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# Analysis of activity of nitrogen metabolism enzymes on grain yield and content of soluble proteins in soybean

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## Abstract

Field and laboratory tests were performed in 1995 in order to find out the effect of interdependance of enzyme activity of nitrogen metabolism on grain yield and content of soluble proteins in soybean. The activities of all three tested enzymes (nitrogenase, NG; nitrate reductase, NR and glutamate dehydrogenase, GDH) were in negative correlation with grain yield and in positive correlation with the content of soluble proteins. Path coefficient analysis showed that direct effect of NG activity was positive when compared to both tested characteristics, while the direct effects of NR and GDH were negative.

**Keywords: soybean, nitrogenase, nitrate-reductase, glutamate-dehydrogenase, path coefficient analysis**

## INTRODUCTION

Considering their metabolism, the plants provide nitrogen from the soil (ionic forms $NO_2^-$, $NO_3^-$ and $NH_4^+$), atmosphere, hydrolisates of proteins, etc. Nitrites have low significance in plant nutrition, while nitrates and ammonium have equal significance which depends on environmental conditions and age of plants. Considering energy, $NH_4^+$ represents more favourable source of nitrogen, due to the fact that it can be directly incorporated into amino acids and other nitrogen compounds by plants.

Assimilation and metabolism of nitrogen in plants are regulated by several enzymes. Most important are nitrate reductase (NR, EC 1.6.6.1-3), nitrite reductase (NR, EC 1.6.6.4), glutamine synthetase (GS, EC 6.3.1.2), glutamate synthase (GOGAT, EC 1.4.7.1), glutamate dehydrogenase (GDH, EC 1.4.1.2.-4) and plant aminotransferases. In symbiotic nitrogen fixation, the enzyme nitrogenase (NG, EC 1.18.2.1) has a key role in nitrogen assimilation in root nodules of plants from the family *Fabaceae*.

The assimilation of $NO_3^-$ in plants is catalysed by the activity of NR and NiR, by successive reduction to $NH_4^+$. The NR activity is increased in the high-yield plant cultivars by increasing nitrogen content, while in the low-yield plants it increases only in the beggining of vegetation. The values for the NR

activity serve as an indicator of the nitrogen state in plants and a biochemical criterion in the selection of protein-rich cultivars. This is due to the fact that positive correlation between the NR activity and protein content in leaves of wheat, sunflower and maize has been found (Gašiæ, 1984). NR and NG co-exist in legume root nodules, and it has been suggested that nodule $NO_3^-$ reduction contributes to N economy in the plant (Caba et al., 1994). The NR is substrate ($NO_3^-$) inducible and the $NO_3^-$ level of the leaf, as well as the NR activity were proposed as suitable parameters for the control of the nitrogen fertilization of grasses (Bergareche and Simon, 1988).

The reactions of the $NH_4^+$ assimilation are catalysed by the enzymes GS, GOGAT and GDH. As depending on the enzyme of assimilation $NH_4^+$, two paths of primary assimilation of nitrogen can be recognised: GS/GOGAT-path and GDH-path. It has been considered that in conditions of low concentration of $NH_4^+$ GS/GOGAT-path is active in a cell, while in conditions of high concentration of $NH_4^+$, GDH-path is being activated (Popoviæ, 1987).

The soybean (*Glycine max* (L.) Merr.) is a leguminous plant, able to uptake nitrogen from the atmosphere, and additionally, from the soil.

The aim of our investigation was to find out the differences between the activities of several enzymes (NG, NR and GDH) of nitrogen assimilation in the soybean and the interdependence of the enzyme activities, content of soluble proteins and grain yield.

## MATERIAL AND METHODS

The studies were conducted in 1995 in experiment fields at the Institute of Field and Vegetable Crops at the location of Rimski Šanèevi, and the enzymes activites were determined at the Biochemical laboratory of the Faculty of Agriculture in Novi Sad. Four new soybean varieties (Ranka, Panonka, Balkan and Vojvodjanka), produced at the Institute of Field and Vegetable Crops in Novi Sad, were included in the study. The trial was established according to a complete randomized block design in four replications, the size of basic plot was 10 $m^2$, i.e., four rows 5 m long, row-to-row distance of 0.5 m. The sowing was performed on April 15, 1995 and harvesting in the period of full maturity of the crop. Seed yield from the basic plot was calculated in kg/ha and 14% moisture as an average value from the four replications. Plant material for the enzymes assays was collected in the R1 phase (Fehr and Caviness, 1977) when the activities of nitrogen assimilation enzymes were at the maximum.

The activity of NG in the root system was determined by the acetylene reduction assay on a gas chromatograph 7600 A Hewlett-Packard (Hardy et al., 1968). The roots of four plants of each cultivar were taken from the soil, shaken slightly to remove most of the soil and transported on ice to the laboratory. The root system and nodules were rinsed with tap and distilled water. The sand adhering to the root surface was preserved. The roots with the adhering sand were incubated with 10% acetylene at 28°C for 24 h. The concentration of the acetylene reduction product - ethylene, in gas sample, was calculated from the pick surface through calibration curve. NG activity was calculated from the concentration of the produced ethylene and was expressed in μM $C_2H_4$ $g^{-1}$ dry nodule $h^{-1}$. Nodule dry weight was recorded after drying for 24 h at 70° C.

The activity of NR and GDH in the leaves, and the content of leaf soluble proteins were determined in a common extract from leaves according to the methods described by Coombs and Hall (1982). The enzyme extract was prepared from 1 g of homogenised sample of fresh leaves in 10 ml of extraction medium (imidazole extraction buffer pH-7.2).

The activity of NADH-dependent NR was determined *in vitro* on the basis of nitrite concentration calculated from the absorbtion of nitrite complex. The change of absorbance was red spectrophotometrically at 540 nm (Guerrero, 1982).

The activity of GDH was determined according to reduced absorbance at 340 nm due to oxidation of NADH. The activity was calculated on the basis of differences of NADH concentration in the presence and absence of ammonium acetate (Coombs and Hall,1982).

The content of the leaf soluble proteins was determined on the basis of the reaction of tyrosine and cysteine residues in protein with the Folin-phenol reagent. The absorbance of the complex produced was measured spectrophotometrically at 500 nm (Lowry et al., 1951).

Three duplicates of measurement were done for each examined parameter.

The results obtained were statistically processed according to the method of path coefficient analysis, that enables the study of direct and indirect effect as well as proportions of common effect (determination) of independent variables ($x_1$, $x_2$...$x_k$) on dependent variable (y) (Li, 1977). In this study, we tested the effect of activity of NG ($x_1$), NR ($x_2$) and GDH ($x_3$) on the yield of soybean grain ($y_1$) and content of soluble proteins ($y_2$).

**Table 1 : Average values of nitrogen metabolism enzymes activities, soluble proteins content and seed yield**

| | NG (mM $C_2H_4$/g d. nod. h) | NR (mM $NO_2^-$/g f. w. h) | GDH (mM NADH/g f. w. h) | soluble proteins (mg prot./g f. w.) | Yield (kg/ha) |
|---|---|---|---|---|---|
| Ranka | 583.40 | 0.85 | 19.14 | 27.57 | 6227 |
| Panonka | 396.42 | 0.18 | 30.48 | 23.57 | 5996 |
| Balkan | 401.78 | 0.55 | 6.64 | 21.25 | 6578 |
| Vojvoðanka | 418.75 | 0.16 | 14.85 | 25.08 | 7114 |

## RESULTS AND DISCUSSION

Significant differences were found studying the activity of assimilation enzyme and nitrogen metabolism in various soybean varieties (Table 1).

The variety Ranka had the highest NG and NR activity, which directly affected the increase of soluble proteins content. This is due to the fact that these enzymes are of key importance in conversion of nitrogen into $NH_4^+$ ions which are incorporated into amino acids and proteins in the course of their

biosynthesis. A lower yield of this variety is an indication of negative correlation between the activity of nitrogen assimilation enzymes and protein

**Table 2:: Correlation coefficients between nitrogen metabolism enzymes activities and seed yield (above diagonal) and soluble protein content (below diagonal)**

| Enzymes | 1. NG | 2. NR | 3. GDH | y. Yield |
|---|---|---|---|---|
| 1. NG | o | 0.799** | 0.028 | -0.217 |
| 2. NR | 0.799** | o | -0.280 | -0.256 |
| 3. GDH | 0.028 | -0.280 | o | -0.349 |
| y. Soluble proteins | 0.674** | 0.270 | 0.226 | o |

Significantly at level 0.05 (*) and 0.01 (**)

production, from one side, and the achieved yield, which is in agreement with data reported in the literature (Leffel, 1988; Holbrook et al., 1989). The varieties Panonka, Balkan and Vojvodjanka showed lower NG and NR activities (except the variety Balkan) and lower content of soluble proteins when compared to the variety Ranka. Grain yield ranged at approximately same level except in the variety Vojvodjanka, in which it was higher. The results of GDH activity showed that all varieties posesses GDH-path of nitrogen assimilation which indicates that the varieties are well provided with $NH_4^+$, produced by the activity of NG and NR. The GDH activity was the lowest in the variety Balkan which had the lowest yield of proteins as well.

According to the results, the method of path coefficient analysis was used for studying the interdependence of the characters studied.

The method of path coefficient analysis requires first the calculation of all correlations between dependent variable and independent variables ($r_{y_1}$, $r_{y_2}$,...$r_{y_k}$) and between independent variables ($r_{12}$, $r_{13}$,...$r_{k-1,k}$). All correlation coefficients are presented in Table 2.

The correlation coefficient (Table 2) indicates that there is no significant interdependence between the activity of nitrogen metabolism and yield. All correlation coefficients are insignificantly negative, which could bring about a conclusion that the activities of nitrogen metabolism enzymes have no effect on yield. In order to verify the corectness of these unusual interdependences, results were further processed using path-coefficient analysis of total effects of the nitrogen metabolism enzymes on yield which brings about a different picture (Table 3).

**Table 3: Path coefficient analysis for seed yield**

| DIRECT EFFECTS | INDIRECT EFFECTS | |
|---|---|---|
| $p_{y1} = 0.3498$ | $r_{12};p_{y2} = -0.5514^*$ | $r_{13};p_{y3} = -0.0155$ |
| $p_{y2} = -0.6901^*$ | $r_{21};p_{y1} = 0.2795$ | $r_{23};\ p_{y3} = 0.1546$ |
| $p_{y3} = -0.552$ | $r_{31};p_{y1} = 0.0098$ | $r_{32};p_{y2} = 0.1932$ |
| $R^2_{y123} = 0.2934$ | | |

Significantly at level 0.05 (*) and 0.01 (**)

Significantly negative direct effect of NR on grain yield was observed, and, at the same time, significantly negative indirect effect of NG *via* NR. The direct effect of NG on yield was positive. Since the soybean belongs to a group of plants having a capacity for nitrogen fixation, in which the activity of NG enzyme has dominant role with respect to nitrogen provision, it seems that grain yield would be even higher in the case that NG activity would increase. As NR is inducible enzyme that depends directly on the concentration of $NO_3^-$ in the medium, the reduced concentration of these iones in the soil, would reduce the activity of NR in the plant, and, as the inhibitory effect of $NO_3^-$ on the nodulation of the root system of soybean is well known (Harper, 1989), this could be an explanation of the significantly negative effect of NR on grain yield. The direct effect of GDH on grain yield was negative but not significant.

Correlation coefficient between the activity of nitrogen metabolism enzyme and the content of soluble proteins is presented in Table 2. Significant positive correlation was found between NG activity and soluble proteins content which was expected regarding the significance of this enzyme in nitrogen assimilation in soybean and its further incorporation into proteins. The activity of the other two enzymes is also in positive correlation with the content of soluble proteins but not significant.

**Table 4: Path coefficient analysis for soluble protein content**

| DIRECT EFFECTS | INDIRECT EFFECTS | |
|---|---|---|
| $p_{y1} = 1.2836^{**}$ | $r_{12};p_{y2} = -0.6089$ | $r_{13};p_{y3} = -0.0007$ |
| $p_{y2} = -0.7621^{**}$ | $r_{21};p_{y1} = 1.0256^{**}$ | $r_{23};\ p_{y3} = 0.0065$ |
| $p_{y3} = -0.0233$ | $r_{31};p_{y1} = 0.0359^*$ | $r_{32};p_{y2} = 0.2134^*$ |
| $R^2_{y123} = 0.6541$ | | |

Significantly at level 0.05 (*) and 0.01 (**)

However, path coefficient of analysis showed that only the NG activity has direct positive effect on the content of soluble proteins (Table 4).

Although there are NR and GDH activities in the studied varieties of soybean, their direct effect on the soluble protein biosynthesis was negative. The NR enzyme catalysed the reaction of $NO_3^-$

reduction, while the GDH enzyme catalysed the reaction of biosynthesis of glutamate, which is an initial compound in other amino acids and protein biosynthesis. Their activity, although present, was of secondary significance for the biosynthesis of soluble proteins as the highest amount of $NH_4^+$ was produced by the enzyme activity of NG.

## CONCLUSION

Negative correlations between grain yield and the activity of studied enzymes have been found.

The content of soluble proteins is in positive correlation with the activity of all three enzymes studied.

Direct effect of NG activity on both examined features was positive.

The activity of NG is of the highest significance for soybean plants, not only with respect to the protein yield, but most likely, for grain yield. Future studies of the effect of interdependance of the nitrogen assimilation and metabolism enzymes on the content of total proteins, will give a complete picture of this problem. The results of this study, could be also applied in soybean selection from the aspect of selecting lines that show higher NG activity, with aim to achieve higher yield of grains and increased protein content.

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