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## NITROGEN METABOLISM ENZYMES, SOLUBLE PROTEIN AND FREE PROLINE CONTENT IN SOYBEAN GENOTYPES AND THEIR F1 HYBRIDS

**ABSTRACT:** Nitrate reductase and glutamine synthetase activity, as well as free proline and soluble protein content were measured in eight soybean parent genotypes and six F1 hybrids. The aim of this study was to determine variability and the mode of inheritance for these parameters, and point out the genotypes of interest for future breeding programs. Analysed genotypes and their F1 hybrids expressed significant differences in activities of nitrate reductase and glutamine synthetase enzymes, as well as in soluble proteins and free proline contents. Since mode of inheritance for all investigated traits was in most cases dominance or heterosis, it can be concluded that these parameters are under control of dominant genes. The obtained results suggest that genotypes with favorable traits, such as variety Linda, line 1511, and F1 hybrids (Linda x LN92-7369) and (Balkan x BL-8), could be of interest as a good starting material for further breeding programs.

**KEY WORDS:** Nitrate reductase, glutamine synthetase, free proline, soluble protein, inheritance, soybean

### INTRODUCTION

The assimilation and metabolism of inorganic nitrogen in plants is a complex process involving a series of enzymes. Nitrate is reduced to  $\text{NH}_4^+$  by the reaction of nitrate reductase (NR) and nitrite reductase (NiR). The conversion of  $\text{NH}_4^+$  into glutamate proceeds *via* two pathways. In the GS/GOGAT pathway,  $\text{NH}_4^+$  is incorporated into glutamine by glutamine synthetase (GS), which is then converted with 2-oxoglutarate (2-OG) to glutamate by glutamate synthase (GOGAT). Glutamate dehydrogenase (GDH) catalyzes the incorporation of  $\text{NH}_4^+$  into glutamate by reversible reductive amination of 2-OG (Inokuchi and Okada, 2001; Cruz et al., 2004).

The cytosolic NR is the first enzyme in the pathway of nitrate assimilation, and its activity is highly regulated. Sufficient NR activity is a prerequisite for optimal utilization of soil N. NR has a major role in incorporation of N for plant yields under field conditions and it is widely known to be substrate inducible (Bergareche and Simon, 1988).

GS is the key enzyme responsible for the assimilation and re-assimilation of ammonia. In higher plants GS is one of the major enzymes responsible for the assimilation of ammonium absorbed from the growth medium, generated by nitrate reduction or re-assimilated after release of endogenous  $\text{NH}_4^+$  by ammonium-evolving processes such as photorespiration (Lea and Milfin, 1974; Fentem et al., 1983; Claussen and Lenz, 1999).

Glutamine is preferred as an N source in various biosynthetic reactions, to amino acids, purine and pyrimidine nucleotides, and other N-containing metabolites (Rao and Venkateswelu, 1986). Although  $\text{NH}_4^+$  is the immediate substrate of GS its effect on GS is still a matter of debate. Genes of cytosolic GS 1 were found to be  $\text{NO}_3^-$ , but not  $\text{NH}_4^+$ , inducible in some plant species, while in some other the GS 1 promoter was  $\text{NH}_4^+$ -inducible. Similar discrepancies are reported for chloroplastic GS 2. Conflicting results are also reported on the effect of  $\text{NH}_4^+$  on GS activity that has been found to be stimulatory or inhibitory. Accumulation of GS product glutamine acts as one of the signals for NR inactivation.

Prolonged water shortages affect practically all metabolic processes and often result in severe reductions in plant productivity. One of most common responses in plants undergoing water deficit is production and accumulation of amino acid proline in its free form. A strong correlation has been established between increased cellular proline levels and the capacity to survive water and high salinity stress as well, in barley (Singh and Paleg, 1972), maize (Pinter et al., 1979) and sugar beet (Štajner et al., 1995).

In higher plants, proline can be synthesized by two possible pathways using either glutamate or ornithine as precursor (Armengaud et al., 2004). Regulation of the glutamate pathway is well documented and has been shown to be the predominant pathway in response to osmotic stress.

The aim of this study was to determine the mode of inheritance for the activity of nitrogen assimilating enzymes, NR and GS, and free proline and soluble protein content in soybean parent genotypes and their F1 hybrids.

## MATERIAL AND METHODS

Plot area utilized for this experiment was located on the experimental fields at the Institute of Field and Vegetable Crops in Novi Sad. The experimental design was a randomized, complete block with four replications.

Biochemical analyses were performed at the Biochemical laboratory of the Faculty of Agriculture in Novi Sad. Eight soybean genotypes and six of their F1 hybrids were chosen, and plant leaves were used for the analysis. The samples were taken in R1 period, at the beginning of plant flowering.

The activity of NR and GS was determined according to C o o m b s and H a l l (1982). NR activity was expressed in  $\mu\text{M NO}_2^- \text{ g}^{-1}$  fresh matter  $\text{h}^{-1}$ , and GS activity was calculated from the hydroxamate content produced and expressed in  $\mu\text{M } \gamma\text{-GH } \text{g}^{-1}$  fresh matter  $\text{h}^{-1}$ . The activity of the enzymes was monitored spectrophotometrically at 540 nm.

Soluble proteins were extracted from soybean laves with 0.1 M phosphate buffer, pH 7, and the protein content was determined according to L o w r y et al. (1951), using bovine serum albumin as standard, and expressed as mg proteins  $\text{g}^{-1}$  fresh matter.

Free proline content was determined according to B a t e s et al. (1973), and expressed as  $\mu\text{g proline } \text{g}^{-1}$  fresh matter.

Each measurement was performed in triplicate.

Statistical evaluation was performed using software Statistica, Version 7.0. The experiments were repeated three times, and differences between genotypes were determined using LSD test for 0.05 significance level. The mode of inheritance was determined compared to mean parents value, for 0.05 significance level (Š t a j n e r et al., 1993, K o b i l j s k i and D e n č i ć, 1999).

## RESULTS AND DISCUSSION

The analysed genotypes and their F1 hybrids differed significantly in enzyme activities, as well as in soluble proteins and free proline content (Tab. 1).

The highest activity of NR was recorded in line 1511, while the lowest was in Lori. Mode of inheritance for this trait was dominance, either positive or negative, in all analysed crosses (Tab. 1).

NR gene expression and activity can be influenced by many factors, such as light and temperature (J i a n g and H u l l, 1998). In this work, these environmental factors should have no significant effects on comparison of NR activity because they were essentially the same for all experimental groups. The level and the distribution of activity of NR between the different plant organs, however, vary among species apparently due to their natural habitat and are also dependent upon the development stage of the plant (G h o s h et al., 2004).

The highest NR activities were expressed in lines 1511 and 1581/99, both grown in genotype collection for their high seed protein levels. Obtained results support the positive correlation previously established between NR activity and protein level in soybean seed (M i l a d i n o v i ć et al., 1996). As the values for the NR activity could serve as an indicator of the nitrogen state in plants and a biochemical criterion in the selection of protein rich cultivars, we can assume that F1 hybrid (1499/99 x 1511), with its high NR activity, will also have high protein content, and recommend it for future studies.

Tab. 1 — Average values and mode of inheritance for NR and GS activity and soluble protein and free proline content in soybean parent genotypes and F1 hybrids

PARENTS AND F1 HYBRIDS	NITRATE REDUCTASE ( $\mu\text{mol NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$ )	GLUTHAMINE SYNTHETASE ( $\mu\text{M } \gamma\text{-GH } \text{ g}^{-1} \text{ h}^{-1}$ )	SOLUBLE PROTEINS (mg protein $\text{g}^{-1}$ fresh matter)	PROLINE CONTENT ( $\mu\text{g proline } \text{g}^{-1}$ fresh matter)
LN92-7369	0.182	59.53	14.19	0.522
1581/99	0.939	62.51	15.92	0.286
1511	1.352	40.68	16.33	0.306
1499/99	0.491	49.61	15.92	0.375
Lori	0.074	58.05	16.66	0.468
Linda	0.159	90.05	18.21	0.442
Balkan	0.848	44.15	15.06	0.266
BL-8	0.753	59.04	16.12	0.404
LN92-7369x 1581/99	0.272 <sup>d-</sup>	57.30 <sup>d+</sup>	16.06 <sup>d+</sup>	0.313 <sup>d-</sup>
1499/99 x 1581/99	0.654 <sup>d-</sup>	70.45 <sup>h+</sup>	17.16 <sup>h+</sup>	0.322 <sup>i</sup>
1499/99 x 1511	1.013 <sup>d+</sup>	49.61 <sup>d+</sup>	14.11 <sup>h-</sup>	0.316 <sup>d-</sup>
Lori x LN92-7369	0.184 <sup>d+</sup>	57.80	17.85 <sup>h+</sup>	0.276 <sup>h-</sup>
Linda x LN92-7369	0.165 <sup>d+</sup>	154.73 <sup>h+</sup>	17.88 <sup>d+</sup>	0.531 <sup>d+</sup>
Balkan x BL-8	0.846 <sup>d+</sup>	156.03 <sup>h+</sup>	18.84 <sup>h+</sup>	0.571 <sup>h+</sup>

i = intermediate inheritance  
d = dominant inheritance  
h = heterosis occurred

The variety Linda had the highest, and the line 1511 the lowest GS activity. In F1 hybrids, the cross (Linda x LN92-7369) had the highest, and (1499/99 x 1511) the lowest GS activity. The mode of inheritance for this trait was positive dominance or heterosis (Tab. 1).

The content of soluble proteins in leaves of soybean genotypes investigated also varied significantly. Variety Linda had the highest, and the line LN92-7369 the lowest protein content among parents, while in F1 generation the highest level of soluble proteins showed F1 hybrid (Balkan x BL-8), and the lowest (1499/99 x 1511). The mode of inheritance was dominance, or, in the most cases, heterosis (Tab.1).

The free proline content was the highest in parent genotypes LN92-7369, Lori, and Linda, and the lowest was in Balkan and 1581/99 genotypes. In F1 generation, however, the highest proline content had (Linda x LN92-7369) and (Balkan x BL-8) hybrids, which expressed dominant and heterotic mode of inheritance, potentially and could be used in further breeding programs as potentially drought resistant lines.

## CONCLUSION

Our study showed that analysed genotypes and their F1 hybrids expressed significant differences in NR and GS enzyme activities, as well as in soluble proteins and free proline content. Since mode of inheritance for all investigated traits was in the most cases dominance or heterosis, it can be concluded that these parameters are under control of dominant genes. The obtained re-

sults suggest that genotypes with favorable traits, such as variety Linda, line 1511, and F1 hybrids (Linda x LN92-7369) and (Balkan x BL-8), could be used in breeding drought more tolerant genotypes with higher nitrogen-fixing capacity and high protein content.

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АКТИВНОСТ ЕНЗИМА МЕТАБОЛИЗМА АЗОТА,  
САДРЖАЈ РАСТВОРЉИВИХ ПРОТЕИНА И ПРОЛИНА  
У ГЕНОТИПОВИМА СОЈЕ И ЊИХОВИМ F1 ХИБРИДИМА

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Резиме

У раду је одређена активност ензима метаболизма азота нитрат редуктазе и глутамин синтетазе, као и садржај растворљивих протеина и слободног пролина у осам родитељских генотипова и шест F1 хибрида. Циљ истраживања је био да се утврде варијабилност наведених генотипова за одабране параметре и начин њиховог наслеђивања. Установљено је постојање статистички значајних разлика између генотипова, како у погледу ензимске активности, тако и у количини растворљивих протеина и слободног пролина. Будући да су се ова својства наслеђивала претежно доминантно, уз појаву хетерозиса, може се претпоставити да су под контролом гена са доминантним дејством. Као генотипови са повољним особинама издвојили су се родитељска сорта Linda и линија 1511, и хибриди (Linda x LN7369) и (Balkan x BL-8), који би могли послужити у узгоју генотипова соје повећане толерантности на сушу са повећаним капацитетом за усвајање азота и садржај протеина.