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Research article

Effects of Nitrogen and Phosphorus Application on Growth and Root Nodules of Mungbean under Sandy Soil Conditions

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Abstract

Keywords

nitrogen;
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root nodules;
mung bean;
sandy soil

Legume production under infertile sandy soil may be limited by low nitrogen (N) and phosphorus (P) availability in the soil. A study was conducted to assess the effects of N and P on growth, yield, and root nodules of mung bean under sandy soil conditions. Two pot experiments were conducted under a greenhouse environment and were laid out in a completely randomized design (CRD) with five replications. Experiment 1 consisted of two types of soils (sandy and loamy sand) and two P application rates at 0 and 13 mg P kg^{-1}. Experiment 2 consisted of 10 fertilizer treatments that included two N treatments of 0 and 53 mg N kg^{-1} in combination with four P treatments of 0, 17, 34, 68 mg P kg^{-1}, P only at 17 mg P kg^{-1}, and a cattle manure that was applied at a rate of 5 t ha^{-1} which was equivalent to 52:07:56 (N:P:K mg kg^{-1}). The results showed that crop growth and root nodules improved with P application in the loamy sand soil (containing medium P), but not in the sandy soil (containing low P). Nitrogen application also improved the crop growth and grain yield significantly in loamy sand soil. However, N application suppressed root nodule formation, an effect that was significant for the N_2 fixation of the crop. Increase in the P rate did not alleviate the inhibitory effect of N on nodule formation. Since N and P fertilizer had opposite effects on root nodules, interactions between N and P need to be further investigated when considering the economic benefits of using these fertilizers.

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1. Introduction

Sandy soils are considered infertile, and this infertility is a major constraint on crop production [1, 2]. Specifically, the soils often have levels of nitrogen (N) and phosphorus (P) [3] that are too low for crop growth, resulting in significant yield reduction in crops, particularly in legumes [4, 5]. Diversified cropping systems that include legumes are of interest for improving soil fertility [6]. Mung bean is a potential legume crop that can be adopted into a crop production system because of its ability to fix N, increase N availability, and provide N for the subsequent crops. The bean appears to be a best-fit as it has a short-duration of cultivation and offers better market-returns than other legumes. Therefore, it is considered to be a suitable post-rice crop in the lowland paddy fields with supplementary irrigation [7, 8].

Low nutrient availability in the soils is a major constraint for legume establishment in Cambodia's sand-dominant soils [9, 10]. Insufficient P for optimum biological nitrogen fixation and yield reduction in legumes is common in most agricultural soil [11]. About 90% of soils in Cambodia have low available P (<7 mg kg^{-1}) [9]. Sufficient level of plant available-P is a vital factor for legume performance [12, 13]. The P-deficient Cambodian sandy soils [9] may also be limiting for leguminous N_2 fixation [13, 14]. Phosphorus is important for nodule development in legume crops [11]. P has been reported to increase the yield components and grain yield of mung bean [15-17]. The low N in rainfed sandy soils [9] may also limit mung bean performance. The positive response of growth and yield to N application was reported. However, N application can affect legume nodulation by reducing the number of nodules [11] and N_2 fixation [18].

The objectives of these experiments were: 1) to determine the effects of fertilizer (N and P) and their interaction on the grain yield, crop performance, and root nodules of mung bean, and 2) to confirm whether P is a limiting nutrient for lowland-rainfed soil.

2. Materials and Methods

2.1 Experimental conditions

The experiments were conducted under greenhouse conditions at the Royal University of Agriculture (RUA), Cambodia from February, 2020 to April, 2020. The daily temperature and relative humidity during the experiments are shown in Figure 1. The data were measured using a simple thermometer. The daily temperature and relative humidity were calculated as an average of three measurements per day (at 8 am, 12 pm, and 8pm).

2.2 Soil characteristics

Sandy soil and loamy sand used in this study were taken at 0-20 cm depth from a farmer's field (11º4'34"N, 184º32'14"E) and research station (11º30'47"N, 104º54'2"E), respectively. The soils were then air-dried and homogenously mixed in each soil type. The characteristics of each soil type are shown in Table 1.

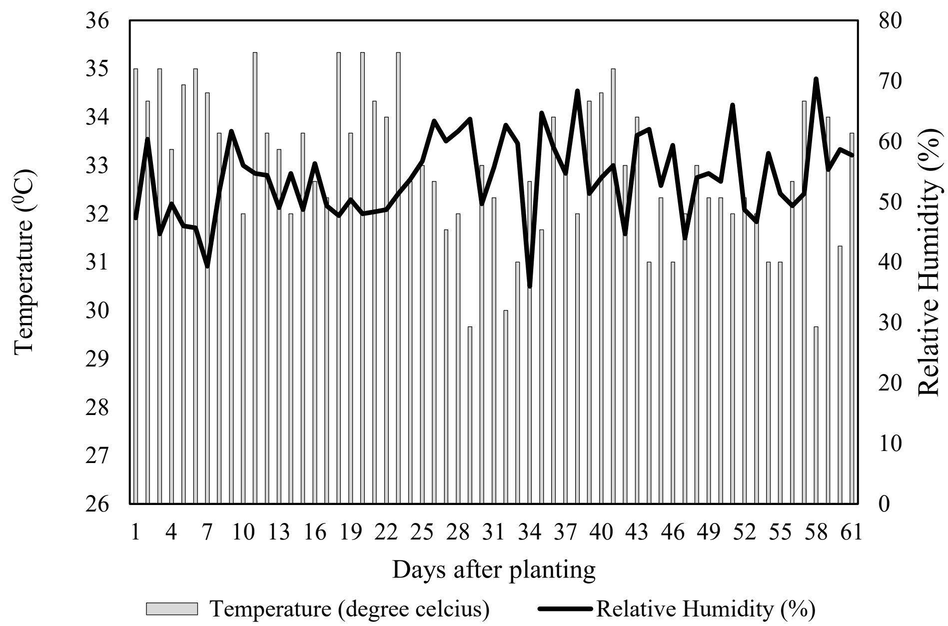

Figure 1. Temperature and relative humidity in the greenhouse during growing period

Table 1. Soil characteristics of the experimental soils

Soil characteristics	Sandy soil	Loamy sand
Preceding crop	**Rice**	**Rice**
pH (1:5 of soil:water)	5.5	6.12
CEC (Ammonium acetate pH 7.0)	0.30 $cmol_c$/kg	3.72 $cmol_c$/kg
Total N (Kjeldahl digestion)	0.019 %	0.067 %
Total Organic C (Black and Walkey)	0.226 %	0.729 %
Available P (Olsen method)	4.23 mg kg^{-1}	12.73 mg kg^{-1}
Available K	0.07 mg kg^{-1}	0.30 mg kg^{-1}
Sand	91.24 %	79.65 %
Silt	3.88 %	6.93 %
Clay	4.88 %	13.42 %

2.3 Experiment setup

2.3.1 Experiment 1: Effect of P application on P-deficit sandy soil

The experiment consisted of four treatments with two types of soils (sandy and loamy sand) and two P application rates at 0 and 10 kg P ha^{-1} (equal to 0 and 13 mg P kg^{-1}) on a plant density basis. The P was applied on the top and incorporated to a depth of 5 cm to simulate the field placement of fertilizer. The characteristics of the two soils are mentioned in Table 1. The fertilizers N and K were applied at the recommended rates of 40 and 25 kg K ha^{-1}, respectively, which were equivalent to 18 mg N and 11 mg K kg^{-1} on a soil weight basis.

2.3.2 Experiment 2: Crop performance responses to nitrogen and phosphorus application

A loamy sand was used in this experiment, and soil characteristics are described in Table 1. The experiment consisted of eight fertilizer treatments including two N treatments of 0 and 40 kg N ha^{-1} (53 mg N kg^{-1}) in combination with four P treatments of 0, 13, 26, 52 kg P ha^{-1} (equal to 0, 17, 34, 68 mg P kg^{-1}; respectively). It is noted that the rate at 40:26:25 (N:P:K kg ha^{-1}) was a common rate for typical mung bean crop grown on sandy soils in Cambodia. Both experiments were laid out in CRD and were replicated with five pots per treatment.

2.4 Fertilizer application

Urea, triple super phosphate (TSP) and muriate of potash (KCl) were applied as the nitrogen, phosphorus and potassium sources, respectively. The fertilizer rates were adjusted to the equivalent amount per pot. All fertilizers were applied pre-plant and were based on plant density calculation (Table 2).

Planting spacing was 20 x 20 cm, and the total number of plants per ha was calculated to be 250000 plants. Thus, the amount of fertilizer needed to be applied per kg of soil was the division of fertilizer used in a hectare to the number of plants per hectare. The amount of N:P:K needed per 1 kg of soil was used to calculate the amount of fertilizer need per pot (3 kg of soil per pot). Fertilizers were broadcast applied and incorporated into the soil to a depth of 5 cm to simulate the farmer practices (plow flowing fertilizer application and before planting).

Table 2. Fertilizer (N:P:K) applied in each treatment

Treatment	N:P:K (kg ha^{-1})	N^1:P^1:K^2 (mg kg^{-1}soil)
Urea 0+TSP 0	00:00:25	00:00:11
Urea 0+TSP 13	00:13:25	00:17:11
Urea 0+TSP 26	00:26:25	00:34:11
Urea 0+TSP 52	00:52:25	00:68:11
Urea 40+TSP 0	40:00:25	53:00:11
Urea 40+TSP 13	40:13:25	53:17:11
Urea 40+TSP 26	40:26:25	53:34:11
Urea 40+TSP 52	40:52:25	53:68:11

[1] calculated by plant density basis
[2] calculated by soil weight basis
[3] containing N (0.78%), P (0.09%) and K (0.83%)

2.5 Planting and crop management

Irrigation water was applied daily to maintain a soil moisture level of 80% of field capacity based on visual observation, and if needed, the plants were watered twice per day.

Plastic rounded-pots (20 cm diameter X 17 cm height) were filled with 3 kg of soil (air-dried and sieved with a 2 mm sieve) to approximate 11 cm of soil depth. The soil was seeded with 2 seeds. After two weeks of seedling growth, one seedling was thinned to keep 1 healthy plant per pot.

2.6 Measurement and calculation

Grain yield and 100-grain weight*:* The grain yield was harvested from the five replicated pots to find the average grain yield per plant. Grain yield was adjusted to a standard 12% grain moisture content. The 100-grain weight measurement was determined by randomly selecting a subsample from the total harvested kernels.

Following harvesting, roots were carefully removed from the pots and washed with clean water with a sieve underneath to catch falling nodules. All root nodules were hand counted regardless of size and were averaged from the five replicated pots for each treatment.

Harvest index (HI) was calculated using the following formula:

$$\text{HI: } \frac{Grain\ yield\ per\ plant}{Grain\ yield\ per\ plant + plant\ biomass\ per\ plant}$$

where the plant biomass was the whole plant after removing pods for grain collection and counting of the root nodules. The samples were oven-dried at 70^0 C for 48 h and weighed.

2.7 Statistical analysis

All the data were analyzed using ANOVA (Analyses of Variance) and mean comparison tests were performed using Fisher's Least Significant Difference (LSD) with Statistix 8 (Version 8.0, Analytical Software, 1985-2003).

3. Results and Discussion

3.1 Phosphorus application on the performance of mung bean

The interactions between soil types and P application were significant, except 100 grain weight (Table 3). Application of 13 mg P kg^{-1} did not significantly affect the crop performance, grain yield and nodule number in the sandy soil treatment. In contrast, amendment with P significantly increased pod number but not 100 grain weight for the loamy sand treatment. The nodule count was significantly greater with P amendment in the loamy sand treatment.

Table 3. Effect of phosphorus on crop growth and nodulation

Soil types	P (mg kg^{-1})	100 grain weight (g)	Grain yield (g pot^{-1})	Harvest index (HI)	Nodules count $plant^{-1}$
Sandy	0	5.04	2.86^a	0.28^a	96^b
	13	5.30	2.63^a	0.26^a	100^{ab}
Loamy sand	0	4.98	1.33^c	0.14^b	78^b
	13	4.82	1.71^b	0.18^b	192^a
Soil types		ns	**	**	ns
P		ns	ns	ns	*
Soil types X P		ns	**	*	*

The same letters in each column represent non-significant differences among treatments at $P < 0.05$ by Tukey HSD's test. ** and * denote significant difference at $P < 0.01$ and $0.01 < P < 0.05$, respectively.

This experiment was conducted to determine if residual soil-P levels were a limiting factor for mung bean establishment under loamy sand soils. Application of P at 13 mg kg^{-1} significantly improved the crop performance and nodulation in the loamy sand soil used in this study. The initial P (Olsen method) of the sandy soil was 4.23 mg kg^{-1} (Table 1), which was rated as "low" [19] and also below the critical value for mung bean (7 mg kg^{-1}) [20]. The loamy sand soil had a higher Olsen-P (12.73 mg kg^{-1}) and was classified as "medium" which was above the critical value for mung bean [20]. The current rates of P application may not be sufficient to enhance the performance, yield and nodulation of mung bean under sandy soils. This may explain why mung bean showed no increase in growth and root nodules with P application for the sandy soil treatments. As nodulation needs energy for its development process, P is needed to generate the energy for fixation processes [21].

Phosphorus is an important constituent of ATP and plays an important role in energy transformation. The response of some yield components, grain yield and nodules due to P application were reported [15, 22]. Several authors also reported an improvement of legume nodulation with P amendment [21, 23]. In the meta-analysis by [24], pooled data from multiple studies showed the reduction of both nodule growth and number with P deficiency. This result implies that the low residual P levels in Cambodia's rainfed, lowland sandy soils is a crop limiting factor.

3.2 Effect of fertilization on mung bean performance

Application of N significantly increased grain yield and 100-grain weight, compared to non-N application (Table 4). Without N, the effect of increasing P rates (0-68 mg kg^{-1}) showed an increase in 100-grain weight. With N, there was no clear significant difference among different P rates for 100 grain weight, but P application had significant interaction with N fertilizer on grain yield. For the 0 kg N ha^{-1} treatments, increasing P rates tended to increase the grain yield. However, for the 40 kg N ha^{-1} (equivalent to 53 mg N kg^{-1}) treatments, maximum P fertilizer application at 68 mg P kg^{-1} did not produce higher grain yield over the 0-N application. However, application of 17 and 34 mg P kg^{-1} increased the grain yield compared to non-P amendment. In the absence of N, application of P increased the root nodules, compared to non-P application. However, application N fertilizer reduced the number of nodules significantly. Increasing P did not increase the root nodules under N-amendment

Nitrogen application increased the grain yield significantly. Nitrogen fertilization also improved harvest index, but reduced the number of nodules. The increase of growth and grain yield from N and P fertilizer additions was also reported by Jamro *et al.* [25]. Higher 100-grain weight and grain yield of mung bean with N application was reported by Yin *et al.* [26]. The increase in 100-grain weight with N application showed the need for N for seed filling. Omran *et al.* [27] reported the optimum grain yield of mung bean was at 40 kg N ha^{-1} and 60 kg P_2O_5 ha^{-1}. As the initial soil N was poor, the response to N was high. Even though legumes can obtain some or most of their needed N through symbiotic N fixation, supplemental N can increase their growth and yield [28]. These results show the importance of N for seed formation of legumes, leading to higher grain yield. In this study, mineral N reduced the number of nodules. In general, supplemental-N reduced nodule counts in many legume crops [20]. Additional N also decreased the number of nodules per plant. The inhibitory effect on legume root nodulation by N application has been well-documented in multiple studies [21, 29-31].

The effect of N on mung bean growth and yield was associated with application of P. Phosphorus fertilizer treatments with O-N showed that harvest index did not respond to increasing P rates, but 100-grain weight did increase. The variation of grain yield caused by P rates when no N

Table 4. Effects of N and P on mung bean growth and nodulation

N (mg kg^{-1})	P (mg kg^{-1})	100 grain weight (g)	Grain yield (g pot^{-1})	Harvest index (HI)	Nodule count per plant
00	00	4.38^{c}	2.14^{h}	0.28^{bc}	15^{b}
	17	4.56^{bc}	2.37^{g}	0.25^{bc}	28^{a}
	34	5.07^{ab}	2.94^{f}	0.29^{abc}	30^{a}
	68	4.98^{abc}	3.72^{e}	0.34^{ab}	25^{a}
53	00	5.08^{ab}	3.97^{c}	0.32^{ab}	16^{b}
	17	5.14^{ab}	4.85^{b}	0.39^{ab}	13^{b}
	34	5.35^{a}	4.76^{a}	0.42^{a}	7^{c}
	68	5.09^{ab}	2.74^{d}	0.26^{c}	7^{c}
Overall sig.		**	**	ns	*
N		**	**	ns	**
P		**	**	ns	**
N X P		ns	**	**	**

The same letters in each column represent non-significant differences among treatments at $P < 0.05$ by Tukey HSD's test. ** and * denote significant difference at $P < 0.01$ and $0.01 < P < 0.05$, respectively

fertilizer was applied was likely a function of 100-grain weight. An increase of 1000-grain weight by P application was reported by Ali *et al.* [32]. It is not clear whether increasing P beyond 53 mg kg^{-1} would continue to increase grain yield. In treatments that included N addition, the application of P did not benefit the grain yield, and for the highest P application treatment, grain yields were reduced. Similarly, rising P application reduced some of grain yield parameters and grain yield when high N (50 kg ha^{-1}) was applied [33].

The effect of N-urea on legume nodulation is poorly documented. The previous findings reported that the reduction of nodulation and N_2 fixation were caused by nitrate [30] rather than urea-N. Studies have shown that either NO_3^- or NH_4^+ can suppress both nodulation and symbiotic N_2 fixation. Low N application has been shown to enhance nodule formation, nitrogenase activity and plant growth of common bean [34]. Tsai *et al.* [35] further reported that high N rates stimulated N_2 fixation if no other nutrients were limited. Elahi *et al.* [36] also reported stimulation of mung bean nodule development under low nitrate, but with higher nitrate the nodules decreased.

In this study, N from urea inhibited nodulation and N_2 fixation. The relationship between nodulation and N source is complex and involves many mechanisms, but the most plausible explanation is that nodule surface absorbs most of the nitrate with only a small amount being transported to the upper part of the plant [34]. However, the nitrate accumulated in the nodule results in respiration inhibition, decreasing O_2 permeability and suppression of photoassimilate import into bacteroid.

In this study, the number of nodules increased with P application when 0 kg N ha^{-1} was applied (no urea). Moreever, in this study, it was found that nodulation was restricted with N fertilization. Leidi and Rodríguez-Navarro [37] found that increasing P enhanced the nodule formation only at low N concentration. In the case of high N (nitrate), high amounts of applied P did not improve nodulation.

4. Conclusions

Crop growth and nodulation improved with P application in loamy sand, but not in sandy soil. Nitrogen application of 40 kg N ha^{-1}, which is equivalent to 53 mg N kg^{-1}, also improved the crop growth and grain yield significantly in the loamy sand. Conversely, N application suppressed nodulation and probably also symbiotic N_2 fixation. Increasing the P rates did not alleviate the inhibitory effect of N on nodule formation. Whether the additional N application is economical is a key question, and economical optimum grain yield purpose should be considered when deciding on high N input.

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