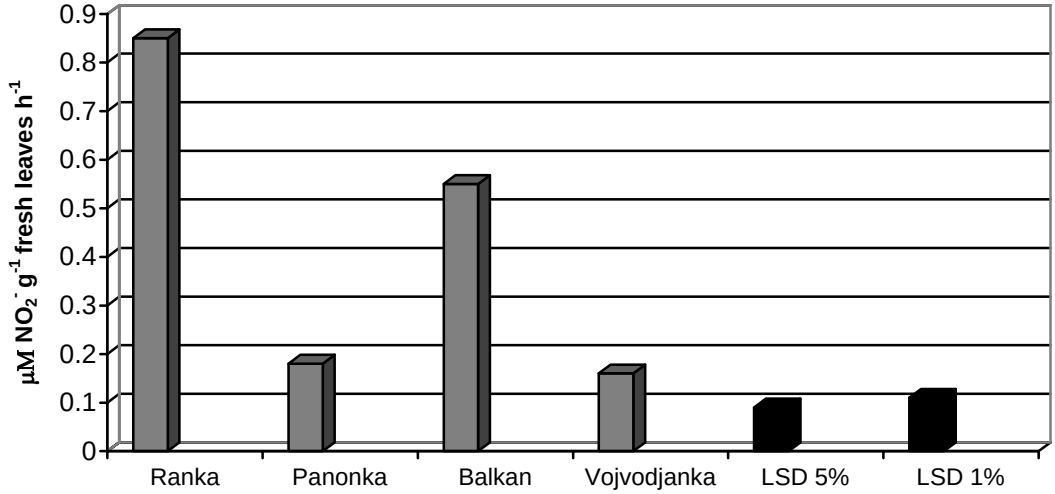


Fig. 2. Nitrate reductase (NR) activity
in different soybean cultivars

The NG activity, expressed as nM $C_2H_4$ $g^{-1}$ dry nodules $h^{-1}$, ranged from 396.42 to 583.4. The highest activity was in the Ranka cultivar. There were statistical differences among all cultivars examined; the lowest activity was recorded in the Panonka cultivar.

Comparing the NR activites, which were 0.85 µM $NO_2^-$ $g^{-1}$ fresh leaves $h^{-1}$ in the Ranka, while only 0.16 and 0.18 µM $NO_2^-$ $g^{-1}$ fresh leaves $h^{-1}$, in Vojvodjanka and Panonka, respectively, it was established that the activity of NR in Ranka was significantly higher than in any other cultivar. In the same time, there were no difference between Panonka and Vojvodjanka NR activities. The NR activity in the Balkan cultivar was significantly lower compared to Ranka but three times higher than in other two cultivars.

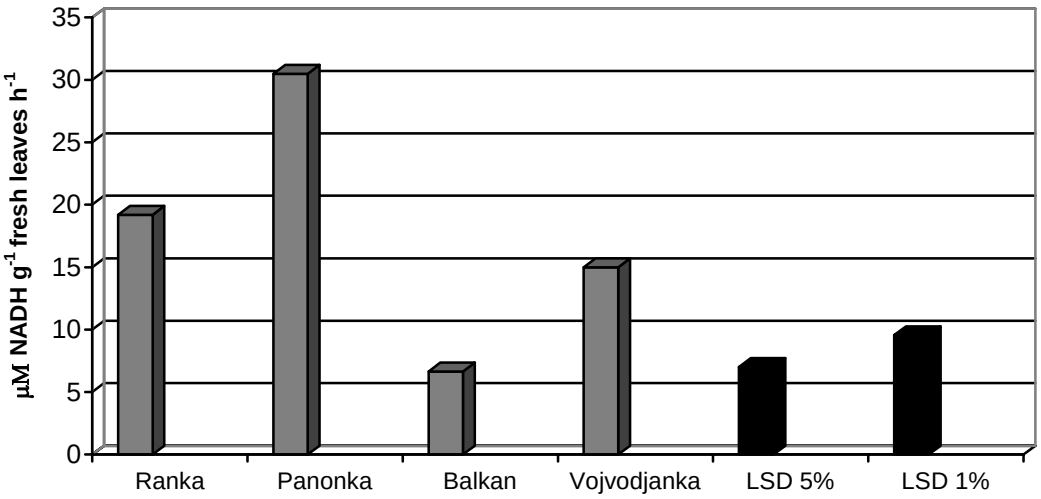


Fig. 3. Glutamate dehydrogenase (GDH) activity
in different soybean cultivars

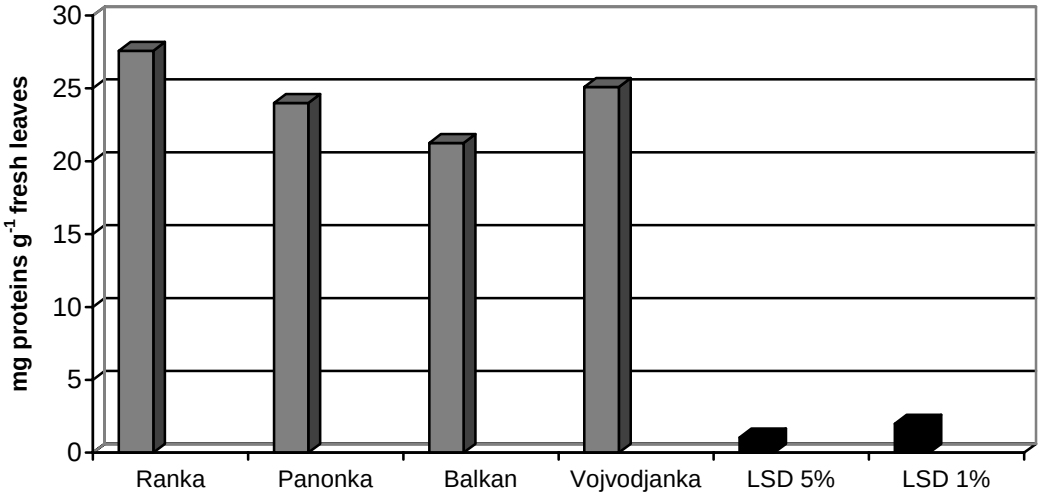


Fig. 4. Soluble proteins content
in different soybean cultivars

The GDH activities varied among investigated cultivars. The highest activity was in Panonka cultivar (30.48 μM NADH $g^{-1}$ fresh leaves $h^{-1}$) while the lowest was in Balkan (6.64 μM NADH $g^{-1}$ fresh leaves $h^{-1}$). There was no significant difference in GDH activity between Ranka and Vojvodjanka cultivars.

The content of leaf proteins ranged from 21.25 (in Balkan) to 27.57 mg proteins $g^{-1}$ (in Ranka genotype). There have been established a significant differences among investigated cultivars.

Results showed a significant differences in enzyme activities. In all investigated cultivars, the activities of NG, NR and GDH have been detected. Ranka cultivar expressed the highest NG activity which means that its symbiosis with Bradyrhizobium supplies plants with considerable amount of $NH_4^+$. In this cultivar, $NH_4^+$ is also produced by the activity of the NR/NiR-biosynthetic pathway, considering the highest NR activity.

While NR is an inducibile enzyme, very dependent on $NO_3^-$ content in the soil [8], it has been proved that $NO_3^-$ plays significant role in soybean nutrition. This result is in agreement with the results of other authors [11] and our previous findings [12, 13] which also have proved that NG is not the only enzyme responsible for N assimilation in soybean. Results showed that the incorporation of $NH_4^+$ in all investigated cultivars was enabled also by the GDH activity. GDH catalyzes the reaction of reverse amination/deamination of $\alpha$-ketoglutarate into glutamate.

Kinetic studies have shown that GDH has low afinity for $NH_4^+$ (Km = 5-100 mM $dm^{-3}$) which means that GDH can effectively act in the nitrogen metabolism only when $NH_4^+$ concentration is high. According to high NG activity it seems that investigated soybean cultivars were well supplied with $NH_4^+$. Investigation of the protein content showed that Ranka, possesing the highest NG and NR activity and a high GDH activity, contains more proteins compared to other cultivars. Panonka and Vojvodjanka cultivars showed lower activity of NG but higher activity of GDH so the biosynthesis of leaf proteins was enabled through nitrogen assimilation both from the soil and atmosphere. This suggests that the activity of NG may play a key role in the assimilation of nitrogen in soybean plants, but it also shows that the activities of some other nitrogen metabolism enzymes, such NR and GDH, are very important. Previous studies of NR activity, carried out on wheat (Triticum aestivum L.) cultivars, showed a positive correlation between the NR activity and the protein content in leaves [14], and therefore, it was suggested that NR activity could serve as a biochemical criterion in the selection of the protein-rich wheat. In the same time, it has been shown that some other biochemical parameters of importance to water and oxidative stress tolerance could be used in soybean production and selection [15].

Our current investigations have shown that the activity of nitrogen metabolism enzymes could be of potential use in soybean growing as well. Results obtained suggest that cultivars such as Ranka, with high enzymes activities, could be of interest in breeding, selection and field production due to its higher protein content. At the same time, the activity of nitrogen metabolism enzymes extracted from leaves in earlier stages of development, could be a suitable biochemical parameter for anticipating the protein yield of soybean grains.

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