

Original Research Article

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Influence of Basal and Top Dressing Nitrogen Fertilization on Symbiotic Traits and Microbial Population in Soybean

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ABSTRACT

Soybean being a leguminous crop has the ability to fix atmospheric nitrogen (N) but the crop N demand during the later growth stages is not fulfilled by biological nitrogen fixation (BNF) alone. Hence, there is a need to supply inorganic N to soybean at reproductive stages for increasing the seed yield. Therefore, this experiment was planned with the objective to study the effect of N application at different stages on symbiotic traits and biological (microbial population) properties of soil at two locations. The experiment consisted of ten treatments of nitrogen application at different stages in a randomized complete block design replicated four times. The application of nitrogen at sowing increased the nodule count and dry weight at both the locations. The symbiotic traits like nodule count, nodule dry weight and leg haemoglobin was highest in the treatment of seed inoculation (SI) + 40 kg N ha⁻¹ at sowing which was however, statistically at par with SI + 20 kg N ha⁻¹ at sowing + 10 kg N ha⁻¹ flower initiation (FI) + 10 kg N ha⁻¹ pod initiation (PI). The basal application of recommended dose of nitrogen improved the nodulation, leg haemoglobin content and soil rhizobial population.

Keywords

Leghaemoglobin,
Microbial
population,
Nitrogen, Soybean,
Symbiotic traits

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Introduction

Soybean [*Glycine max* (L.) Merrill] is an important pulse-cum-oilseed crop which belongs to family Papilionaceae. It is grown in tropical, sub-tropical and temperate regions of the world and is believed to be originated in

East Asia. The area under soybean has been increasing rapidly since 1970's as compared to other crops. At present the crop is grown on 6% of the world's arable land (Hartman *et al.*, 2011). In India during 2015-16, soybean was grown on an area of 11.6 million hectare, with a production of 8.5 million tonnes and

productivity of 738 kg ha⁻¹. In comparison to other leguminous crops the nutritional requirement of soybean is higher. For higher yields in legume crops, relatively high amount of nitrogen (N) is required due to their high protein content (Bellaloui *et al.*, 2015).

Generally, application of N is not recommended in legumes under favourable conditions as they are able to grow well on soil N, symbiotic N and N stored within the cotyledons. It was reported that 50-60% of soybean N demand is fulfilled by BNF (Salvagiotti *et al.*, 2008).

Harper *et al.*, (1974) reported that BNF contributes 25-60% N and the remaining 40-75% N comes from soil. Soybean being a leguminous crop has the ability to fix atmospheric N but the crop N demand during the later growth stages is not fulfilled by biological nitrogen fixation (BNF) alone (Kumawat *et al.*, 2000). Limited supply of N during this period reduces the photosynthetic capacity of leaves and restricts the seed-filling period and the plant moves towards senescence, thus reducing yield. Gan *et al.*, (1997) observed that nodule formation and flowering stages were optimum for application of nitrogen in soybean.

Many researchers have reported two crucial stages for application of nitrogen in soybean which were seedling development and pod filling stages (Yadav and Chandel, 2010; Singh and Singh, 2013; Niranjana *et al.*, 2015; Yadravi and Angadi, 2016). Basal application of N in soybean helps in the formation of nodules and thus, helps in increasing the symbiotic nitrogen fixation rate. Application of N as starter is necessary to increase soybean yield (Gai *et al.*, 2017; Virk *et al.*, 2018) and also improved the nodulation (Mrkovacki *et al.*, 2008; Virk *et al.*, 2018). However, Kaschuk *et al.*, (2016) reported negative effect of N fertilizer on nodulation of soybean.

It has been observed that nitrogen application at the time of sowing is unable to increase the yield of soybean as it reduces the nodulation and results in increased vegetative growth. Hence, starter nitrogen and biological fixation alone becomes insufficient to meet the crop N requirements. Furthermore, it was observed that application of inorganic nitrogen at reproductive stages (R1-R5) helps in increasing the nitrogen utilization period (Barker and Sawyer, 2005). Since, nitrogen is prone to leaching losses so it needs to be applied in split doses. Gan *et al.*, (1997) observed that nodule formation and flowering stages were optimum for application of nitrogen in soybean. Gan *et al.*, (2003) reported that application of nitrogen as starter and top dressing significantly increased nodulation. Many researchers have reported two crucial stages for application of nitrogen in soybean which were seedling development and pod filling stages (Yadav and Chandel, 2010; Singh and Singh, 2013; Niranjana *et al.*, 2015; Yadravi and Angadi, 2016).

N requirement is more during seed development stage due reduction in nitrogen fixation between R5 and R7 stages. Mourtzinis *et al.*, (2018) reported that split application of N between planting and reproductive stages provided higher yield than zero N and single application of N. The split application of N (at sowing + stage R4) provided an increase in yield, as compared to control (Moreno *et al.*, 2018).

Furthermore, addition of N also affects bacterial count (Zhou *et al.*, 2017), nutrient uptake by soybean crop and N status in soil (Niranjana *et al.*, 2015; Yadravi and Angadi, 2016). However, the effects of different time of application and its combination with different levels of N application in reproductive stages along with biofertilizer have not been widely studied. There is a need to study basal and top dressing of N

fertilization at different reproductive stages along with seed inoculation on symbiotic traits, nutrient uptake and biological properties of soybean. Therefore, an experiment was planned with the objective to study the effect of N application at different stages on symbiotic traits, nutrient uptake by the crop and chemical (organic carbon, available nitrogen, available phosphorus and available potassium) and biological (microbial population) properties of soil at two locations.

Materials and Methods

Experimental site

The experiment was conducted at Punjab Agricultural University, Ludhiana (LDH) and Regional Research Station, Faridkot (FDK) during *kharif* 2017.

The soil of experimental site at Ludhiana was loamy sand with low organic carbon and available nitrogen, high available phosphorus and medium available potassium, whereas, at Faridkot, soil texture was loam with medium organic carbon, low available nitrogen, medium available phosphorus and high available potassium.

Experimental layout and treatments

The experiment comprised of ten treatments viz. control (zero nitrogen), seed inoculation (SI) with *Bradyrhizobium* sp, recommended dose of nitrogen (RDN), RDN + SI, SI + 15 kg N ha⁻¹ at sowing + 15 kg N ha⁻¹ at flower initiation (FI), SI + 10 kg N ha⁻¹ (sowing) + 10 kg N ha⁻¹ (FI) + 10 kg N ha⁻¹ at pod initiation (PI), SI + 40 kg N ha⁻¹ (sowing), SI + 20 kg N ha⁻¹ (sowing) + 20 kg N ha⁻¹ (FI), SI + 20 kg N ha⁻¹ (sowing) + 10 kg N ha⁻¹ (FI) + 10 kg N ha⁻¹ (PI), RDN + SI + 2% urea spray at 60 and 75 days after sowing (DAS). The experiment was laid out in randomized complete block design replicated four times.

Crop management

Sowing of soybean variety SL 958 was done on 9 June and 14 June, 2017 at Ludhiana and Faridkot, respectively. Sowing was done with *kera* method with good soil moisture conditions at a depth of 50 mm with row to row spacing of 450 mm and plant to plant of 50 mm by using seed rate of 75 kg ha⁻¹. The recommended dose of nitrogen @ 39.1 kg ha⁻¹ was applied as per the treatments in the form of urea (46% N) and a uniform basal dose of phosphorus (P₂O₅) @ 60 kg ha⁻¹ was applied as single super phosphate (16% P₂O₅) at the time of sowing at Ludhiana and Faridkot. The seed was inoculated with *Bradyrhizobium* sp. (LSBR3) culture @ 5g kg⁻¹ seed. The seed were first moistened with little amount of water and then mixed thoroughly with one packet of the *Rhizobium* culture (containing 1 × 10⁸ cells g⁻¹ of carrier) and were sown immediately after drying it in shade. To control weeds, pre-emergence application of Stomp 30 EC (pendimethalin) @ 1500 ml ha⁻¹ within 24 hours of sowing and post-emergence application of Parimaze (imazethapyr) @ 750 ml ha⁻¹ was done at 20 DAS by using 500 litres of water. According to the weather conditions and crop requirement five irrigations were scheduled at both the locations. The crop was harvested on 28 October and 2 November, 2017 at Ludhiana and Faridkot, respectively. The crop was harvested when the colour changed from green to yellow and the plants dried up. Threshing was done with the help of soybean thresher.

Data collection and analysis

Periodic nodule count was taken at 60, 90 and 120 DAS. Five plants were uprooted carefully from each plot. The adhering soil was washed off with water from the roots and the nodules were separated and counted. Dry weight of nodules was also recorded periodically at 60, 90 and 120 DAS. The nodules were dried in an

oven at 70°C for 48 hours. The leg haemoglobin content was determined by the method given by Wilson &Reisenauer,1963. Leg haemoglobin content in the nodules was determined by Drabkin's solution.

The soil samples taken from 0-15 cm soil depth were analysed for total bacterial count and *Rhizobium* count in soil. The analysis for total bacterial count and *Rhizobium* count was done by serial dilution plating method (Subba Rao, 1988) by using nutrient agar medium and on yeast extract mannitol agar (YEMA) media by pour plate technique, respectively. Bacterial populations were expressed as colony forming units per gram of soil (cfu g⁻¹ of soil).

Statistical analysis

The data was collected for various aspects and was statistically analyzed per the procedure given by Gomez &Gomez, 1984. The comparisons were made at 5% level of significance.

Results and Discussion

Symbiotic traits

The numbers of nodules were significantly affected by application of nitrogen at different growth stages (Table 1). Application of nitrogen as SI + 40 kg N ha⁻¹ at sowing recorded significantly higher number of nodules plant⁻¹ (35.5) as compared to other treatments and was found statistically at par with RDN, RDN + SI and RDN + SI + 2% urea spray at 60 and 75 DAS at Ludhiana. There was further increase of about 40.3% in number of nodules as compared to control at 90 DAS in treatment SI + 40 kg N ha⁻¹ (sowing) which was found statistically at par with SI + 20 kg N ha⁻¹ (sowing) + 10 kg N ha⁻¹ (FI) + 10 kg N ha⁻¹ (PI), RDN, RDN + SI and RDN + SI + 2% urea spray at 60 and 75 DAS at Ludhiana. At 120

DAS, decrease in nodulation was observed. The number of nodules plant⁻¹ was non-significantly affected by different treatments of nitrogen application.

At Faridkot, no significant effect of nitrogen application was observed at 60, 90 and 120 DAS on number of nodules plant⁻¹ and nodulation was relatively less as compared to Ludhiana. This might be due to the reason that soybean was sown for the first time in that field and there was no built up of *Bradyrhizobium* sp.

The effect of nitrogen application was found significant on nodule dry weight at 60 DAS at Ludhiana (Table 2). The maximum nodule dry weight of 150.1 mg plant⁻¹ was recorded in SI + 40 kg N ha⁻¹ (sowing) at Ludhiana which was 41.6 % higher than the control. The nodule dry weight increased as the number of nodules increased at 90 DAS. However, the differences among different treatments were non-significant at 90 and 120 DAS at Ludhiana and at 60, 90 and 120 DAS at Faridkot.

Leg haemoglobin content has a positive correlation with the nitrogen fixation and nitrogenase activity of nodules. Application of nitrogen had significant effect on leg haemoglobin content of soybean (Table 3). Different nitrogen treatments significantly affected the leg haemoglobin content at 60 and 90 DAS at Ludhiana. At 60 DAS, the maximum leg haemoglobin content was recorded in SI + 40 kg N ha⁻¹ sowing treatment which was at par with RDN + SI, SI + 15 kg N ha⁻¹ (sowing) + 15 kg N ha⁻¹ (FI), SI + 10 kg N ha⁻¹ (sowing) + 10 kg N ha⁻¹ at (FI) + 10 kg N ha⁻¹ (PI), SI + 20 kg N ha⁻¹ (sowing) + 20 kg N ha⁻¹ (FI) and RDN + SI + 2% urea spray at 60 and 75 DAS at Ludhiana, whereas, no significant effect of nitrogen application on leg haemoglobin content was observed at Faridkot.

There was increase in leg haemoglobin content at 90 DAS at both the locations. Treatment of SI + 40 kg N ha⁻¹ (sowing) recorded the maximum leg haemoglobin content which was at par with RDN + SI, SI + 15 kg N ha⁻¹ (sowing) + 15 kg N ha⁻¹ (FI), SI + 10 kg N ha⁻¹ (sowing) + 10 kg N ha⁻¹ at (FI) + 10 kg N ha⁻¹ (PI), SI + 20 kg N ha⁻¹ (sowing) + 20 kg N ha⁻¹ (FI), SI + 20 kg N ha⁻¹ (sowing) + 10 kg N ha⁻¹ (FI) + 10 kg N ha⁻¹ (PI) and RDN + SI + 2% urea spray at 60 and 75 DAS at both the locations.

Microbial population

At harvest microbial count of rhizobias was significantly increased by application of nitrogen as starter dose (Table 4). The soil rhizobia were increased by 21.8% over control at Ludhiana and by 35% at Faridkot, in treatment SI + 40 kg N ha⁻¹ (sowing). However, application of nitrogen did not affect total bacterial count at both the locations.

The application of nitrogen in soybean helps in the formation of nodules and thus, helps in increasing the symbiotic nitrogen fixation rate. Application of small amount of starter dose of nitrogen at sowing not only increases plant growth but also stimulates nodule formation and nitrogen fixation (Singh and Saxena, 1972).

The study revealed that nodule number and nodule dry weight increased significantly with application of SI + 40 kg N ha⁻¹ (sowing). The application of starter dose helped in stimulating the nodule formation. Pandey *et al.*, (1995) also reported that number and dry weight of nodules increased with application of 40 kg N ha⁻¹ and decreased afterwards. There was not much increase in nodulation with top dressing of nitrogen at reproductive stages. Similar results also reported by (Gan *et al.*, 2002;

Niranjan *et al.*, 2015). A decrease in nodulation was observed at 120 DAS. The decrease in nodule number might be due to degeneration of the nodules as the crop moved towards maturity. These observations are in accordance with Niranjan *et al.*, (2015).

The leg haemoglobin content was recorded maximum in treatment of SI + 40 kg N ha⁻¹ (sowing) which might be due to more number of effective nodules with application of nitrogen as basal dose. Increase in leg haemoglobin content with application of nitrogen was also reported by Virk *et al.*, (2018).

Microbial population

The application of nitrogen as starter dose might have resulted in increase in rhizobial population, which however, also enhanced formation of root nodules (Table 1). The application of nitrogen increased the microbial quantity in soybean rhizosphere as reported by Zhang *et al.*, (2013). Application of nitrogen enhanced the concentration of soil N nutrients, but decreased bacterial count (Zhou *et al.*, 2017).

This study investigated the effect of basal and top dressing of nitrogen at different stages on symbiotic traits, nutrient uptake and soil properties. The nodule count and nodule dry weight plant⁻¹ were significantly increased in treatment SI + 40 kg N ha⁻¹ (sowing) which was, however, at par with SI + 20 kg N ha⁻¹ (sowing) + 10 kg N ha⁻¹ (FI) + 10 kg N ha⁻¹ (PI) at Ludhiana. The leg haemoglobin content was also improved with application of nitrogen as SI + 40 kg N ha⁻¹ (sowing). Therefore, application of nitrogen at sowing and reproductive stages fulfilled the high N demand by the crop and thus, improved symbiotic traits.

Table.1 Effect of nitrogen application at different stages on number of nodules plant⁻¹ of soybean

Treatments	Number of nodules plant ⁻¹					
	60 DAS		90 DAS		120 DAS	
	LDH	FDK	LDH	FDK	LDH	FDK
Control	20.8	3.8	41.1	8.7	20.3	2.4
SI with <i>Bradyrhizobium</i> sp.	28.0	5.4	51.4	11.8	22.8	3.1
RDN	30.1	4.3	53.5	11.5	22.2	3.3
RDN + SI	33.3	4.4	53.1	11.8	22.7	3.0
SI + 15 kg N ha ⁻¹ (sowing) + 15 kg N ha ⁻¹ (FI)	26.8	4.3	50.8	11.6	24.2	3.3
SI + 10 kg N ha ⁻¹ (sowing) + 10 kg N ha ⁻¹ (FI) + 10 kg N ha ⁻¹ (PI)	29.3	4.4	48.8	11.1	21.9	3.2
SI + 40 kg N ha ⁻¹ (sowing)	35.5	5.2	57.7	12.7	25.2	3.5
SI + 20 kg N ha ⁻¹ (sowing) + 20 kg N ha ⁻¹ (FI)	28.8	4.2	52.6	11.8	20.5	3.2
SI + 20 kg N ha ⁻¹ (sowing) + 10 kg N ha ⁻¹ (FI) + 10 kg N ha ⁻¹ (PI)	28.6	5.0	54.0	12.3	20.1	3.1
RDN + SI + 2% urea spray at 60 and 75 DAS	30.8	4.8	52.8	12.0	20.8	3.1
LSD (<i>p</i> =0.05)	6.0	NS	5.0	NS	NS	NS

*Ludhiana **Faridkot

Table.2 Effect of nitrogen application at different stages on nodule dry weight of soybean

Treatments	Nodule dry weight (mg plant ⁻¹)					
	60 DAS		90 DAS		120 DAS	
	LDH	FDK	LDH	FDK	LDH	FDK
Control	106.0	16.0	195.6	38.5	110.1	9.6
SI with <i>Bradyrhizobium</i> sp.	133.0	18.4	218.7	48.0	115.1	11.1
RDN	137.3	19.2	223.7	47.5	116.0	11.1
RDN + SI	143.1	19.5	226.7	48.9	112.1	12.0
SI + 15 kg N ha ⁻¹ (sowing) + 15 kg N ha ⁻¹ (FI)	132.3	18.1	215.1	46.8	120.5	12.3
SI + 10 kg N ha ⁻¹ (sowing) + 10 kg N ha ⁻¹ (FI) + 10 kg N ha ⁻¹ (PI)	130.2	19.4	205.5	45.6	117.3	11.6
SI + 40 kg N ha ⁻¹ (sowing)	150.1	20.2	236.5	48.5	122.7	12.3
SI + 20 kg N ha ⁻¹ (sowing) + 20 kg N ha ⁻¹ (FI)	134.5	18.5	221.4	46.0	115.3	11.8
SI + 20 kg N ha ⁻¹ (sowing) + 10 kg N ha ⁻¹ (FI) + 10 kg N ha ⁻¹ (PI)	132.7	18.5	221.7	44.2	118.2	11.4
RDN + SI + 2% urea spray at 60 and 75 DAS	132.8	18.6	223.6	47.1	120.2	11.3
LSD (<i>p</i> =0.05)	15.6	NS	NS	NS	NS	NS

Table.3 Effect of nitrogen application at different stages on leghaemoglobin content of soybean

Treatments	Leghaemoglobin content (mg g ⁻¹ fresh weight of nodules)			
	60 DAS		90 DAS	
	LDH	FDK	LDH	FDK
Control	3.0	2.1	4.2	2.3
SI with <i>Bradyrhizobium</i> sp.	4.3	2.7	4.9	2.9
RDN	3.3	2.6	4.3	2.7
RDN + SI	5.3	2.9	6.2	3.1
SI + 15 kg N ha ⁻¹ (sowing) + 15 kg N ha ⁻¹ (FI)	5.5	2.8	6.2	3.1
SI + 10 kg N ha ⁻¹ (sowing) + 10 kg N ha ⁻¹ (FI) + 10 kg N ha ⁻¹ (PI)	5.4	3.0	6.2	3.4
SI + 40 kg N ha ⁻¹ (sowing)	5.6	3.2	6.3	3.6
SI + 20 kg N ha ⁻¹ (sowing) + 20 kg N ha ⁻¹ (FI)	5.4	3.1	6.0	3.4
SI + 20 kg N ha ⁻¹ (sowing) + 10 kg N ha ⁻¹ (FI) + 10 kg N ha ⁻¹ (PI)	5.2	3.2	6.0	3.4
RDN + SI + 2% urea spray at 60 and 75 DAS	5.4	3.0	6.3	3.4
LSD (p=0.05)	0.3	NS	0.3	0.5

Table.4 Effect of nitrogen application at different stages on microbial population at harvest

Treatments	Soil bacterial population (× 10 ⁶ cfu g ⁻¹ soil)		Soil rhizobia (× 10 ⁶ cfu g ⁻¹ soil)	
	LDH	FDK	LDH	FDK
	Control	3.7	3.7	3.2
SI with <i>Bradyrhizobium</i> sp.	3.8	3.5	3.6	2.5
RDN	3.8	3.7	3.6	2.5
RDN + SI	3.7	3.6	3.8	2.7
SI + 15 kg N ha ⁻¹ (sowing) + 15 kg N ha ⁻¹ (FI)	3.8	3.7	3.5	2.3
SI + 10 kg N ha ⁻¹ (sowing) + 10 kg N ha ⁻¹ (FI) + 10 kg N ha ⁻¹ (PI)	3.9	3.7	3.6	2.4
SI + 40 kg N ha ⁻¹ (sowing)	3.8	3.7	3.9	2.7
SI + 20 kg N ha ⁻¹ (sowing) + 20 kg N ha ⁻¹ (FI)	3.8	3.6	3.5	2.4
SI + 20 kg N ha ⁻¹ (sowing) + 10 kg N ha ⁻¹ (FI) + 10 kg N ha ⁻¹ (PI)	3.7	3.6	3.5	2.4
RDN + SI + 2% urea spray at 60 and 75 DAS	3.8	3.7	3.7	2.2
LSD (p=0.05)	NS	NS	0.2	0.3

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Competing interest

The authors declare no conflicts of interest.

Abbreviations used

SI-seed inoculation, RDN- recommended dose of nitrogen, FI- flower initiation, PI- pod initiation, DAS- days after sowing, LDH- Ludhiana, FDK-Faridkot

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