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Effects of P on *Vigna unguiculata* Cv. 305 and *Stylosanthes hamata* Cv. Verano Symbiosis in the Field of a Rubber-tree Plantation

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Abstract: In order to assess the effects of P addition and NP addition on nodulation and N fixation this study examines the nodulation of *Vigna unguiculata* cv. 305 (cowpea) and *Stylosanthes hamata* cv. Verano in fertilized and unfertilized conditions in NE Thailand. *V. unguiculata* cv. 305 and *S. hamata* cv. Verano were studied for their adaptation to low P on the top of the toposequence. The results showed that P addition not only increases above-ground biomass but also nodulation and N_2 fixation of *V. unguiculata* cv. 305 and *S. hamata* cv. Verano. Nitrogen fixation represented between 50 and 58% of total plant N with P applications. The soil organic carbon increased strongly the weight of the nodules for *V. unguiculata* cv. 305 when no fertilizer was added. Our results pointed out a positive correlation between N content inside the plant and nodule dry weight for *V. unguiculata* cv. 305. We noticed that an increase of 1 mg in nodule dry weight produced an increased in total plant N at flowering stage of 1.29 mg plant^{-1} in the control and of 1.48 mg plant^{-1} in the PK treatment. Regarding *S. hamata* cv. Verano, an increase of 1 mg in nodule dry weight at 183 days after sowing (DAS) produced an increase in N content of 3.18 mg plant^{-1}.

Key words: Effects of P, rubber plantation, *S. hamata*, symbiosis, *V. unguiculata*

INTRODUCTION

Cowpeas are an important source of protein, as they contain an average of 23% protein, 60% starch and 2% oil (Aykroyd and Doughty, 1964). Besides the human consumption of cowpea seeds, the whole plant is used as a livestock food and for soil improvement (Fageria *et al*., 1997). *Stylosanthes hamata* also is used as a lay in cropping systems, with nitrogen benefits up to 90 kg ha^{-1} recorded in West Africa and northern Australia under experimental conditions (Skerman and Riveros, 1977).

Tropical and subtropical soils are P deficient. The sandy soils of NE Thailand, developed on blown sands, are especially P deficient (Suriya-Arunroj *et al*., 2000). The limitation of symbiotic nitrogen fixation (BNF) by environmental constraints, especially phosphorus deficiency, restricts the extension of the legume cultivation and the development of a sustainable agriculture. Furthermore, P deficiency is more likely to affect N_2-dependent legumes than other species because symbiotic nitrogen fixation is an energetic process which requires more inorganic P than mineral nitrogen assimilation (Ribet and Drevon, 1995).

N and P deficiencies are limiting factor for the production of beans in many part of the world. The objectives of this study were i) to characterize the effects of soil P on the growth and nodulation of *V. unguiculata* cv. 305 (cowpea) and *S. hamata* cv. Verano, ii) to test the use of the weight of the nodules to estimate N fixation.

MATERIALS AND METHODS

Site characteristics: The experiment was conducted within a 112 hectares catchment located at Ban Non Tun, 30 km from Khon Kaen (16°19'43.90" N, 102°45'07.91" E; (Hammecker *et al*., 2006). Northeast Thailand is characterized by a semi-arid tropical climate with a distinct rainy season from mid May to mid October and a dry season from mid October to mid May. The mean annual rainfall for Khon Kaen was 1208 mm for the period 1961-1990.

Experimental design: A completely randomized block design with five replications was used. Each plot had an area of 5.76 m^2. Seeds of *V. unguiculata* cv. 305 and *Stylosanthes hamata* cv. Verano were sampled to measure

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their average weight and to determine their N and P content before the start of the experiment. The seeds were then inoculated with *Bradyrhizobium sp.* (*V. unguiculata*) strain TAL 169+NC 92 which is the *Bradyrhizobium* selected for Thailand.

They were sown at the end of May and the experiment was carried until mid December. *V. unguiculata* cv. 305 was grown twice and *S. hamata* cv. Verano was cut 10 cm above soil surface every three months during the rainy season. Potassium nitrate and TSP were applied at emergence (Table 1). For the NP treatment, urea was applied as follows: 20 kg N ha^{-1} at emergence, 50 kg N ha^{-1} at the beginning of flowering and 50 kg N ha^{-1} as urea at grain filling. PK treatment was a bare plot with 30 kg N ha^{-1} as potassium nitrate and 90 kg P ha^{-1} triple super phosphate (TSP) at emergence of the seedlings on the sown plots.

Biomass measurements: At flowering for *V. unguiculata* cv. 305, 10 plants per plot were randomly selected in the control and the P treatment. For each plant the above-ground part was cut and the roots were carefully removed from the soil. For each cut for *S. hamata* cv. Verano a pit was dug to 30 cm to collect the roots. The number of nodules was counted. The plant samples were dried at 70°C for 48 h before the above-ground biomass, the weight of the roots and the weight of the nodules were determined.

Nitrogen measurements: For each sample of above-ground biomass N content was measured by micro-Kjeldahl with indophenol blue. The above ground biomass of each plot was bulked to obtain a representative sample (2 species ×2 treatments ×5 replicates = 20 samples). N and P were determined for each of these samples. The roots of each plot were bulked and N and P were determined on the bulked samples (2 species ×2 treatments ×5 replicates = 20 samples). For the control and the P treatment, the grains and the rest of the plant were analyzed to determine N and P content (2 species ×2 treatments ×5 replicates = 20 samples). The p-value = 0.05 is considered to be significant.

RESULTS

Effect of P fertilization on the growth of *V. unguiculata* and *S. hamata*: To assess the effect of P on the symbioses, we compared the control with the P and NP treatments for *V. unguiculata* cv. 305 and *S. hamata* cv. Verano (Fig. 1). For *V. unguiculata* cv. 305, the data in Fig. 1 show that P addition resulted in a significantly better shoot growth at flowering for crops 1 and 2 and at harvest for crop 2 (20.1, 2.1 and 1.1 g $plant^{-1}$ against 12, 1.2 and 0.7 g $plant^{-1}$, respectively). By contrast, seed weight and root growth were not significantly increased by P addition, even though the values for P were consistently slightly higher. N and P addition (NP) resulted in a significant increase compared to the control in the above-ground biomass for crop 2 (2.3 against 1.4 g $plant^{-1}$). N and P addition (NP) did not significantly improve any biomass parameter compared to P addition (P). The differences in growth between crop 1 and crop 2 was due to water saturation of the soil during the rainy season that has induced a decrease in soil oxygen for crop 2. In addition to the impact on the respiration of the roots and of the nodules, this may have also caused a loss of nitrate from the soil by denitrification which can result in nitrogen deficiency.

For *S. hamata* cv. Verano, the data in Fig. 2 show that P addition resulted in higher values of shoot and root growth than the control for all the DAS, but the difference was significant only at 158 DAS (7.1 against 4.9 g $plant^{-1}$). By contrast, N and P addition (NP) at 92 and 183 DAS (12.9 and 8.4 g $plant^{-1}$, respectively) increased significantly the shoot growth compared to control treatment (9.8 and 6.8 g $plant^{-1}$, respectively). The NP treatment did not improve significantly plant growth, compared to the P treatment. At 92 DAS the plant was harvested and cut 10 cm above the soil surface. This explains the lower shoot biomass at 158 DAS than at 92 DAS.

Effect of P fertilization on nodulation: For *V. unguiculata* cv. 305, Fig. 3 shows the nodule weight, the number of nodules and the average weight of the nodules at different growth stages for the two crops. The average weight of the nodules (specific NDW) was calculated by dividing the nodule weight by the number of nodules. P addition resulted in a significant increase in nodule weight for all the stages of the two crops. This higher weight was the result of an increase in the number of nodules (significant increase in most cases) rather than an increase in the average weight of the nodules, which was relatively stable (between 5 and 8 mg $nodule^{-1}$). N and P addition (NP treatment) resulted in a weight of nodules not

Table 1: Treatments studied at Ban Non Tun

Treatment	Potassium nitrate (KNO_3)	Triple super phosphate (TSP)	Urea	Plants
Control	30 kg N ha^{-1} 84 kg K ha^{-1}	-	-	Vigna/Stylo
P	30 kg N ha^{-1} 84 kg K ha^{-1}	90 kg P ha^{-1}	-	Vigna/Stylo
NP	30 kg N ha^{-1} 84 kg K ha^{-1}	90 kg P ha^{-1}	120 kg N ha^{-1}	Vigna/Stylo

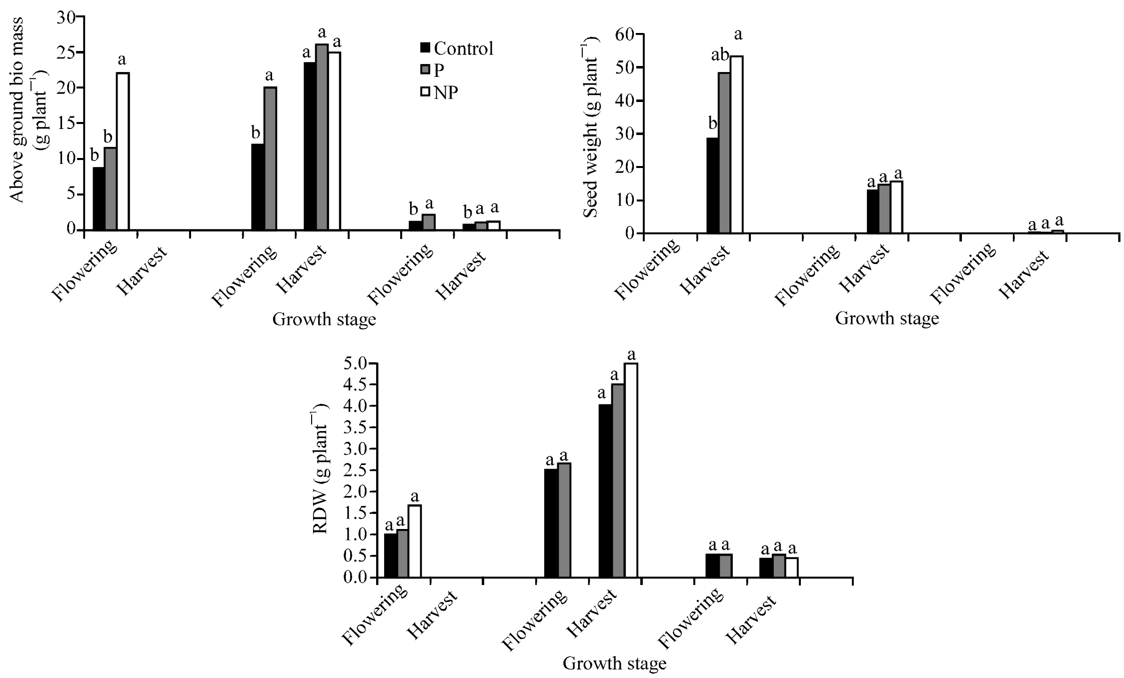

Fig. 1: Above-ground and below-ground biomass of *Vigna unguiculata* cv. 305 in the control, P and NP treatment at different growth stages for the three crops. Bars with different letters show significant effect at $p = 0.05$

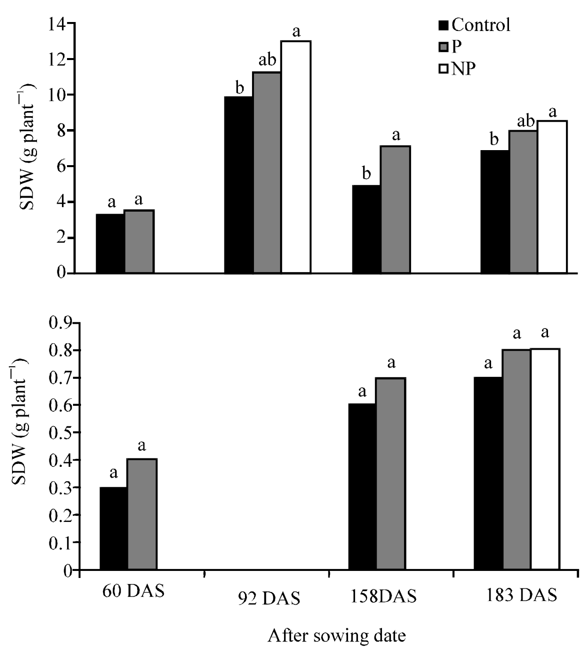

Fig. 2: Shoot and root dry weight of *Stylosanthes hamata* cv. Verano in the control P and NP treatment at different days after sowing (DAS). Bars with different letters show significant effect at $p = 0.05$

significantly different from the control and significantly lower than P addition (P). The lower nodule weight in the NP treatment than in the P treatment was not surprising, as is well known that N fertilizer inhibits nodulation. The decrease in nodule weight resulted mainly from a smaller number of nodules and from a decrease in the average weight of the nodules to a smaller extends (only significant at harvest stage for crop 1).

Figure 4 shows the relationship between shoot growth at flowering stage and nodule weight for *V. unguiculata* cv. 305 in the control and P treatment for crop 1. There was no correlation between shoot growth and nodule weight in each treatment. We then merged the data of the control and P treatment, which revealed a correlation between shoot growth and nodule weight. With an increase of 1 mg in nodule weight, shoot growth at flowering stage increased by 34 mg. There was no correlation between shoot growth and nodule weight at harvest stage for *V. unguiculata* for crop 1, whatever the treatment.

For *S. hamata* cv. Verano did not nodulate before 60 DAS and the nodules were not studied 92 DAS. P addition resulted in a significantly higher nodule weight only at 158 DAS and this increase was due to a higher number of nodules. P and N addition did not result in any change in nodule weight, the number of nodules nor the average weight of the nodules 183 DAS. This means that the application of N fertilizer to perennial legumes does not inhibit nodulation in contrast with annual legumes. Indeed, annual legumes produce nodules in the first 30 cm under soil surface whereas perennial legumes

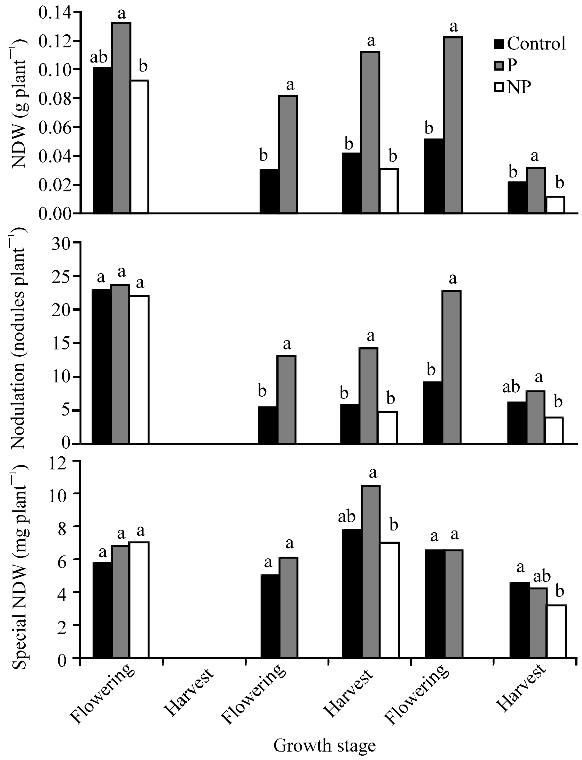

Fig. 3: Nodulation of *Vigna unguiculata* cv. 305 in the control, P and NP treatment at different growth stages for the three crops. Bars with different letters show significant effect p = 0.05

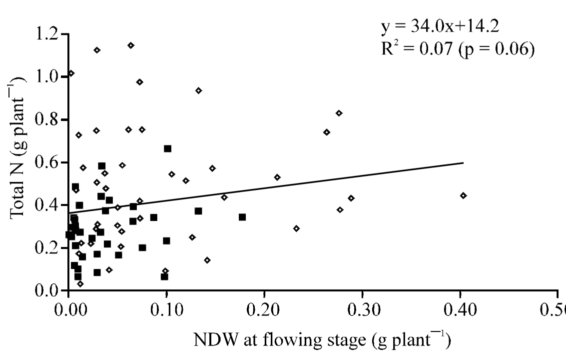

Fig. 4: Correlation between shoot growth and nodule weight at flowering stage for *Vigna unguiculata* cv. 305 in the control and P treatment at Ban Non Tun (crop 1)

such as *S. hamata* cv. Verano can produce nodules as deep as 100 cm below soil surface then N application may not affect nodulation below 30 cm. Compared to *V. unguiculata*, the nodules of S. hamata were much smaller (around 0.8 mg against 5 to 8 mg) and more numerous (30 to 60 against 5 to 25) (Fig. 5).

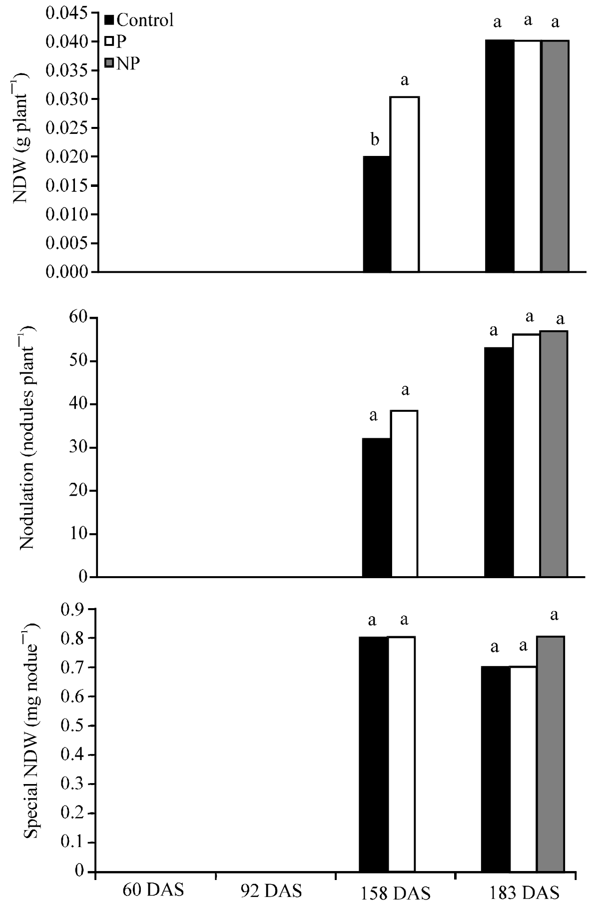

Fig. 5: Nodulation of *Stylosanthes hamata* cv. Verano in control, P and NP treatment at different days after sowing (DAS). Bars with different letters show significant effect at p = 0.05

Effect of P fertilization on plant N content and relation with nodulation: For *V. unguiculata* cv. 305, Fig. 6 shows total plant N at different growth stages for the three crops. P addition increased consistently total N in the plant. However, the difference between the control and the P treatment was significant only for the shoot weight at flowering and harvest in the first crop (0.5 and 0.68 g plant^{-1} against 0.3 and 0.55 g plant^{-1}, respectively). For *S. hamata* cv. Verano, Fig. 7 shows total plant N at 60, 92, 158 and 183 DAS. The addition of P increased consistently total N in the plant and the difference between P and the control was significant 183 DAS.

Figure 8 shows the relationship between total plant N at flowering and the weight of nodules of *V. unguiculata* cv. 305 in the control and P treatment for crop 1. As there was no correlation between shoot growth and nodule weight in each treatment the correlation was studied on all the data together. There was then a

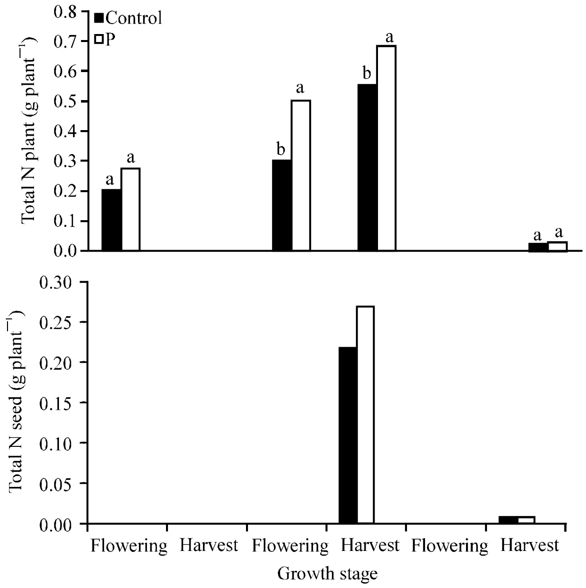

Fig. 6: Total plant N for *Vigna unguiculata* cv. 305 in the control and P treatment at different growth stages for the two crops. Bars with different letters show significant different at p = 0.05

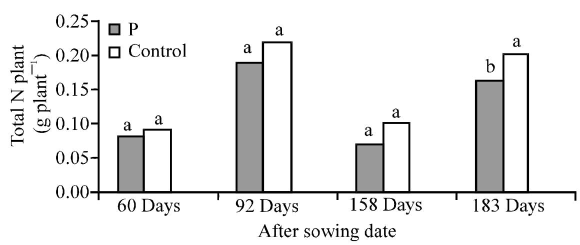

Fig. 7: Total plant N for *Stylosanthes hamata* cv. Verano in the control and P treatment at different days after sowing (DAS). Bars with different letters show significant different at p = 0.05

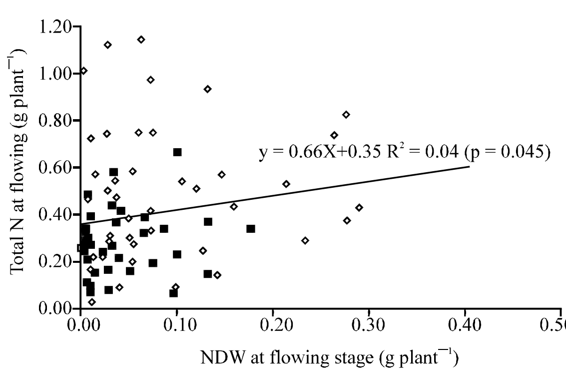

Fig. 8: Correlation between total plant N and nodule weight at flowering stage for *Vigna unguiculata* cv. 305 in the control and P treatment at Ban Non Tun (crop 1)

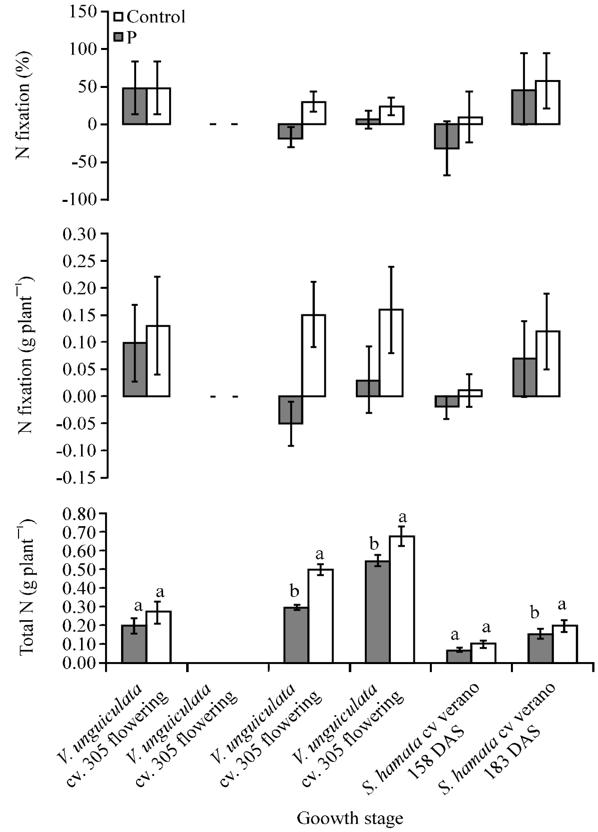

Fig. 9: N content, N fixation and N fixation (%) for *Vigna unguiculata* cv. 305 and *Stylosanthes hamata* cv. Verano in the control and P treatment at different growth stages in Ban Non Tun. Error bars are standard errors. Bars with different letters show significant different at p = 0.05

correlation between total plant N and the weight of the nodules. Every increase of 1 mg in nodule weight increases of total plant N at flowering by 0.66 g.

N fixation can be estimated from the correlation between total plant N and nodule weight. Indeed, the intersection of the correlation line with the y axis gives the quantity of N that originates from the soil (NDW = 0). Knowing the quantity of N originating from the soil, N fixation was calculated as the difference between total plant N and N originating from the soil. Figure 9 shows total N, N fixation and percent of N fixation of *V. unguiculata* cv. 305 and *S. hamata* cv. Verano at different growth stages. The addition of P increased significantly total plant N for *V. unguiculata* cv. 305 at flowering and harvest stage in crop 2 and for *S. hamata* cv. Verano at 183 DAS. In the best cases (*V. unguiculata* at flowering for crop 1 and *Stylosanthes* at 183 DAS), N fixation represented between 50 and 58% of total plant N. The proportion of N fixed varied for one year to another

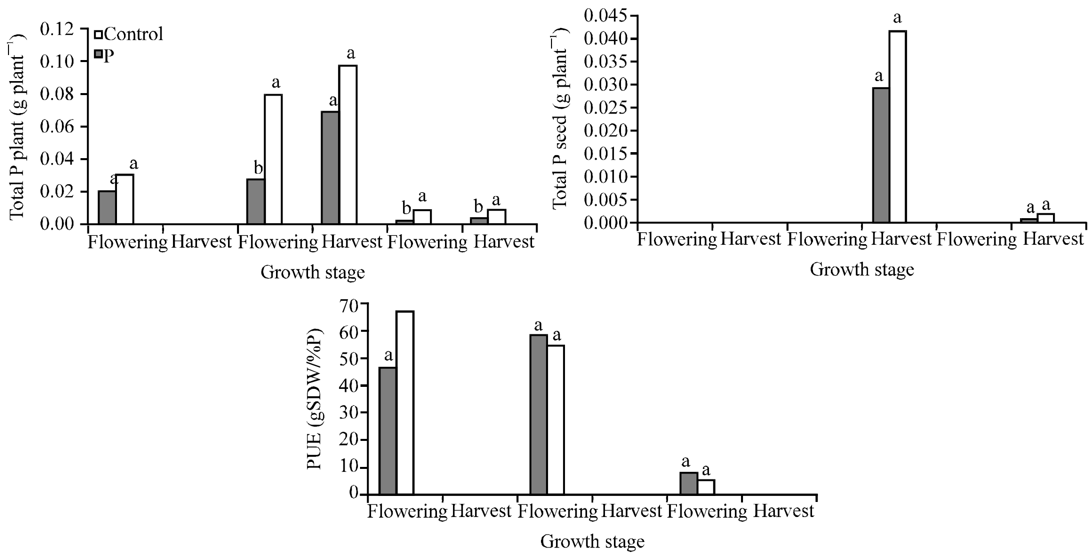

Fig. 10: Total P plant and PUE for *Vigna unguiculata* cv. 305 in the control and P treatment at different growth stages in Ban Non Tun. Bars with different letters show significant different at p = 0.05

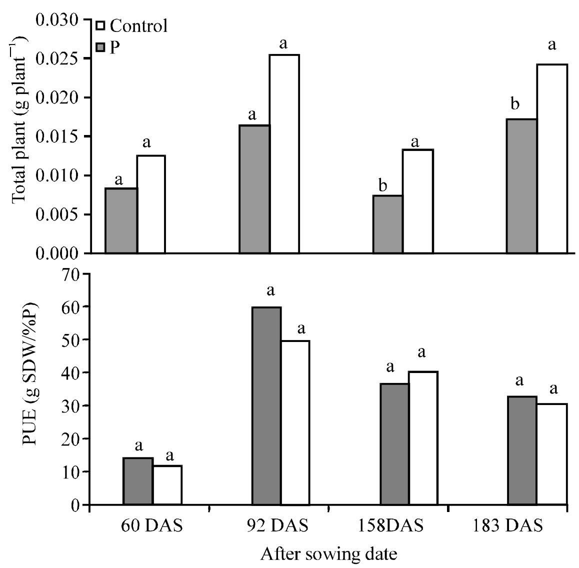

Fig. 11: Total P plant and PUE for *Stylosanthes hamata* cv. Verano in the control and P treatment at different growth stages in Ban Non Tun. Bars with different letters show significant at $p \geq 0.05$

for *V. unguiculata* cv. 305 at flowering, crop 1 showed a higher N fixation than crop 2 (49 against 30%).

Effect of P fertilization on plant P of *V. unguiculata* and *S. Hamata*: Figure 10 shows total plant P and PUE for *V. unguiculata* cv. 305. P addition increased significantly total plant P in crop 1 at flowering (79 against 27 mg plant^{-1}). P addition increased also significantly total plant P for crop 2 both at flowering and harvest (8 and 8 mg plant^{-1} for P against 2 and 3 mg plant^{-1} for the control, respectively). There were no significant differences in total seed P at harvest stage and PUE at flowering stage between the two treatments.

The addition of P increased significantly total plant P of *S. hamata* cv. Verano at 158 and 183 DAS (13 and 24 mg plant^{-1} for P against 7 and 17 mg plant^{-1} for the control, respectively). PUE was not significantly different between the treatments for *S. hamata* cv. Verano, whatever the date (Fig. 11).

DISCUSSION

In this study, we compared the control with the P and NP treatments. P applications resulted in an increase in the number of nodules for both *V. unguiculata* cv. 305 and *S. hamata* cv. Verano (Fig. 3 and 4) which is consistent with results obtained by Cassman *et al.* (1993) who also showed that application of P increased the number of nodules of soybean grown in heavily weathered acid soils. Moreover, following P addition, we noticed an increase in shoot growth (Fig. 1 and 2). These results are supported by Israel (1987, 1993) who noticed that P addition had specific roles in nodule initiation, growth and functioning in addition to its effects on host plant growth processes. He pointed out that P fertilization was especially important for enhancing nodulation and dry matter production of soybean plants. In his trials, the stimulating effect of P on nodule growth and function ultimately resulted in the improve growth of shoots of soybean plants and presumably also in yield. Regarding our experiments, P fertilization also increased total plant P (Fig. 9 and 10) in concordance with previous studies which had proved that P application increased P concentrations in some *Stylosanthes* species (*Stylosanthes scabra* cv. Scca, *Stylosanthes guiunensis* cv. Schofield and *Stylosanthes viscosa* CPI 34904) (Gilbert *et al*., 1989).

In addition, we measured higher values for the total N content in plants and seeds for *V. unguiculata* cv. 305 and total N content in plants for *S. hamata* cv. Verano after P fertilization (Fig. 5 and 6). These results are supported by previous studies which showed that P applications also influenced the contents of other nutrients in cowpea leaves (Kang and Nangju, 1983) and seed (Omueti and Oyenuga, 1970).

More particularly, P addition resulted in a correlation between growth, nodule weight and total N which was used as an estimate of the efficiency in fixing N. Indeed, Muleba and Ezumah (1985) highlighted that P had multiple effects on nutrition and nitrogen fixation. Luse *et al*. (1975) as well as Kang and Nangju (1983) noticed that P fertilizer increased nodulation and N fixation. Our experiments therefore suggest that under deficient conditions, P fertilization results in an enhanced nodule number and mass and greater N_2 fixation per plant and per gram of nodules.

CONCLUSION

P addition not only increased the above-ground biomass but also the nodulation and N_2 fixation of *V. unguiculata* cv. 305 and *S. hamata* cv. Verano. The addition of nitrogen fertilizer did not increase the biomass produced and had a negative effect on nodulation. P applications increased the number of nodules and the overall dry weight of nodules but no significant increase in the weight of single nodules was recorded. In this study, N fixation was deduced from the correlation between total plant N and nodule dry weight. Under P fertilization, an increase in the nodule dry weight of 1 mg resulted in increasing the total plant N by 3.18 mg at 183 DAS in *S. hamata* cv. Verano and by 1.48 mg at flowering stage for *V. unguiculata* cv. 305. N fixation was therefore increased by P applications and provided between 50 and 58% of total plant N. The correlation between total plant N and nodule dry weight seems thus a promising method to estimate N fixation in further researches on the effectiveness of N fixation in nodulated plants.

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