Integrated Effect of Rhizobium Inoculation and Phosphorus Application on Tissue Content, Symbiotic and Phosphorus use Efficiency in Soybean Production

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Abstract—Soybean (Glycine max) is an important legume crop that is cultivated all over the world as livestock feed, food for human consumption, soil fertility improvement and industrial products such as candles and paints. However, Nitrogen (N) and phosphorus (P) nutrient have been attributed to the decline in soybean yields. Furthermore, scanty information is documented on P-efficient soybean genotypes, which are a sustainable P management strategy for enhancing symbiotic efficiency (SEF) and phosphorus use efficiency (PUE). As a solution, field experiment was conducted at Chuka University farm to evaluate the integration effect of rhizobium inoculation (R) and P on tissue nutrient content, SEF and PUE in soybean production in Meru South Sub County. Two cultivations (Trial I and II) were done in 2018. Treatments included; three rates of R (0, 100 and 200 g ha⁻¹), three rates of P (0, 20 and 30 kg ha⁻¹), either applied alone or integrated and soybean genotypes (SB19 and SB24). Both Trials were laid out in a randomized complete block design in split-split plot arrangement with each treatment replicated thrice. Genotypes were assigned main plot, R subplots and P in sub-subplots. Data collected was subjected to analysis of variance using the Scientific Analysis System SAS and significantly different means separated using Tukey test at (p≤0.05). The results showed significant difference in N and P tissue content, SEF and PUE for SB19 and SB24 genotypes in both Trials at (p≤0.05). The highest N tissue content of between 1.73% and 9.10% was observed when integration of R and P were applied at the rate of 200 g and 30 kg for SB19 and SB24 in both Trials. While R and P at the rate of 200 g and 30 kg per ha showed the highest P content of between 849.6 ppm and 955.0 ppm in both Trials. The highest SEF recorded was 207% and 261%, and 201% and 227% in Trials I and II, respectively. The PUE was highest when R and P was applied at the rate of 200 g and 30 kg per ha for SB19 and SB24 soybean in both Trials. Integration of R and P at the rate of 200 g and 30 kg ha⁻¹ and adoption of either SB19 or SB24 showed a potential in enhancing soybean cultivation.

Keywords—Genotypes, phosphorus use efficiency, symbiotic, Tissue content.

1. INTRODUCTION
Soybean (Glycine max) is a crop that is cultivated as a source of livestock feed, human food and soil fertility improvement. It is the world's largest source of protein feed, the second largest source of vegetable oil and the fourth leading crop produced globally [2][3]. Biological nitrogen fixation is crucial and efficient process in improving soil fertility [4]. Application of phosphorus in soybean cultivation enhanced biological nitrogen fixation BNF in soybean-rhizobium interaction [5] increasing nitrogen content in plant tissues [6]. Integration of rhizobium and phosphorus was reported to have improved uptake of N and P in soybean [7]. Elsewhere, intercrop of Soybean/maize resulted to a higher nitrogen content in maize grain, indicating enhanced nitrogen uptake [8]. Phosphorus content in grain and straw was influenced by R and P application [9]. Plant nitrogen concentration in response to increased soil phosphorus supply was observed, with

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phosphorus content in grain and straw being highest in integration of R and P [10]. Phosphorus and nitrogen play specific roles in symbiotic N$_2$-fixation efficiency through their effects on nodulation and N$_2$-fixation process [11]. High yields with subsequent profits, are related to symbiotic efficiency (SEF) of soybean with N-fixing bacteria [12]. Nitrogen fixation is very sensitive to P deficiency due to reduced nodule mass and decreased ureide production [7]. Symbiotic N-fixation has a high P demand for energy provision [13][14]. Nitrogen fixation varied among rhizobia isolates with SEF ranging between 27 and 112% when beans were inoculated with R [15] Isolates from beans had higher SEF compared to the commercial inoculants [15]. Koskey et al. [16] used plant shoot dry weight to estimate SEF which ranged between 86.7 - 123.72% in common beans. In seed potato tuber production, phosphorus use efficiency PUE increased from 0 kg/kg observed with 0 kg P per ha and 0 kg N per ha to a range of 75.9 kg/kg to 186.6 kg/kg when integration of P and N was used [17].

II. MATERIALS AND METHODS

The experiment was conducted at Chuka University farm, Meru South Sub-County in two cultivations (Trial I and II) in 2018. Chuka University lies at an altitude of 1399 m above sea level, at latitude of 0 20'0"S and longitude of 37 39'0"E (Fig. 1). Temperature range of 20.97 °C - 27.25 °C, rainfall of 1178 mm, and nitisol type of soils. Major crops in the area are; Phaseolus vulgaris, Vigna unguiculate, Cajanus cajan, Glycine max, Sorghum spp, Musa spp, Mangifera indica, Coffea arabica and Camellia sinensis [18].

![Fig. 1: Location of Chuka in Tharaka Nithi County in Kenya](image)

III. EXPERIMENTAL DESIGN

The experiment was laid out in a randomized complete block design in a split-split plot arrangement with each treatment replicated thrice. Treatments included; three rates of P (0, 20 and 30 Kg ha$^{-1}$), and three rates of R (0, 100 and 200 g ha$^{-1}$) either applied alone or integrated and two soybean genotypes (SB19 and SB24). The triple superphosphate (0:46:0) was used as the source of phosphorus. The SB19 and SB24 genotypes were assigned the main plot, P rates the sub-plot and R rates to sub-subplots. The size of experimental plot was 1.5 x 1.3 m with 1 m path between main plots and 0.5 m between subplots and sub-subplots.

A. Soil Sampling and Analysis

The soil was sampled across and diagonally in each site at a depth of 25 cm using a soil auger. A kilogram of a homogeneous composite soil sample from each site was packed independently into sterile bags for analysis. Soil analysis was done at Kenya Agricultural Research and Livestock Organization (KALRO) – Embu.

B. Planting Materials, Planting and Crop Management

Certified soybean seeds and inoculant were obtained from KALRO-Kakamega and MEA Limited-Nakuru respectively. The inoculation was done in Plant Science Laboratory of Chuka University. Where soybean seeds were moistened with 4% Gum Arabica solution in a basin and the inoculant was added at the rates of 10 g per Kg and 20 g per Kg of soybean seeds. The mixture was stirred until even coating was attained. The seeds were then spread on flat plywood under a shade and allowed to air dry for 30 minutes. Inoculated seeds were sown early in the morning to avoid its exposure to direct sun rays that could affect the efficacy of the inoculant.

A basal application of phosphorus at the rate of 0 Kg, 20 Kg, and 30 Kg P per ha which was equivalent to 0 g, 3.6 g, and 5.4 g per plot was done during planting to the assigned plots. Two seeds were sown at inter and intra row spacing of 0.5 m and 0.1 m respectively in a plot measuring 1.2 m x 1.5 m. Seedlings were thinned to one per hill one week after emergence giving a plant population of 200,000 plants per ha or 39 plants per plot. All cultural practices recommended for soybean cultivation were done equally to all the plots.

Data Collection

1) Integration Effect of Different Rates of Rhizobium and Phosphorus on Shoot and Grain Nitrogen and Phosphorus Content

Twenty grams of shoot and grain from experimental plants were randomly taken from every plot, placed in khaki papers and dried in the oven at 60 °C for 72 hours. Dry shoot and grain samples were ground into powder using a blender. The powder was then sieved using a laboratory test sieve and packed in khaki paper bags ready for laboratory
analysis. The plant shoots and grain powder were analyzed for nitrogen (% N) and P (ppm) content using Kjeldahl according to Bremner [19].

a) Integration Effect of Different Rates of Rhizobium and Phosphorus on Rhizobium Use Efficiency

The plant shoot dry weight (SDW) was used to estimate the symbiotic nitrogen-fixing efficiency (SEF) of the commercial R. Symbiotic efficiency in this study was determined according to Koskey et al. [16], using the formula below:

\[
SEF = 100 \times \left( \frac{SDW \text{ of inoculated (Kg)}}{SDW \text{ of non inoculated (Kg)}} \right)
\]

b) Integration Effect of Different Rates of Rhizobium and Phosphorus on Phosphorus Use Efficiency

The phosphorus use efficiency (PUE) was computed according to Belete et al. [20] using the formula below:

\[
PUE = \frac{(Seed \text{ yield (kg) of plots with Fert.} - \text{ yield (kg)control})}{\text{Quantity of phosphorus (P) applied in kg per plot}}
\]

Data analysis

The data collected was subjected to analysis of variance using the statistical scientific analysis system for windows V8 1999-2001 by SAS Institute Inc., Cary, NC, USA and significantly different means separated using Tukey test at \(p\leq0.05\).

IV. RESULTS

1) Integration Effect of Different Rates of Rhizobium and Phosphorus on Shoot and Grain Nitrogen Content

There were no significant difference in shoot and grain N content between genotype SB19 and SB24 within and between Trial I and II \((p\leq0.05)\). However, there were significant respond of the integration of R and P in shoot and grain N content within genotypes SB19 and SB24 at \((p\leq0.05)\) in both Trials. Rhizobia application at the highest rate of 200 g per ha, increased the shoot N content from the control to 1.4% and 1.03%, and 1.46% and 1.09% for SB19 and SB24 genotypes in Trials I and II, respectively (Table 1). Application of P at the highest rate of 30 Kg per ha, shoot N content increased from the control treatment to 0.94% and 0.83%, and 0.96% and 0.86% for SB19 and SB24 genotypes in both Trial I and II, respectively (Table 1).

Integration of R and P significantly increased grain N content for SB19 and SB24 genotypes in both Trial I and II whether applied alone or integrated. Rhizobia at the highest rate of 200 g per ha, increased grain N content to 5.97% and 5.11%, and 6.02% and 5.07% for SB19 and SB24 genotypes in both Trial I and II, respectively. Compared to control, highest integration of R and P at the rate of 200 g and 30 Kg per ha, increased grain N content by 3.81% and 2.11%, and 3.53% and 1.93% for SB19 and SB24 genotypes in both Trial, respectively (Table 1).

Table 1: Effect of Rhizobia and Phosphorus on Soybean Shoot and Grain N Content.

| Variety | Trt | Shoot | Grain |
|---------|-----|-------|-------|
|         |     | N (%) | N (%) |
| SB19    | T1  | 0.81** | 5.12** |
|         | T2  | 0.88** | 5.54** |
|         | T3  | 0.94c | 5.69c |
|         | T4  | 0.98c | 5.8c |
|         | T5  | 1.53d | 6.8c |
|         | T6  | 2.2b  | 7.69b |
|         | T7  | 1.4c  | 5.97d |
|         | T8  | 2.15c | 6.82c |
|         | T9  | 2.98a | 8.93a |
| SB24    | T1  | 0.78a | 5.57a |
|         | T2  | 0.92a | 5.62a |
|         | T3  | 0.96a | 5.83a |
|         | T4  | 0.98a | 5.80a |
|         | T5  | 1.63a | 6.80a |
|         | T6  | 2.46b | 7.74b |
|         | T7  | 1.46b | 6.02d |
|         | T8  | 2.10a | 6.98c |
|         | T9  | 3.09a | 9.10a |

*Means with the same letter along the column for the same variety are not significantly different at \(p\leq0.05\); MSD=Mean Significant Difference; Treatments: T1=Control (0 g R and 0 Kg P per ha); T2 and T3=20 Kg and 30 Kg P per ha respectively; T4 and T7=100 g R and 20 Kg P per ha respectively; T5=100 g R and 20 Kg P per ha; T6=100 g R and 30 Kg P per ha; T8=200 g R and 20 Kg P per ha and T9=200 g R and 30 Kg P per ha; R=Rhizobia; P=Phosphorus.

Integration Effect of Different Rates of Rhizobium and Phosphorus on Shoot and Grain Phosphorus Content

There was no significant difference in shoot and grain P content between genotype SB19 and SB24 within and between the two Trials \((p\leq0.05)\). However, there were
significant effect of the integration of R and P in shoot and grain P content within genotypes SB19 and SB24 at \( p \leq 0.05 \) in Trials I and II. Rhizobia application increased shoot P content in SB19 and SB24 genotypes in both Trial I and II. Application of R at the rate of 100 g per ha, significantly increased the Shoot P content from 253.1 ppm and 248.2 ppm, and 256.9 ppm and 251.8 ppm observed with the control treatment to 334.3 ppm and 328.4 ppm, and 337.4 ppm and 332.1 ppm for SB19 and SB24 genotypes in both Trial I and II, respectively (Table 1).

Application of P at the highest rate of 30 Kg per ha, increased Shoot P content to 852.4 ppm and 950 ppm, and 853.3 ppm and 906 ppm, and 849.6 ppm and 913.8 ppm for SB19 and SB24 genotypes in both Trial I and II, respectively (Table 2). Rhizobia application increased grain P content in SB19 and SB24 genotypes in both Trial I and II. For instance, application of R at the rate of 100 g per ha, significantly increased the grain P content from the control treatment to 326.1 ppm and 320.6 ppm, and 333.6 ppm and 327.8 ppm observed with the control treatment to 334.3 ppm and 332.1 ppm, and 337.4 ppm and 332.1 ppm for SB19 and SB24 genotypes in both Trial I and II, respectively (Table 1).

Application of P at the highest rate of 30 Kg per ha, increased grain P content to 849.6 ppm and 955 ppm, and 853.3 ppm and 906 ppm, and 849.6 ppm and 913.8 ppm for SB19 and SB24 genotypes in both Trial I and II. Integration of R and P at the highest rate of 200 g per ha, significantly increased grain P content from the control treatment to 430.1 ppm and 328.4 ppm, and 337.4 ppm and 332.1 ppm for SB19 and SB24 genotypes in both Trial I and II, respectively (Table 2).

For instance, application of R at the rate of 100 g per ha, increased Shoot P content to 430.1 ppm and 328.4 ppm, and 337.4 ppm and 332.1 ppm for SB19 and SB24 genotypes in both Trial I and II, respectively (Table 2). Rhizobia application increased grain P content in SB19 and SB24 genotypes in both Trial I and II. For instance R application at the rate of 100 g per ha, significantly increased the grain P content from the control treatment to 509.9 ppm and 519.6 ppm, and 514.1 ppm and 524.5 ppm for SB19 and SB24 genotypes in both Trial I and II, respectively. Compared to control, application of R at the rate of 200 g per ha significantly increased the grain P content from the control treatment to 340.1 ppm and 350.8 ppm, and 346.4 ppm and 356.4 ppm for SB19 and SB24 genotypes in both Trial I and II, respectively (Table 2).

Table 2: Effect of Rhizobia and Phosphorus on Soybean Shoot and Grain P Content.

| Variety | Trial 1 | Trial 2 |
|---------|---------|---------|
|         | Shoot P (ppm) | Grain P (ppm) | Shoot P (ppm) | Grain P (ppm) |
| SB19    |         |         |         |         |
| T1      | 253.1e  | 225.6c  | 248.2e  | 235.6e  |
| T2      | 290.2e  | 323.3b  | 285.0e  | 333.3b  |
| T3      | 326.1f  | 340.6f  | 320.6f  | 350.8f  |
| T4      | 334.3f  | 509.9e  | 328.4f  | 519.6f  |
| T5      | 488.4d  | 579.8c  | 497.9d  | 589.8d  |
| T6      | 671.6b  | 734.4b  | 737.2b  | 834.3b  |
| T7      | 361.7a  | 430.1f  | 385.4g  | 529.6c  |
| T8      | 542.0c  | 524.6d  | 649.9c  | 624.6c  |
| T9      | 849.6a  | 852.4d  | 906.0a  | 950.0a  |
| SB24    |         |         |         |         |
| T1      | 256.9a  | 241.7d  | 251.8a  | 240.6a  |
| T2      | 294.0e  | 328.4c  | 289.0f  | 334.4b  |
| T3      | 333.6f  | 346.4d  | 327.8e  | 356.4f  |
| T4      | 337.4e  | 514.1c  | 332.1e  | 524.5f  |
| T5      | 490.4a  | 584.3c  | 510.6e  | 594.7c  |
| T6      | 672.2b  | 739.4b  | 738.4b  | 839.3b  |
| T7      | 366.0c  | 432.4c  | 383.0c  | 534.6c  |
| T8      | 535.7c  | 529.7d  | 660.2c  | 629.7c  |
| T9      | 849.6a  | 853.3a  | 913.8a  | 955.0a  |
| MSD     |         |         |         |         |
| C.V.    | 2.01    | 1.8     | 1.31    | 0.85    |

*Means with the same letter along the column for the same variety are not significantly different at \( p \leq 0.05 \); MSD=Mean Significant Difference; Treatments: T1=Control (0 g R and 0 Kg P per ha); T2 and T3=20 Kg and 30 Kg P per ha respectively; T4 and T7=100 g R and 20 Kg P per ha respectively; T5=100 g R and 200 g P per ha respectively; T6=100 g R and 30 Kg P per ha; T8=200 g R and 20 Kg P per ha and T9=200 g R and 30 Kg P per ha; R=Rhizobia; P=Phosphorus.

Integration Effect of Different Rates of Rhizobium and Phosphorus on Symbiotic Efficiency

There was no significant difference in symbiotic efficiency between genotype SB19 and SB24 within and between Trials I and II \( p \leq 0.05 \). However, there was significant influence of the integration of R and P in symbiotic efficiency (SEF) within genotypes SB19 and SB24 at \( p \leq 0.05 \) in Trials I and II. For instance, application of R at the rate of 100 g, increased SEF from 101% and 107%, and 101% and 100% observed with the control treatment to 129% and 165%, and 114% and 116% for SB19 and SB24 genotypes in Trial I and II, respectively (Table 3). When P was applied at the highest rate of 30 Kg per ha, SEF increased from the control treatment to 135% and 157%, and 126% and 131% (Table 3). Compared to control

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Integration of R and P at the highest rate of 200 g and 30 Kg per ha, increased SEF by 106% and 154%, and 101 and 127% for SB19 and SB24 genotypes in Trial I and II, respectively (Table 2).

Table 3: Effect of Rhizobia and Phosphorus on Symbiotic Efficiency (%), Phosphorus (Kg/Kg) and Rhizobium (Kg/Kg)

| Variety | Trt  | SEF (%) | PUE (Kg/Kg) | SEF (%) | PUE (Kg/Kg) |
|---------|------|---------|-------------|---------|-------------|
|         |      |         | Trial I     |         | Trial II    |
|         | T1   | 101d    | 0d          | 107d    | 0d          |
|         | T2   | 108d    | 4.65c       | 119d    | 4.8c        |
|         | T3   | 135ed   | 3.9c        | 157ed   | 3.9c        |
|         | T4   | 129ed   | 0d          | 165ed   | 0d          |
|         | T5   | 153bc   | 8.15a       | 182bc   | 8.15b       |
|         | T6   | 176ab   | 6.49b       | 149bc   | 3.78c       |
|         | T7   | 130cd   | 0d          | 152cd   | 0d          |
|         | T8   | 167ab   | 8.6a        | 200ab   | 8.96a       |
|         | T9   | 207a    | 6.9a        | 261a    | 8.75a       |
| SB24    | T1   | 101d    | 0d          | 100d    | 0d          |
|         | T2   | 103d    | 4.2c        | 104d    | 4.3b        |
|         | T3   | 126cd   | 3.6c        | 131cd   | 3.6c        |
|         | T4   | 114cd   | 0d          | 116cd   | 0d          |
|         | T5   | 145bc   | 7.8a        | 155bc   | 3.8c        |
|         | T6   | 187ab   | 6.6bc       | 209bc   | 4.2b        |
|         | T7   | 119cd   | 0d          | 122cd   | 0d          |
|         | T8   | 179ab   | 8.9a        | 199ab   | 9a          |
|         | T9   | 201a    | 9.4a        | 227a    | 9.5a        |
| MSD     | 31   | 1.3     | 46          | 1.4     |
| CV (%)  | 25   | 58      | 31          | 59      |

*Means with the same letter along the column for the same variety are not significantly different at (p≤0.05); MSD=Mean Significant Difference; Treatments: T1 = Control (0 g R and 0 Kg P per ha); T2 and T3=20 Kg and 30 Kg P per ha respectively; T4 and T7=100 g R and 200 g R per ha respectively; T5=100 g R and 20 Kg P per ha, T6=100 g R and 30 Kg P per ha; T8 =200 g R and 20 Kg P per ha and T9 =200 g R and 30 Kg P per ha; PUE=Phosphorus Use Efficiency; SEF=Symbiosis efficiency

Integration Effect of Different Rates of R and P on Phosphorus Use Efficiency

There were no significant difference in phosphorus use efficiency (PUE) between genotype SB19 and SB24 within and between Trials I and II (p≤0.05). However, there were significant respond of the integration of R and P in PUE within genotypes SB19 and SB24 at (p≤0.05) in Trials I and II. When P was applied at the rate of 20 Kg per ha, the soybean grain yield obtained increased to 4.65 Kg/Kg and 4.8 Kg/Kg, and 4.2 Kg/Kg and 4.28 Kg/Kg for SB19 and SB24 soybean genotypes in Trial I and II, respectively (Table 3). Furthermore, when P was applied at the rate of 30 Kg per ha the soybean grain yields increased from the control treatment to 3.93 Kg/Kg and 3.9 Kg/Kg, and 3.58 Kg/Kg and 3.6 Kg/Kg for SB19 and SB24 genotypes in Trial I and II, respectively. Integration of R and P at the highest rate of 200 g and 30 Kg per ha increased grain yield from the control treatment to 6.9 Kg/Kg and 8.75 Kg/Kg, and 9.37 Kg/Kg and 9.48 Kg/Kg for SB19 and SB24 genotypes in Trial I and II, respectively (Table 3).

V. DISCUSSIONS

There was significant increase of shoot and grain N uptake, these probably, could be associated with R and P. Which enhanced N fixation, root length and root mass resulting to absorption of mineral nutrients from the soil, particularly available N. Similarly, significant increase in root nodules due to integration of R and P increased N2 fixation. This led to increase in N uptake which is in agreement with Bargaz et al. [21] who observed that, P plays a vital role in physiological and developmental process in plants. Similarly, significant increase in root nodulation due to integration of Rand P increased N2 fixation that led to increase in N uptake by shoot and grain of soybean.
Higher P content in shoot and grain was attributed to the root length of soybean. A long root system might have created a greater root-soil contact which increased P uptake hence high P content in shoot and grain. These findings are in agreement with Mathenge [11] who observed that a larger root system enhanced by P provided greater root-soil contact hence higher uptake. Furthermore, high shoot and grain P content can be attributed to the presence of R applied which enhanced the solubilization of the P in the soil increasing available P for the uptake. This is in agreement with Adjei-Nsiah et al. [1] who observed that rhizobium has the ability to solubilize P increasing P concentration in the soil. This concurs with Abbasi et al. [22] who reported that soybean grain/straw P uptake was increased with increased integration of P and R.

High SEF observed in control treatment in both Trials, probably, suggest that native R were active and effective, which consequently increased SDW. Further, the good performance of control, could be associated with native strain adaptation to the ecological conditions of the study area. These results are in agreement with the findings by Kawaka et al. [23] who reported SEF ranging between 67 and 170% when common beans were inoculated with native rhizobia. High performance of commercial R probably, could be associated with the commercial strains being more adapted to the study area compared to the native strains, leading to higher performance in SEF. This is contrary to Mungai and Karubiu [24] who observed native rhizobia isolated from common beans having higher SEF compared to commercial inoculants. Integration of R and P had the highest SEF, probably, this was associated with enhanced energy provision by P which improved the performance. This concurs with the findings by Bargaz et al.[21] who reported BNF having a high P energy demand. Low PUE where P was applied alone in the present study, probably, may be associated with P fixation in the soil (beyond scope of this study) making P less available to the plants. This concurs with findings by Fageria and Barbosa [25] and Singh et al.[26] who observed higher P fixation decreasing PUE in lentil and rice enterprises, respectively. Overall, the present study observed low soybean grain produced for every Kg of P applied for soybean genotype SB19 and SB24 in trial I and II, respectively. This economic production was below findings by Abbasi et al. [22] who reported higher soybean grain yield produced with each Kg of phosphorus applied.

VI. CONCLUSIONS AND RECOMMENDATIONS
This study demonstrated that integration of R and P at the rate of 200 g and 30 kg per ha was the optimum rate of application compared to other treatment levels in soybean performance. It was observed that symbiotic and phosphorus use efficiency was influenced by the R and P for SB19 and SB24 soybean genotypes. The two genotypes, SB19 and SB24 performed equally well. Farmers can adopt integration of R and P at the rate of 200 g and 30 kg per ha and either of the genotypes for sustainable soybean cultivation. Further research by use of other sources of P such as phosphorus solubilizing bacteria to enhance symbiotic and phosphorus use efficiency is recommended.

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