

## **THE EFFECTS OF NITROGEN ON PROTEIN, OIL AND TRYPSIN INHIBITOR CONTENT OF SOYBEAN**

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Nitrogen fertilization have influence on protein, oil and trypsin inhibitor content of different soybean genotypes. Seed protein content was increased over control by 60 kg ha<sup>-1</sup> nitrogen while trypsin inhibitor was reduced by all treatments (30, 60,90 N kg ha<sup>-1</sup>) as compared to controls. Significant genetic variation in TI was found both within the genotype class with the Kunitz inhibitor present as well as within the class lacking this inhibitor. Genotypes containing the Kunitz trypsin inhibitor protein (KTI)

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exhibit a higher TI than genotypes lacking this protein, however, in both groups of genotypes TI was similarly affected by nitrogen application. Oil content was reduced following nitrogen fertilisation.

*Key words:* nitrogen, protein, soybean, trypsin inhibitor

## INTRODUCTION

Nitrogen is an integral component of many compounds, including chlorophyll and enzymes, as well as amino acids and related proteins. It is known that nitrogen is an essential element for achieving high and stable yields and increased grain proteins. Soybean has one of the highest nitrogen requirements among the most agronomic crops. Biological fixation and fertilizer N are the main sources of meeting the N requirement of high-yielding soybeans. As soybean is a legume crop, symbiotic dinitrogen fixation has a huge impact on soybean quality and on seed protein content. However, several studies have shown that the symbiotic N-fixation is not able to meet high N-requirement of this crop particularly under the N-deficient conditions. CVJANOVIC *et al*, 2007, studied the effects of different rates of mineral nitrogen as well as soybean seed bacterisation with the symbiotic species on yield quality and quantity and found that the mixture of diazotrophs and fertilization with 40 kg N ha<sup>-1</sup> resulted in higher values of studied parameters in soybean. A number of workers (DURASAMI and MANI, 2001; KUMAWAT *et al*, 2000; BACHHAV and SABALE, 1996; SHARMA and MISRA, 1997) reported the positive role of nitrogen in increasing yield, protein content and nutrient uptake of soybean.

Mature seeds of soybean contain a number of antinutritional components with various level of biological activity. Among them, proteinase inhibitors are considered most important, as they prevent food and feed utilization of raw soybean and require heat treatment of soybean meal in order to become fully inactivated (LIENER, 1994). Appropriate agronomic management of soybean crop could reduce the content of protease inhibitors, which may open new pathways of post harvest treatment and product utilization. (VOLLMANN *et al*, 2003).

The aim of the present study was to evaluate the effect of nitrogen on protein content, oil and trypsin inhibitor activity in four soybean variety.

## MATERIALS AND METHODS

Four soybean genotypes were evaluated. Laura and Lana, genotypes lack the Kunitz type of trypsin inhibitor, were selected by Maize Research Institute. Vojvodanka selected by the Institute of Field and Vegetable crops, Novi Sad and Galeb selected by Uljarice, Beograd have standard seed quality. A trial was carried out on chernozem in the experimental field of the Maize Research institute „Zemun Polje“, at Zemun polje, during 2008. Soybean seeds were inoculated with the bacteria immediately before sowing. The experiment was laid out in a split plot with five treatments: control, without fertilizer; P60K60 (60 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 60 kg ha<sup>-1</sup>

K<sub>2</sub>O, T<sub>1</sub>), N30P60K60 (30 kg ha<sup>-1</sup> N, 60 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 60 kg ha<sup>-1</sup> K<sub>2</sub>O, T<sub>2</sub>), N60P60K60 (60 kg ha<sup>-1</sup> N, 60 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 60 kg ha<sup>-1</sup> K<sub>2</sub>O, T<sub>3</sub>), N90P60K60 (90 kg ha<sup>-1</sup> N, 60 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 60 kg ha<sup>-1</sup> K<sub>2</sub>O, T<sub>4</sub>). The intention of these treatments was to create different conditions in terms of nitrogen supply during seed filling period which could influence on the expression of seed quality characteristics.

Seed protein and oil contents were determined by near-infrared reflectance spectroscopy (NIRS) using Infratec 1241 Grain analyzer, (Foss Tecator, Sweden). The content of trypsin inhibitors was estimated according to a modified Erlanger method, HAMERSTRAND *et al.* (1981), using Na-benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPA) as substrate. The absorbance was measured at 410nm.

## RESULTS AND DISCUSSION

It is known that protein composition varies among genotypes as well as the levels of trypsin inhibitors (PESIC *et al.*, 2005). However, little information is available about environmental influences and the effects of nitrogen supply on the protein and antinutritional constituents of soybean. Seed protein content was determined in four soybean genotypes grown under different nitrogen treatment. A considerable variation in soybean seed protein content across different treatments is presented in Table 1. The average protein content was from 36, 55 in variety Vojvodanka to 37, 45 in variety Laura. Protein content in seeds of all analyzed genotypes was progressively increased with increasing levels of nitrogen up to 60 kg N ha<sup>-1</sup>. Laura has the highest protein content in all treatments. Increase in protein content of soybean with increasing level of nitrogen was also reported by many workers (MORSHAD *et al.*, 2008; EMAN, 2002; KUMAWAT *et al.*, 2000; BACHHAV and SABALE, 1996, EL-SHAMY *et al.*, 2001).

The availability of nitrogen during the seed-filling period is important prerequisite of high seed protein content. PEAK *et al.* (1997) demonstrated that the increase in seed protein content after late nitrogen application was associated with poorer protein quality, because the low-sulphur beta subunit of 7S protein was more strongly expressed than other fraction of seed storage protein. According to VOLMAN *et al.* (2000), nitrogen fertilization at the flowering stage was superior to control and rhizobium inoculation in increasing seed protein content in a set of genotype. The low magnitude of interaction between genotypes and nitrogen treatment in seed quality characters suggests that different genotypes would react similarly to those management variants and that selection of genotypes would give similar results under each of the nitrogen treatments.

MORSHAD *et al.* (2008) found that nitrogen uptake by seeds increased with increasing level of N up to certain level. DURAISAMI and MANI (2001) found that the uptake of N by soybean was favorably affected by the residual effect of N levels. KUMAWAT *et al.* (2000) reported that the N uptake by seeds of soybean significantly increased with the increase in N up to 60 kg ha<sup>-1</sup>.

Table 1. Protein, oil and trypsin inhibitor content of soybean seed under different N treatments

Genotype / treatment	Proteins %	Oil %	Total N %	Trypsin inhibitor mgg <sup>-1</sup>
Vojvodanka P60 K60	36,49	19,98	5,92	31,04
Vojvodanka N30 P60 K60	36,57	19,94	6,00	30,07
Vojvodanka N60 P60 K60	36,89	19,89	6,06	29,9
Vojvodanka N90 P60 K60	36,77	19,89	5,92	29,37
Vojvodankla control	36,02	20,01	5,83	31,21
average	36,55	19,94	5,95	30,32
Lana P60 K60	36,57	21,3	6,12	15,26
Lana N30 P60 K60	36,75	21,2	6,13	15,24
Lana N60 P60 K60	37,18	21,05	6,18	14,67
Lana N90 P60 K60	36,58	21,34	6,20	14,54
Lana control	36,56	21,43	6,12	15,35
average	36,73	21,26	6,15	15,01
Laura P60 K60	37,37	19,53	6,22	14,73
Laura N30 P60 K60	37,42	19,51	6,25	14,38
Laura N60 P60 K60	37,64	19,22	6,28	14,28
Laura N90 P60 K60	37,51	19,57	6,43	14,05
Laura control	37,31	19,62	6,18	15,01
average	37,45	19,49	6,27	14,49
Galeb P60 K60	37,34	19,95	6,14	29,85
Galeb N30 P60 K60	37,4	19,92	6,17	29,49
Galeb N60 P60 K60	37,56	19,91	6,22	29,25
Galeb N90 P60 K60	37,38	19,93	6,20	29,01
Galeb control	37,28	19,98	6,11	30,25
average	37,39	19,94	6,17	29,57

Nitrogen concentration in soybean seed is also under genetic control (BRIM and BURTON, 1979). Total seed N ranged from 5, 83% in Vojvodanka to 6, 18% in Laura. Slightly changes in the amount of N in seed were found with varying amount of N fertilizer.

Apart from seed protein content, oil and trypsin inhibitor content were also influenced by the nitrogen regime. Protein and oil content are negatively correlated (BURTON, 1985) and the protein content shows more variability than oil content (WEILENMANN and LUQUEZ, 1999). Seed oil content decreased while seed protein content increased with increased amounts of applied nitrogen thereby supporting previous findings (BLAMEY & CHAPMEN, 1981, LOUBSER & GRIMBEEK, 1985, STEER *et al.*, 1986).

The presence of protease inhibitors in soybean prohibits the utilization of the raw beans for food and feed. Two major classes of protease inhibitors are present

in soybean seeds, the Kunitz trypsin inhibitor and the Bowman-Birk inhibitor. In agronomic and plant breeding research trypsin inhibitor activity has rarely been monitored in field trials, although it could be treated as a quantitative characteristic and target for selection. Laura and Lana, genotypes lack the Kunitz type of trypsin inhibitor, were selected by Maize Research institute. Another two genotypes, Vojvodanka and Galeb, have standard trypsin inhibitor concentration. The trypsin inhibitor content of the studied genotypes is shown in Table 1. The investigated soybean varieties displayed different TI level. As expected, the lowest TI content was detected in Laura and Lana, a KTI-lacking cultivar. Trypsin inhibitor activity was influenced by the applied treatments. TI concentration of different genotypes under different N treatments is presented in Fig 1. Genotypes containing the Kunitz trypsin inhibitor protein (KTI) exhibit a higher TI than genotypes lacking this protein but in both groups of genotypes TI was similarly affected by nitrogen application. VOLLMAN *et al* (2003) found that TI was reduced by nitrogen or nitroge+sulphur application. Soybean genotypes segregated according to the presence or absence of the Kunitz trypsin inhibitor has been evaluated for trypsin inhibitor activity (TIA) in field trials. The authors found that TIA was affected significantly by environment (geographical location), fertilization treatment and genotype. Nitrogen application, which caused an increase in seed protein content, resulted in a reduction in TIA by about 15% as compared with the control. PAEK (1997) demonstrated that the low-sulphur subunits of 7S protein are more strongly expressed than other fractions of soybean seed storage proteins at late nitrogen application.

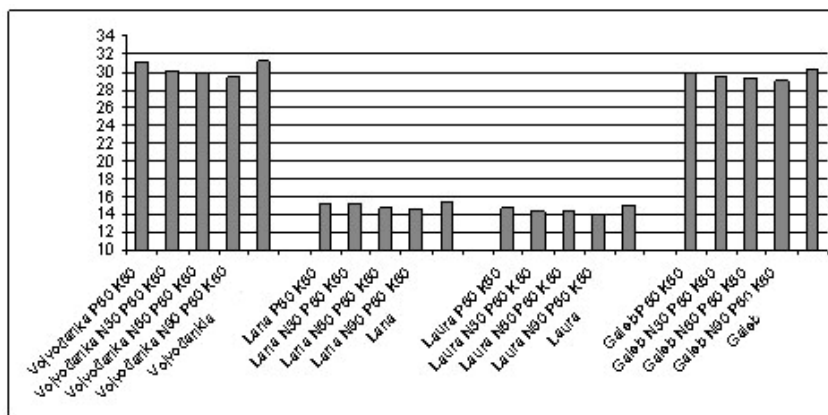


Fig1. Trypsin inhibitor activity under different N treatments

Many studies have shown an increase in yield and associated dry matter accumulation as a result of nitrogen application to soybean (AFZA *et al.*, 1987; MICHAEL *et al.*, 2001; OSBORNE and RIEDELL, 2006; WOOD *et al.*, 1993). In our study the seed yield was increased with increasing rates of N application up to 60 kg ha<sup>-1</sup>

and there after it reduced (date no shown). REDDY *et al* (1990) reported that application of 60 kg ha<sup>-1</sup> resulted in highest seed yield of soybean. According to BOROOMANDAN *et al.*, (2009) seed yield was significantly increased in 40 kg N ha<sup>-1</sup> application compared to control but application of 80 kg N ha<sup>-1</sup> decreased seed yield.

### CONCLUSION

Soybean seed quality and seed protein content in particular, is subject to considerable variation which may affect the processing value of the soybean. So further research is necessary in order to fine-tune soybean nutrition towards achieving a high quality soybean production.

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## UTICAJ AZOTA NA SADRŽAJ PROTEINA, ULJA I TRIPSIN INHIBITORA U ZRNU SOJE

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### I z v o d

Đubrenje azotom ima uticaj na sadržaj proteina, ulja i tripsin inhibitora u zrnu različitih genotipova soje. Sadržaj proteina se povećao iznad vrednosti kontrole primenom 60 kg ha<sup>-1</sup> dok se sadržaj tripsin inhibitora smanjio u svim tretmanima (30, 60, 90 N kg ha<sup>-1</sup>) u poređenju sa kontrolom. Značajno genetičko variranje tripsin inhibitora je utvrđeno unutargenotipova koji sadrže Kunic tripsin inhibitor kao i genotipova bez ovog inhibitora. Genotipovi koji sadrže KTI su ispoljili veće vrednosti za tripsin inhibitore nego genotipovi bez ovog proteina, dok je kod obe grupe genotipova uticaj azota na sadržaj tripsin inhibitora bio isti. Sadržaj ulja se smanjio nakon primene azotnog đubriva.

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