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Effect of N Fertilizer Application on Growth and Yield of Inoculated Soybean

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Abstract

The paper studied the effects of different nitrogen rates (0, 30, 60, 90 kg N/ha) on the soybean cultivar Proteinka, inoculated with the NS–Nitragin microbial fertilizer. Trials were set up at Bački Petrovac on a soil with no previous history of soybean cultivation. Samples of plant material were taken twice, once at soybean flowering and once at maturity. At flowering, the largest mass and length of the above-ground plant parts were recorded in the treatment with 60 kg N/ha, while the largest nodule number, mass and N content were obtained with 30 kg N/ha. The largest pod, grain numbers and grain mass and N content were produced by the inoculated treatment with no N added, and that was the treatment that produced the largest yield as well.

Keywords*: B. japonicum*, cultivar, NS–Nitragin, total microbial abundance

Introduction

Soybean (*Glycine max* (L) Mer) is an economically important leguminous crop on a worldwide scale and also the most important legume in China (Gan et al., 2003). Soybean nitrogen (N) requirements are met in a complex manner, as this crop is capable of utilizing both soil N (mostly in the form of nitrate) and atmospheric N (through symbiotic nitrogen fixation) (Harper 1974; Milić et al., 2002). Studies of the effects combined N (soil N + mineral fertilizer N) has on the physiology of the Rhizobium-legume symbiosis have shown that a large amount of N in the fertilizer reduces root infections (Dazzo and Brill, 1978), nodule mass and number (Streeter, 1988), levels of N-fixing activity in the nodules (Gibson, 1974), and the total amount of fixed N. The degree of N-fixation inhibition varies depending on the form of N applied (Dart and Wildou, 1970), season (Pate and Dart, 1961), light intensity, temperature (Gipson, 1974), and other environmental factors (Pankhurst, 1981). Symbiotic nitrogen fixation begins after the formation of nodules, reaches maximum activity in the early stages of pod filling, and subsides in the late reproductive stage (Imsande, 1988; George and Singleton, 1992). For increased legume yields and nitrogen fixation levels, it is essential to understand the interactions taking place between inorganic N supply and plant growth throughout the entire period of crop development.

In order for us to obtain information needed for the formulation of optimal N addition levels for soybean cultivation, experiments were carried out to determine:

- the effects adding different N amounts has on nodulation and nitrogen fixation at flowering;

- the effects of incorporation of different N amounts on yield parameters and grain yield.

Materials and methods

Used in this paper were *Bradyrhizobium japonicum* microbial strains that are used for the production of the NS–Nitragin microbial fertilizer. The *Bradyrhizobium japonicum* strains were grown on a Demolon liquid medium in a thermostat on a shaker at 30^0 C for four days and injected onto a sterile peat carrier. The trials were carried out on an experimental plot at Bački Petrovac in 2005 and 2006. A randomized block design with four replications was used in each of the treatments, which were as follows:

No N added
30 kg N/ha
60 kg N/ha
90 kg N/ha

Seeds from all the treatments were inoculated with NS–Nitragin just before sowing.

The seeds were of the soybean cultivar Proteinka, developed at the Institute of Field and Vegetable Crops in Novi Sad.

At flowering, plant samples were taken from five plants per replicate in each treatment. Nodule mass and number per plant, length and mass of the above-ground plant parts and root, and the N content of nodules and above-ground plant parts were determined. At the end of the growing season, pod and grain number, grain N content, and grain mass and yield were analyzed.

N content was determined using the standard Kjeltec equipment.

Table 1 Effect of N levels on the length, mass and N content of the above-ground plant parts and nodule number, mass and N content at soybean flowering

N level kg/ha	Above-ground length (cm)	Above-ground mass (g)	Above-ground N Content (mg/plant)	Nodule number	Nodule mass (mg)	Nodele N content (mg/plant)
0	99.16	17.87	543.69	60.29	342.00	1.739
30	100.25	15.99	483.35	80.87	398.50	2.214
60	101.29	19.51	619.65	40.08	313.00	1.767
90	91.04	19.46	574.04	30.75	171.50	0.519

At flowering and at the end of the vegetation period, soil samples for microbiological and chemical analyses were taken as well. They were used to determine total microbial abundance on soil agar, the number of ammonifiers on MPA, the number of free N-fixing bacteria, and Azotobacter abundance on Fjodor's medium.

Results and discussion

Results obtained at flowering

The results shown in the tables are the average for the years 2005 and 2006 godina. Table 1 shows the length, mass and N content of the above-ground plant parts and nodule number, mass and N content at flowering. The greatest length, mass and N content of the above-ground plant parts were found in Treatment 3 (60 kg N/ha), while the largest nodule number, mass and N content were recorded in Treatment 2 (30 kg N/ha).

Table 2 shows total microbial abundance, the abundance of ammonifiers and oligonitrophiles, and Azotobacter abundance at flowering. The largest total number of microorganisms and Azotobacter abundance were obtained with 60 kg N/ha, which correlates with the length and mass of the above-ground parts of the plant.

Results obtained at maturity

Table 3 shows pod number, grain number, grain mass and N content, and yield at 14% moisture. The greatest number of pods and grains and the largest grain mass were obtained in treatment 1 (no N), while the smallest values of these traits were recorded in treatment 3 (60 kg N/ha). Table 4 shows abundance at soybean maturity of the microbial groups studied. The largest total number of microorganisms and *Azotobacter* abundance were found in the treatment with no N added, which correlates with the indicators shown in Table 3.

According to these findings concerning pod and grain number and grain mass and N content as indicators of yield and total microbial abundance and *Azotobacter* numbers as indicators of soil biogenicity, the inoculated treatment with no N added was the most effective.

The largest average yield in the 2005/2006 period was produced by the inoculated treatment without N, which correlates with pod and grain numbers, grain mass, and total microbial and Azotobacter abundance at maturity (Table 4). A decline in yield with increasing N rate was observed as well. This leads to the conclusion that inoculated soybean can be successfully grown without the addition of N fertilizer, especially on soils such as the one from

Table 2 Effect of N levels on total microbial abundance and the number of ammonifiers, oligonitrophiles and *Azotobacters* at soybean flowering

N level kg/ha	Total number of microorganisms x 10^7	Number of ammonifiers x 10^7	Number of oligonitrophiles x10^6	Number of Azotobacters x 10^2
0	248.13	150.75	242.32	145.20
30	203.88	158.69	257.36	156.69
60	285.53	184.48	313.13	181.95
90	284.23	147.99	358.81	173.46

Table 3 Effect of N levels on pod and grain numbers, grain mass and N content and yield

N level kg/ha	Pod number	Grain number	Grain N content (mg/plant)	Grain mass	Yield (kg/ha)
0	60.67	137.37	197.28	25.41	4350
30	59.83	134.66	162.38	24.74	4251
60	53.87	123.88	185.93	22.84	4073
90	59.96	136.04	193.28	25.01	4014

Table 4 Effect of N levels on total microbial abundance and the number of ammonifiers, oligonitrophiles and *Azotobacters* at soybean maturity

N level kg/ha	Total number of microorganisms x 10^7	Number of ammonifiers x 10^7	Number of oligonitrophiles x10^6	Number of Azotobacters x 10^2
0	364.35	189.98	337.89	167.50
30	343.08	239.70	330.81	158.67
60	316.86	226.81	348.65	156.89
90	340.34	209.92	321.05	149.30

our study. The soil in question is a moderately calcareous, humus-rich chernozem with a medium to good supply of N (leaning more towards the latter) and a pH of 7.25-8.2 (depending on analyses) (Table 5).

Smith et al., (1986) have pointed out that «legumes seeded fields do not need nitrogen», while other authors have recomended that small quantities of N should be applied to reach good nodulation and maximum yields (Streeter, 1988; Cherney and Duxbury, 1994).

It is known that mineral N and fixed N are necessary for maximum N accumulation and yield in legumes (Harper, 1974; Bhangoo and Albritton, 1976; Franco et al., 1979) due to the high energy requirements in the development, maintenance and functioning of the symbiotic system compared with mineral N assimilation (Ryle et al., 1978; Pate et al., 1979; Finke et al., 1982).

A review by Imsande, (1989) indicates that soybean plants growing with an abundance of mineral N in the medium may accumulate less total N than soybeans that are well nodulated. The results of George and Singleton, (1992), however, have shown the opposite, as in that study the dry matter mass and total N of soybean and string bean grown in two locations increased significantly when increased amounts of mineral N were incorporated (9 – 120 – 900 kg N/ha).

The findings of Seneviratne et al., (2000) have shown that inoculation and fertilizer use promote plant growth and increase grain yields in soybean. Incorporating 23 kg N/ha as the primary fertilizer application and adding 23 kg N/ha at the end of flowering does not inhibit soybean nodulation. This shows the importance of nitrogen fixation in tropical conditions even with fertilizer incorporation included. In all the locations in said study, grain yields increased as a result of inoculation and fertilizer use when compared to uninoculated check treatments.

It has been shown that legumes have a highly significant response to small N rates (20 – 30 kg N/ha) in semi–arid tropics, as BNF does not operate at the beginning of plant growth and not all plant N requirements can be met by nitrogen fixation, especially in low soil N conditions (Wani et al., 1995).

In Chinese conditions, according to Gan et al., (2002), the addition of 50 kg N/ha-1 at the reproductive stages R1 through R5 instead of 75 N/ha before sowing or at the V4 stage is recommended for the soybean genotypes studied. The field trial results showed that the addition of N at the reproductive stage, especially at the R5 stage (pod filling), was the most effective in increasing total fixed N amounts, whereas adding high N amounts prior to sowing or at the V4 stage had a negative effect on the yield of soybean grains.

Conclusions

Results obtained at maturity showed that the largest total number of microorganisms and Azotobacter abundance were found in the treatment with no N added.

According to these findings concerning pod and grain number and grain mass and N content as indicators of yield and total microbial abundance and Azotobacter numbers as indicators of soil biogenicity, the inoculated treatment with no N added was the most effective.

The largest average yield in the 2005/2006 period was produced by the inoculated treatment without N.

This leads to the conclusion that inoculated soybean can be successfully grown without the addition of N fertilizer.

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Table 5 Chemical soil analysis

N level kg/ha	pH KCl	pH H_2O	$CaCO_3$ %	Humus	Total N %	P_2O_5	K_2O
0	7.27	8.23	4.49	2.90	0.190	46.00	31.68
30	7.25	8.20	4.24	2.90	0.195	50.69	33.61
60	7.26	8.19	4.13	2.95	0.195	45.29	32.47
90	7.23	8.20	4.33	2.90	0.194	46.56	32.27
Average	7.25	8.20	4.30	2.91	0.193	47.13	32.51

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