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**Response of added P, S and microbial inoculant  
on biological nitrogen fixation and yield of  
soybean**

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**Abstract**

A field experiment was conducted at experimental farm of Birsa Agricultural University during the *kharif* season, 2016 to study the response of added P, S and microbial inoculant on biological nitrogen fixation and yield of soybean with 18 treatment combinations having two levels of inoculation ( $I_0$  and  $I_1$ ), three levels of phosphorus ( $P_{40}$ ,  $P_{60}$  and  $P_{80}$  kg ha<sup>-1</sup>) and three levels of sulphur ( $S_0$ ,  $S_{15}$  and  $S_{30}$  kg ha<sup>-1</sup>) in a split-split plot design replicated thrice. The soil of the experimental site was sandy clay loam in texture having good drainage and fairly moisture retention capacity with acidic pH (5.2), EC (0.08dS m<sup>-1</sup>), low in organic carbon (2.6 g kg<sup>-1</sup>), CEC (8.5 cmol (p<sup>+</sup>) kg<sup>-1</sup>), total nitrogen (0.157%) and available nitrogen (181.5 kg ha<sup>-1</sup>), medium in available phosphorus (23.9 kg ha<sup>-1</sup>) and available sulphur (17.0 ppm) was above the critical range. Microbial population in initial soil was  $29.33 \times 10^4$  Propagules g<sup>-1</sup>,  $22 \times 10^6$  CFU g<sup>-1</sup>,  $8.9 \times 10^6$  CFU g<sup>-1</sup> for fungi, bacteria and actinomycetes, respectively. The results revealed that different levels of phosphorus and sulphur along with microbial inoculation significantly influenced the nodulation parameters and yield such as number of nodules (33.0plant<sup>-1</sup>), fresh weight of nodule (0.69 gplant<sup>-1</sup>) and dry weight of nodule (0.31 gplant<sup>-1</sup>) were recorded maximum with application of phosphorus @ 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> while having statistical equivalence with 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> for number of nodules per plant. BNF and yield of soybean remarkably increased with increasing doses of phosphorus and recorded maximum of 84.0 kg ha<sup>-1</sup>, 24.0 q and 27.1 q ha<sup>-1</sup> BNF, grain and straw yield, respectively with 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Application of 30 kg S ha<sup>-1</sup> was found equally effective with application of 15 kg S ha<sup>-1</sup> in respect to number of nodules, fresh weight, dry weight of nodule and grain yield and recorded maximum value of 31.4plant<sup>-1</sup>, 0.67 gplant<sup>-1</sup> and 0.30 gplant<sup>-1</sup>, and 23.6 q ha<sup>-1</sup>, respectively with application of 30 kg S ha<sup>-1</sup>. BNF increased with increasing doses of sulphur and recorded maximum of 77.0 kg ha<sup>-1</sup> with 30 kg S ha<sup>-1</sup>. Microbial inoculation significantly increased the number of nodules (34.0plant<sup>-1</sup>), fresh weight (0.69 gplant<sup>-1</sup>), dry weight of nodule (0.30 gplant<sup>-1</sup>), BNF (83.4 kg N ha<sup>-1</sup>), grain yield (23.4 q ha<sup>-1</sup>) and straw yield (26.4 q ha<sup>-1</sup>).

**Keywords:** Biological nitrogen fixation and yield of Soybean, P, S and microbial inoculant

**Introduction**

Soybean (*Glycine max* (L.) Merrill) is a leguminous oil seed crop, occupying 2<sup>nd</sup> rank for oil seed production (8569.80 MT, 2015-16, MOA, GOI) in India. It is an important high-quality protein source for human and animal nutrition. In terms of food components, soybean contains 35 - 40% protein, 19- 22% oil, 35% carbohydrate (17% of which is a dietary fibre), 5% minerals and several other components including vitamins (Liu, 1997). Soybean with the soil bacteria *Bradyrhizobium japonicum* symbiotically colonizing the plant's roots and are able to fix atmospheric nitrogen (N<sub>2</sub>). Soybean can biologically fix nitrogen in the range of 85 - 154 kg N ha<sup>-1</sup> (Giller, 2001) [5]. Phosphorus (P) and sulphur (S) are major nutrient elements for grain legumes. Phosphorus has very positive effects on nodule formation and nitrogen fixation in legume crops (Sepetolu, 2002) [14]. Phosphorus in the soil has developmental activity in the plant's root growth.

Depending on phosphorus applications, the contact area of the root expands with the growth of root which in turn, gives rise to a flourishing in productivity, also making it easier for the plant to benefit from the other nutritional elements in higher proportions (Marschner, 1995) [11]. Sulphur plays a vital role in plant metabolism. It constitutes the main element of amino acids such as cysteine and methionine, which are of essential nutrient value. In addition to these functions, ferro-sulphur proteins play an important role in nitrogen fixation and electron movement in photosynthesis (Kadiolu, 2004) [8]. Sulphur has positive effects on root growth in plants in general. This element positively affects nodulation in legume crops (Kacar, 1984) [7].

### Materials and methods

The experiment was conducted under field condition at experimental farm of Soil Science and Agricultural Chemistry, B.A.U, Ranchi. The soil of the experimental site was sandy clay loam in texture having good drainage and fairly moisture retention capacity with acidic pH (5.2), EC (0.08dS m<sup>-1</sup>), low in organic carbon (2.6 g kg<sup>-1</sup>), CEC (8.5 cmol (p+) kg<sup>-1</sup>), total nitrogen (0.157%) and available nitrogen (181.5 kg ha<sup>-1</sup>), medium in available phosphorus (23.9 kg ha<sup>-1</sup>) and available sulphur (17.0 ppm) was above the critical range. Soybean (var. JS-335) was taken as a test crop during the Kharif season, 2016 with 18 treatment combinations comprising two levels of inoculation (I<sub>0</sub> and I<sub>1</sub>), three levels of phosphorus (40, 60 and 80 kg ha<sup>-1</sup>) and three levels of sulphur (0, 15 and 30 kg ha<sup>-1</sup>) in split-split plot design with three replications. The data on nodulation and yield parameters were recorded by following standard methods. Assessment of nodulation by five randomly selected whole plants from each treatment and replications were uprooted at 50% flowering stage. The roots were washed carefully to remove soil particles and the nodules were separated from the roots and counted, fresh weight of nodules were taken and then root nodules were oven dried at 70°C for 72 hours and their dry weight was measured. Total nitrogen content in nodule was determined by Kjeldahl's method (Jackson, 1973) [6]. BNF (kg N ha<sup>-1</sup>) potential was calculated based on number of nodules per plant, dry weight of nodules (g), N content in nodules (%) and plant population (350,000 ha<sup>-1</sup>). Grain and straw yield were recorded at harvest and expressed in q ha<sup>-1</sup>. The harvest index is a ratio of economic yield to the biological yield per hectare and expressed in percentage.

### Results and Discussion

#### Nodulation parameters

Data as indicated in the table 1 shows that number of nodules per plant, fresh weight and dry weight of nodules and biological nitrogen fixation were significantly affected by the different level of phosphorus. Among the levels of phosphorus, application of 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> produced the highest number of nodules (33.0 plant<sup>-1</sup>) and was found to be

significantly superior over 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> that produced minimum number of nodules (26.4plant<sup>-1</sup>). However, it was remained at par with 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (30.3plant<sup>-1</sup>) while in case of fresh weight and dry weight of nodules per plant, application of 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was found to be significant over 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and recorded maximum fresh weight and dry weight of nodules (0.69g and 0.31g plant<sup>-1</sup>, respectively) followed by 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (0.66g and 0.29g plant<sup>-1</sup>, respectively) whereas, the lowest fresh weight and dry weight of nodules (0.61g and 0.26g plant<sup>-1</sup>, respectively) were registered with 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Application of phosphorus had significant effect on biological nitrogen fixation and followed the sequence of P<sub>80</sub> (84.0 kg N ha<sup>-1</sup>) > P<sub>60</sub> (73.0kg N ha<sup>-1</sup>) > P<sub>40</sub>(55.5kg N ha<sup>-1</sup>). During nodulation, phosphorus is an essential ingredient for *Rhizobium* bacteria to convert atmospheric nitrogen into an ammonium (NH<sub>4</sub><sup>+</sup>) form usable by plants. Adequate supply of phosphorus seems to have promoted root growth, resulting in higher production and development of root nodules. The increase in number of nodules may also resulted in increase in fresh and dry weight of nodules. (Tahir *et al.*, 2009; Abbasi *et al.*, 2012) [15, 1]. BNF varied from 55.5 to 84.0kg N ha<sup>-1</sup> in acid soils under sub-humid condition however, several authors reported for BNF variation starting from 80 to 154 kg ha<sup>-1</sup> (Giller, 2001) [5]. It is apparent from data that the number of nodules per plant was significantly affected by different level of sulphur application (Table 1). Application of 30 kg S ha<sup>-1</sup> gave the highest number of nodules (31.4plant<sup>-1</sup>) and found significantly superior over 0 kg S ha<sup>-1</sup> (28.5plant<sup>-1</sup>) which was at par with 15 kg S ha<sup>-1</sup> (30.0plant<sup>-1</sup>). Fresh weight and dry weight of nodules per plant were observed significantly high under the treatment receiving 30 kg S ha<sup>-1</sup> (0.67g and 0.30g plant<sup>-1</sup>, respectively) over 0 kg S ha<sup>-1</sup> (0.63g and 0.28g plant<sup>-1</sup>, respectively) which was however, at par with 15 kg S ha<sup>-1</sup> (0.66g and 0.29g plant<sup>-1</sup>, respectively) while in case of BNF, doses of sulphur followed the significant sequence of S<sub>30</sub>(77.0kg N ha<sup>-1</sup>) > S<sub>15</sub> (70.5kg N ha<sup>-1</sup>) > S<sub>0</sub> (65.0kg N ha<sup>-1</sup>). The increase in number of nodules and weight of nodule might be due to vital role of sulphur in formation of ferredoxin iron containing plant protein that act as electron carrier in photosynthesis and involved in N-fixation by nodule bacteria. (Dhage *et al.*, 2014; Long Kumar *et al.*, 2017) [4, 10]. The data pertaining to number of nodules, fresh weight, dry weight of nodules per plant and BNF as influenced by the levels of microbial inoculation is presented in table 1. Inoculated plot produces significantly more number of nodules (34.0 plant<sup>-1</sup>), fresh weight (0.69g plant<sup>-1</sup>) and dry weight (0.30 gplant<sup>-1</sup>) and BNF (83.3 kg N ha<sup>-1</sup>) than uninoculated (26.0 plant<sup>-1</sup>, 0.62g plant<sup>-1</sup>, 0.28g plant<sup>-1</sup> and 58.2 kg N ha<sup>-1</sup>, respectively). It may be because *Rhizobium* involves in BNF and provide the better root growth which facilitated more area for nodule formation. (Zerpa *et al.*, 2013; Aminu *et al.*, 2015) [17, 3]. Anon significant deviation in N content of nodule was observed due to application of phosphorus, sulphur and microbial inoculation. (Table 1).

**Table 1:** Effect of P, S and microbial inoculation on number, fresh weight, dry weight, N content of nodules and BNF of soybean

Treatment	No. of nodules per plant	Fresh weight of nodules per plant (g)	Dry weight of nodules per plant (g)	N content in nodule (%)	BNF (kg N ha <sup>-1</sup> )
<b>Phosphorus level (kg ha<sup>-1</sup>)</b>					
P <sub>1</sub>	26.4	0.61	0.26	2.22	55.5
P <sub>2</sub>	30.3	0.66	0.29	2.25	73.0
P <sub>3</sub>	33.0	0.69	0.31	2.26	84.0
S.Em (±)	1.36	0.01	0.005	0.02	3.79
CD (P=0.05)	4.43	0.04	0.015	NS	12.37

Sulphur level (kg ha <sup>-1</sup> )					
S <sub>1</sub>	28.5	0.63	0.28	2.24	65.0
S <sub>2</sub>	30.0	0.66	0.29	2.25	70.5
S <sub>3</sub>	31.4	0.67	0.30	2.25	77.0
S.Em (±)	0.71	0.01	0.004	0.01	1.95
CD (P=0.05)	2.07	0.03	0.012	NS	5.68
Inoculation level					
I <sub>0</sub>	26.0	0.62	0.28	2.24	58.2
I <sub>1</sub>	34.0	0.69	0.30	2.26	83.4
S.Em (±)	1.26	0.01	0.003	0.01	3.48
CD (P=0.05)	7.67	0.06	0.01	NS	21.20
C.V. (%)	10.1	6.01	5.77	2.54	11.7

## Yield

Critical examination of data given in table 2 reveals that phosphorus levels significantly influenced the grain and straw yield. The application of 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> produced significantly higher grain yield (24.0 q ha<sup>-1</sup>) and straw yield (27.1 q ha<sup>-1</sup>) over 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (21.0 q 23.6 q ha<sup>-1</sup> for grain and straw yield, respectively). It was also evident that application of 60 kg and 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> differed significantly with each other. The increase in grain and straw yield of soybean over lower dose of phosphorus might be due to increase translocation of photosynthates towards the sink development have occurred (Wu *et al.*, 2012; Dhage *et al.*, 2014) [16, 4]. The perusal of data presented in table 2 shows that the different doses of sulphur influenced significantly the grain yield of soybean. Significantly higher grain yield (23.6 q ha<sup>-1</sup>) was recorded with the application of 30 kg S ha<sup>-1</sup> as compared to 0 kg S ha<sup>-1</sup> (21.5 q ha<sup>-1</sup>) which was at par with 15 kg S ha<sup>-1</sup> (22.3 q ha<sup>-1</sup>). The increase in grain yield of soybean by sulphur application might be due to the effect of sulphur in utilizing large quantities of nutrients through their well

developed root system, which resulted in better utilization of plant nutrients from soil (Parakhia *et al.*, 2016 and Longkumer *et al.*, 2017) [13, 10]. The results further revealed in table 2 that apparently the straw yield of soybean was found non significant with different doses of sulphur application. Inoculation had pronounced and significantly affected the grain and straw yield of soybean. The inoculation produced significantly higher grain and straw yield 23.4 q and 26.4 q ha<sup>-1</sup>, respectively as compared to uninoculated plot (21.6 q and 24.5 q ha<sup>-1</sup> of grain and straw yield, respectively). This might be due proper establishment and greater infection of N-fixers and use of specific rhizobial strain homologous for the test plant may have influenced to develop healthy and efficient nodules supplemented with P and S in adequate number on root biomass, resulting in efficient dinitrogen reduction and its assimilation leading to increase in yield (Morad *et al.*, 2013 and Adeyeye *et al.*, 2017) [12, 2]. Harvest index was observed to be around 47% and there was non significant difference among the treatments.

**Table 2:** Effect of P, S and microbial inoculation on yield of soybean

Treatment	Grain yield (q ha <sup>-1</sup> )	Straw yield (q ha <sup>-1</sup> )	Harvest index (%)
Phosphorus level (kg ha <sup>-1</sup> )			
P <sub>1</sub>	21.0	23.7	47.0
P <sub>2</sub>	22.6	25.6	46.8
P <sub>3</sub>	24.0	27.1	46.8
S.Em (±)	0.39	0.43	0.14
CD (P=0.05)	1.30	1.42	NS
Sulphur level (kg ha <sup>-1</sup> )			
S <sub>1</sub>	21.5	24.5	46.7
S <sub>2</sub>	22.3	25.4	46.7
S <sub>3</sub>	23.6	26.7	47.1
S.Em (±)	0.53	0.61	0.14
CD (P=0.05)	1.55	NS	NS
Inoculation level			
I <sub>0</sub>	21.6	24.5	46.8
I <sub>1</sub>	23.4	26.4	47.0
S.Em (±)	0.16	0.10	0.11
CD (P=0.05)	0.99	0.61	NS
CV (%)	10.0	10.3	1.28

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