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NOTE

EFFECT OF RHIZOBIUM AND NITROGEN ON SOYBEAN[1]

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Abstract – Two separate experiments were conducted to investigate the effects of *Rhizobium japonicum* and N on yield and other agronomic traits in soybeans [*Glycine max* (L.) *Merrill*]. In both experiments, two levels of rhizobia (with and without rhizobia) were used. In experiment 1, 5 levels of N (0, 30, 60, 90 and 120 kg/ha) and in experiment 2, 7 levels of N (0, 30, 60, 90, 120, 150 and 180 kg/ha) were applied. Seeds for rhizobium-treated plots were treated with Nitragin inoculum prior to planting. Variety Clark 63 was used in this study.

The results obtained showed that: (a) an increased amount of N caused plants to flower later in experiment 1; (b) soybean had a negligible yield response to N fertilization; (c) application of rhizobium increased the number of nodules per plant, height, seed weight, the number of pods per plant, the yield and amount of oil; however, it reduced the number of days to flowering.

INTRODUCTION

It has been well established by previous studies that soil inoculation has a direct effect on yield increase in soybean. Extensive field trials have shown negligible response of nodulated soybeans to N fertilizers [1, 3, 4, 6]. If normal nodulation occurs, there may be no need for the use of N fertilizer in soybean [1]; however, responses from N have been reported in some cases [2]. A number of investigators have shown responses of soybeans to both inoculation and N fertilizer [5, 7–9].

In soils containing *Rhizobium japonicum*, there is little need for inoculation [3, 6, 10]. However, in most areas of Iran, where soybean is a new crop, inoculation can be a substitute for N application. Either soil or seed inoculation may be used in such cases. Although seed inoculation is easier to do and is more economical, it is confronted with certain limitations. For example, seeds inoculated with bacteria must be sown without delay to prevent heat damage to the bacteria or that enough bacteria may not attach to the seed.

This study was conducted to determine the effects of seed inoculation on yield and other agronomic traits of soybean and to find out the effectiveness of seed inoculation as a substitute for N application.

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MATERIALS AND METHODS

Two experiments were conducted on variety Clark 63 of soybean at the College of Agriculture, Karaj, Iran.

Experiment 1

Two levels of *Rhizobium japonicum* (r_0 = without rhizobia, and r_1 = with rhizobia) and 5 levels of N fertilizer ($n_0 = 0$, $n_1 = 30$, $n_2 = 60$, $n_3 = 90$ and $n_4 = 120$ kg/ha N) were used. Urea was used as a source of N and applied to the soil a few days before planting. In the r_1 plots, seeds were treated with Nitrogen prior to planting. Planting was done on 15 May 1974. A split-plot design with 3 replications in which N levels were as main plots and rhizobium levels as sub-plots was used. Each plot consisted of four 4 m long rows with 80 plants in each row. Rows were 0.5 m apart. The stand was sprayed once with a solution of 0.2% Malathion (57%) against aphids. The field was hoed twice and irrigated frequently to prevent moisture stress.

Traits studied were: flowering time (number of days from planting to 50% bloom), height in cm, number of nodules per plant at the beginning of flowering, number of pods per plant and number of seeds per pod, all obtained from 10 randomly selected plants; seed weight in g found from 100 seeds, yield in kg/ha, oil percentage found from a 4-g sample using a soxhlet apparatus, and amount of oil (kg/ha). Plants were harvested on 11 September 1974. Data for each trait were subjected to analysis of variance and mean values were compared by non-orthogonal *t*-tests.

Experiment 2

The material and methods used in this experiment were the same as in experiment 1 with the following exceptions: (a) 7 levels of N ($n_0 = 0$, $n_1 = 30$, $n_2 = 60$, $n_3 = 90$, $n_4 = 120$, $n_5 = 150$ and $n_6 = 180$ kg/ha) were used; (b) each plot consisted of four 5 m long rows with 100 plants in each row; (c) during the growth period, the field was hoed 3 times, sprayed twice and irrigated 16 times; (d) planting was done on 14 May and harvesting was completed by 4 October 1975; (e) nodule counts were made at the beginning of flowering and 2 weeks after the first count.

RESULTS AND DISCUSSION

Results of analyses of variance in experiment 1 revealed the following: (a) nitrogen levels were significantly different for flowering time and for number of seeds per pod; (b) the two rhizobium levels showed significant differences for height, number of nodules per plant, seed weight, yield and amount of oil; (c) significant interaction effects were observed between N and rhizobium for number of nodules per plant and for seed weight. In experiment 2, however, analyses of variance showed (a) a significant difference between N levels for flowering time only, (b) rhizobium levels to be significantly different for nodule counts and flowering time at the 0.01 level, for number of pods per plant at the 0.05 level, and for yield and amount of oil at the 0.10 level of significance, and (c) a significant interaction effect between N and rhizobium for flowering time only.

Table 1 shows mean values for N levels for flowering time and number of seeds per pod in experiment 1 and for flowering time in experiment 2. It is clear that the increased

Table 1. Mean values for N levels for flowering time and the number of seeds per pod in experiment 1 and for the flowering time in experiment 2

N level	Days to flower			No. seeds/pod
	Experiment 1	Experiment 2	Mean	Experiment 1
n_0	48.2	54.7	51.5	2.6
n_1	48.6	55.2	51.9	2.6
n_2	48.0	55.3	51.7	2.5
n_3	49.9	54.3	52.1	2.6
n_4	50.7	54.5	52.6	2.5
n_5		52.2	–	
n_6		53.3	–	
LSD	0.9	1.2		0.08

amount of N in experiment 1 caused the plants to flower later. However, no conclusive evidence was derived of the effect of N on the number of seeds per pod in experiment 1 and on flowering time in experiment 2. It thus appears that Clark 63 soybeans show negligible yield response to N fertilizer.

Mean values for rhizobium levels for various traits under study in experiments 1 and 2 are presented in Table 2. The application of rhizobium increased the number of nodules per plant, height, seed weight, the number of pods per plant, yield and the amount of oil; however, it reduced the number of days to flowering.

Table 2. Mean values for rhizobium levels for various traits under study in experiments 1 and 2

Experiment 1

Rhizobium levels	No. nodules per plant	Height (cm)	Yield (kg/ha)	Amount of oil (kg/ha)	Seed weight (mg/seed)
r_0	0.06	67.4	897	212	126
r_1	3.18	75.8	1277	286	142

Experiment 2

Rhizobium levels	No. nodules/plant		Flowering time (days)	No. pods per plant	Yield (kg/ha)	Amount of oil (kg/ha)
	1st count	2nd count				
r_0	0.37	0.89	55.4	27.2	999	215
r_1	3.52	6.44	53.0	35.2	1170	252

Table 3. Mean values for different N and rhizobium levels for seed weight and number of nodules/plant in experiment 1 and for the flowering time in experiment 2

	Experiment 1				Experiment 2	
N level	Seed weight (mg/seed)		No. nodules/plant		Days to flower	
	r_0	r_1	r_0	r_1	r_0	r_1
n_0	125	137	0.00	2.33	55.3	54.0
n_1	128	141	0.17	4.53	56.7	53.7
n_2	123	141	0.00	3.20	56.0	54.7
n_3	132	147	0.13	2.43	56.7	52.0
n_4	125	143	0.00	3.40	56.3	52.7
n_5					53.3	51.0
n_6					53.3	53.3
Mean	127	140	0.06	3.18	55.4	53.1
LSD	10		1.36		1.3	

In Table 3, mean values are given for N X rhizobia interaction for seed weight and for the number of nodules per plant in experiment 1 and for the flowering time in experiment 2. The heaviest seeds are produced in $n_3\ r_1$, the highest number of nodules per plant in $n_1\ r_1$ and the earliest flowering plants in $n_5\ r_0$. It appears that N suppresses nodulation (Table 3). This result is in agreement to that of Baard and Hoover [1].

Some physical and chemical properties of the soil in which experiment 1 was carried out are given in Table 4. Experiment 2 was done in the same field but in another site. Although the original amount of N in the soil was low, there was no yield response to N in either experiment. This may be due to the unavailability of N, which was given to the soil in the form of urea. Application of some other forms of N such as ammonium sulfate may have been of more use to the plant.

Table 4. Some physical and chemical properties of the soil in experiment 1

Soil property	Soil depth (cm)	
	0–20	20–40
Field capacity	19.8	19.7
Wilting point	10.2	10.3
Soil density	2.60	2.58
ECe ($m\Omega^{-1}$ per cm^2)	0.6	0.4
Total N (0.1%)	0.69	0.54
P (mg/100 g)	1.50	0.80
Na (mg/1) in a 1 : 1 extract	17.50	17.00
K (mg/1) in a 1 : 1 extract	8.00	4.00
pH	7.5	7.6

The following conclusions can be drawn from this study: (a) rhizobium causes significant effects on several agronomic traits of soybean including yield and amount of oil; and (b) seed inoculation in soybean appears to be superior to N fertilization and is also much cheaper.

An interesting question may be in respect to the effectiveness of seed inoculation as compared to soil inoculation. This may be answered by further experiments in future.

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