Effects of Nitrogen Fertilizer and Tropical Legume Residues on Nitrogen Utilization of Rice-Legumes Rotation

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Abstract: Nitrogen (N) is the major nutrient limiting yield of rice (*Oryza sativa*) and adequate quantity of nitrogenous fertilizer is one of the important strategies for increasing yield. In this study two tropical legumes such as long bean and mung bean were used as precedence crops of rice. Nitrogen fertilizer was applied in both legumes at rates of 0, 2, 4 and 6 g m^{-2}. No chemical fertilizer was applied in 2nd year for both legumes and rice crop. First crop cycle of rice was also fertilized with different levels of N fertilizer (0, 4, 8 and 12 g m^{-2}) to assess the ability of long bean and mung bean to supply nitrogen to wetland rice and to determine the amount of fertilizer N required to optimize rice yield when long bean and mung bean were grown in the rice crop rotation. Mung bean added 4.7-5.7 g N m^{-2} of which 0.3 to 1.1 g fixed N m^{-2} while long bean added 4.6-5.5 g N m^{-2} of which 0.2 to 1.0 g fixed N m^{-2} to the soil when legumes residue was incorporated in 2010. In the 2nd cycle of cropping mung bean added 4.6-5.4 g N m^{-2} of which 0.5 to 1.2 g fixed N m^{-2} while long bean added 4.4-5.3 g N m^{-2} of which 0.5 to 1.1 g fixed N m^{-2} to the soil when both legume plant residue was incorporated in 2011. Rice after long bean and rice after mung bean with N at rates of 8 and 12 g N m^{-2} produced higher yield of rice in both years although no N fertilizer was applied in 2nd year rice crop. This superior performance of rice after long bean or mung bean is likely linked to higher N uptake along with N fixation of mung bean and long bean which can be a possible supplement N source to boost soil fertility. Such tropical legumes that improve productivity of rice might be attractive to farmers who are generally resource-poor farmers. The results reveal that mung bean and long bean can supply >50% of N required for rice and can be a feasible alternative organic N source to enhance soil fertility.
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1. Introduction

Nitrogen (N) is an essential macronutrient for plants and constituent of amino acids, proteins, chlorophyll, several plant hormones and improving grain yields of cereal crops (Ladha and Reddy, 2003; Zhang et al., 2004). However, excessive amounts and inappropriate application methods lead to low N efficiency and high fertilizer losses through runoff, leaching, denitrification, and volatilization (Richter and Roelcke, 2000; Zhu et al., 2000), resulting in a series of environmental problems. Low N efficiency also increases production costs, leading to lower net returns for farmers (Wang et al., 2001). Thus, efficient N utilization should be realized in agriculture for environmental and economic reasons (Stevens et al., 2005; Delin et al., 2008). Rational N application is an important measure to improve nitrogen use efficiency, while the coordination between soil N supply and crop N demand is the one of the key to rational N application (Fageria and Baligar, 2005). Soil N and biological nitrogen fixation (BNF) by associated organisms are major sources of N for lowland rice (George et al., 1992). More than 50% of the N used by flooded rice receiving fertilizer N is derived from the combination of soil organic N (Bouldin, 1986; Broadbent, 1984) and BNF by free-living and rice plant-associated bacteria (Roger and Ladha, 1992). The remaining N requirement is normally met with fertilizer. Soil organic N is continually lost through plant removal, leaching, denitrification and ammonia volatilization (Kirda et al., 2001). The additional concern is that the capacity of soil to supply N may decline with continuous intensive rice cropping under wetland conditions, unless it is replenished by biological N fixation (Kundu and Ladha, 1995). Reducing fertilizer N use in lowland rice systems while maintaining the native soil N resource and enhancing crop N output is desirable from both environmental and economic perspectives. This may be possible by increasing BNF by using legume crops, minimizing soil N losses, and by improved recycling of N through plant residues (Buresh and De Datta, 1991). Thus the management of indigenous soil N and N derived in situ through legume BNF has the potential to enhance the N nutrition and N use efficiency of crops and total N output from a lowland rice-based cropping system (Ladha and Reddy, 2003). The ability of legumes to fix N and their residual impact on soil N status has long been recognized, but many farmers also realize that the accrued N benefits will vary between different legume systems (Rochester and Peoples, 2005). To date the fate of N in green manure and productivity of dual-purpose legumes and their effects on soil N dynamics and their contributions to the yield and N uptake (Ladha et al., 1996) of the following rice crop has been studied only a few instances (George et al., 1998).

Leguminous green manures played a role in conserving NO_3 in addition to fixing atmospheric N_2. Grain and forage legumes and their residues could supplement available N for succeeding rice (Ladha et al., 1996). Nitrogen production from legumes is a key benefit of growing cover crops and green manures (Schmid and Klay, 1984).

Fertilizers are an important component to sustain high yields under mono cropping systems in Malaysia and the government spent RM 1.14 billion to import mineral fertilizers in 2001 (DOA, 2003). To minimize the dependence on mineral fertilizers, the government has focused towards natural and healthier methods of food production (Faridah, 2001). The department of agriculture has also been encouraging farmers to employ an integrated approach of rice cultivation with vegetables, intercropping practices of sweet corn, maize, crop rotation and organic farming (Wan, 2003). The optimal use of fertilizer, fuel and pesticides, through improved management practices can help increase the profitability of agricultural production while helping to meet society's environmental goals. Inclusion of annual or perennial legumes or cover crops in rotation with cereals will improve soil N fertility levels (Derksen et al., 2001), improve soil structure, water holding capacity (Russell et al., 2006), soil organic C and N (Campbell and Zentner, 1993), mineralizable C and N (Biederbeck et al., 1994), higher grain yield, economic returns, while reducing production risk and increasing long-term sustainability as well as to achieve agronomic and environmental benefits (Peterson et al., 1993; Anderson et al., 1999).

Productivity is slowly declining as well as environmental quality is deteriorating by injudicious applications of chemical fertilizer under intensive monoculture system such as paddy rice in Malaysia. Healthy and pleasant ecosystems are inevitable in order to make an environment safe crop production system (Khairuddin, 2002). Grain legumes or cover crops can play a significant role to add mineralize N to the soil by decomposing legume residue to the principal crops (Motior et al., 2009); and improve nutrient status of soil as well as to protect bare soil against erosion losses in the warm tropical climate. However grain legumes are produced on a small scale and crop rotation practices are not well practiced especially in rice producing areas in Malaysia, where double cropping per year or sometimes five crops in a two year period (Khairuddin, 2002) causes an alarming soil degradation and great threatens the environment through intensive use of chemical fertilizers. Undoubtedly, the use of legumes as grain or cover crops is desirable in terms of the environment and economy. Both long bean and mung bean are widely used in Malaysia as popular vegetables and practiced as mono crop but there is an ample opportunity to fit these crops in upland rice crop rotation system. However in recent years, sustainability or use of natural resources rather than increase in the food production has attracted the attention of many people and there has been a trend towards use of legumes to improve the soil N fertility for the following rice crop. The use of long bean and mung bean alone or with inorganic N fertilizers offer promising opportunities to evaluate N contribution to rice crop rotation systems in Malaysia. Therefore the present study was undertaken with the following objectives: (i) to quantify N fixed by long bean and mung bean using the total N difference method (ii) to assess the ability of long bean and mung bean to supply N to rice and (iii) to determine the amount of fertilizer N required to optimize rice yield when long bean and mung bean are included in the system

2. Material and Methods

Experimental Site and Plan

The experiment was conducted in a glass house at the University of Malaya, Kuala Lumpur, Malaysia during 2010-2011. Soil was collected from rice field in Selangor (1° 28' 0" N, 103° 45' 0" E), Malaysia. The top 30-cm soil layer had an air dried pH (1:5 w/v water) of 6.55±0.20, cation-exchange capacity of 15 ($cmol_c$ kg^{-1} soil), and contained 1.75±0.48 % organic carbon, 0.18±0.04 % total N, NH_4-N 6.37±1.25 (mg 100^{-1} g soil), exchangeable CaO 171.0±20.15 (mg 100^{-1} g soil), exchangeable MgO 10.8±2.75 (mg 100^{-1} g soil) and exchangeable K_2O 14.9±9.06 (mg 100^{-1} g soil). The soil texture of experimental pot was clay loam. Soils were air-dried before being used in the experimental pots. Polyethylene pots (height 46 cm x diameter 54 cm = surface area 1 m^2) were filled with soil up to about 40 cm height of each pot and kept 30 days to settle soil depth. The seeds of mung bean, long bean and corn were sown in soil maintaining saturated moisture until germination. The experiment was conducted under completely randomized design with four replications.

Cultivation and sampling of legume, corn and rice crops

In the 2010, mung bean, long bean and corn were randomly assigned to pots. In the first cycle of the experiment, N fertilizer at rates of 2, 4 and 6 gm^{-2} was applied in mung bean and long bean while 4, 8 and 12 g m^{-2} was applied in corn and rice crop. N fertilizer was applied in soil before sowing of mung bean, long bean and corn. In addition, 16 pots filled with soil were placed as the requirement to fulfill of rice after fallow crop rotation to compare mono cropping. In rice crops four rates of fertilizer (0, 4, 8 and 12 g N m^{-2}) were superimposed onto the fallow 16 pots and each crop pots. After harvesting of corn and or incorporation of legume residues, rice was planted as 2nd crop. Zero-N checks were also included in all crops for the first cycle. After harvesting of rice again mung bean, long bean and corn was grown in the same pot as third crop in 2nd cycle but no chemical fertilizer was applied to see the residual effect of legume residue for the subsequent crop. Nitrogen fertilizer (urea) was used only in first year rice crop and no fertilizer N and other chemical fertilizer was applied in second year rice crop. Simultaneously fallow pot was also used for rice crop cycle. In early May of 2010 and 2011, mung bean, long bean and corn was planted in pots. After 70 days of crop age, all crops were harvested. Mung bean and long bean plant residues were cut and manually chopped into 10-to 12-cm pieces and uniformly spread onto the pots and incorporated to a depth of about 10 cm into soil with hand mulching following flooding of the pot and kept for 30 days in preparation for rice planting. About 100 g fresh plant samples were taken and kept in oven at 72^0C for 48 hours then dry weight into whole plants of dry samples were converted. Out of 12 plants per pot, four plant samples were taken from each pot for above ground dry matter and N determination for each crop at final harvest.

Two-week-old seedlings of rice were transplanted at pots on July 15 in 2010 and July 16 in 2011. In 2010, first year rice crop was fertilized with urea and applied in three splits: one third at transplanting, one third at tillering and one third at panicle primordial initiation stages, respectively. In both years rice was harvested during the second week of December. Total biomass and grain yield were determined from experimental pot. Rice grain, culm and leaf were dried to constant weight at 70^0C and analyzed for total N by the micro-Kjeldahl method (Bremner and Mulvaney, 1982; Ladha et al., 1996).

Estimation of Biological Nitrogen Fixation

The contributions of biological nitrogen fixation (BNF) to total N accumulation in legume were estimated by the N difference method (Peoples and Herridge, 1990; Peoples et al., 2002). Plant materials were dried at 70^0C for at least 48 h, weighed; milled and total N concentration was determined by Kjeldahl digestion. Sources of N for non-fixing and fixing crops are different and corn was used as non-fixing control or reference crop. It is assumed that sources of N for non-fixing crops are soil and fertilizer. For non-fixing crops, the proportions of N from all available sources can be expressed (IAEA, 2001):

$$\%\ Ndff_{NF} + \%\ Ndfs_{NF} = 100\ \%$$

Where, $Ndff_{NF}$ stands for nitrogen derived from fertilizer for non-fixing crops, $Ndfs_{NF}$ stands for nitrogen derived from soil for non-fixing crops. On the contrary, sources of N for fixing crops (F) are soil, fertilizer and atmosphere and it can be expressed:

$$\%\ Ndff_F + \%\ Ndfs_F + \%\ Ndfa_F = 100\ \%$$

$$\%\ Ndfa = 100 - (\%\ Ndff_F + \%\ Ndfs_F)$$

Where, $Ndff_F$ stands for nitrogen derived from fertilizer for fixing crops, $Ndfs_F$ stands for nitrogen derived from soil for fixing crops and $Ndfa_F$ stands for nitrogen derived from atmosphere for fixing crops.

Estimates of the proportion of legume N derived from N_2 fixation (% Ndfa) with the total N difference procedure were calculated by comparing N accumulated in the legume with N accumulated in the non-legume reference as follows: % Ndfa= 100[(Legume N – Reference N)]/(Legume N).

Statistical analysis

Statistical analysis was carried out by one-way ANOVA using general linear model to evaluate significant differences between means at 95% level of confidence (SAS, 2003). Further statistical validity of the differences among treatment means was estimated using the least significant differences (LSD) comparison method.

3. Results and Discussion

Biomass production and nitrogen accumulation of legume crops

In 2010, aboveground biomass yield at harvest was 138-162 g m^{-2} for mung bean and 135-158 g m^{-2} for long bean with corresponding N uptake of 4.7-5.7 g m^{-2} for mung bean and 4.6-5.5 g m^{-2} for long bean, respectively. In 2011, aboveground biomass yield at harvest was 137-156 g m^{-2} for mung bean and 132-158 g m^{-2} for long bean (Table 1) with corresponding N uptake of 4.6-5.4 g m^{-2} for mung bean and 4.4-5.3 g m^{-2} for long bean, respectively (Table 2). In both years, long bean and mung bean produced consistently similar biomass with N accumulation. The amount of nitrogen available from legumes depends on the species of legumes grown, the total biomass production and the

Table 1. Biomass accumulation of mung bean, long bean, and corn as affected by nitrogen fertilizer

Fertilizer N (g m^{-2})		Biomass accumulation (g m^{-2})					
	corn	Mung bean 2010	Mung bean 2011	Long bean 2010	Long bean 2011	corn 2010	corn 2011
0	0	138 c	137 b	135 c	132 c	463 d	450 d
2	4	145 b	141 b	145 b	142 b	538 c	515 c
4	8	156 a	149 a	152 ab	148 ab	580 a	560 b
6	12	162 a	156 a	158 a	158 a	635 a	603 a

Means followed by the same letters are not significantly different at the 5% level

percentage of N in the plant tissue. Motior et al., (2011) observed that broad bean produced >10 kg dry matter m^{-2} at physiological maturity which produced >35 g N m^{-2}. Nitrogen production from legumes is a key benefit of growing cover crops and green manures. Nitrogen accumulations by leguminous cover crops ranged from 4.5 to 22.5 g of nitrogen per m^{-2} (Evans et al., 2001).

Rochester and Peoples (2005) reported that total N inputs from faba bean crop residues (11.6 to 19.9 g m^{-2}) which were lower than those achieved by green manure vetch (16.4 to 26.4 g m^{-2}). In our study, total N inputs from dwarf long bean residues were similar to mung bean residues because of similar growth nature.

Legume crops effect on nitrogen fixation

In this study the plant N derived from N_2 fixation (% Ndfa) in mung bean was 7-23% and long bean was 4-22% in 2010 as calculated by the total N difference method. Regardless of N fertilizer applied in rice crops in 2010 plant N derived from N_2 fixation (% Ndfa) in mung bean was 9-26% and long bean was 8-23% of total plant N in 2011 as calculated by the total N difference method (Table 2). Maximum N_2 fixation was derived from mung bean (23-26%) and long bean (22-23) when both legume was grown with zero N fertilizer. Estimates of % Ndfa for other forage legumes and *Cajanus cajan* were with in the range of 44 to 95 % (Peoples and Herridge, 1990). Nitrogen fixation by broad bean and hairy vetch was 41 and 78% of total plant nitrogen (Motior et al., 2009).

Table 2. Nitrogen uptake and N recovery efficiency (NCE) of long bean and mung bean as affected by fertilizer N and estimates of the proportion of plant N derived from N_2 fixation of long bean and mung bean determined by N-difference method

Crops & N g m^{-2}	N uptake		Legume N[a]		N fixation (%) [b]		NCE (%)	
	2010	2011	2010	2011	2010	2011	2010	2011
Mung bean								
0	4.7 c	4.6 c	1.1 a	1.2 a	23 a	26 a	0	0
2	5.0 b	4.8 c	0.7 b	0.8 b	14 b	17 b	20 a	20 a
4	5.4 ab	5.1 b	0.6 b	0.6 b	11 c	12 c	17 b	16 b
6	5.7 a	5.4 a	0.3 c	0.5 b	7 d	9 c	16 b	15 b
Long bean								
0	4.6 c	4.4 c	1.0 a	1.1 a	22 a	23 a	0	0
2	5.0 b	4.8 b	0.7 b	0.9 a	14 b	17 b	16 b	10 b
4	5.2 b	5.1 a	0.5 b	0.6 b	8 c	12 c	17 a	14 a
6	5.5 a	5.3 a	0.2 c	0.5 b	4 d	8 d	16 b	14 a
Corn								
0	3.6 c	3.4 c	-	-	-	-	-	-
4	4.3 b	4.0 b	-	-	-	-	-	-
8	4.8 a	4.5 a	-	-	-	-	-	-
12	5.3 a	4.9 a	-	-	-	-	-	-

Means followed by the same letters are not significantly different at the 5% level

[a]Data collected from average percentage of total N derived from N_2 fixation (%Ndfa) values derived from columns 2^{nd} and 6^{th}; 3^{rd} and 7^{th} columns of table 2 as N fixed = 1/100 (% Ndfa X total N).

[b]N fixed by legumes was calculated based on N-difference method. Corn used as reference plants for estimation of N_2 fixation by N-difference method.

Nitrogen recovery efficiency (NRE) was significantly higher (20%) when mung bean was grown with 2 g N m^{-2} in 2010 and similar trend was also observed in 2011 (Table 2). Application of a lower rate of N fertilizer was associated with the highest N_2 fixation NRE in both long bean and mung bean. Nitrogen recovery efficiency was appreciably higher in long bean when grown with 4 g N m^{-2} in 2010 while in 2011 NRE was higher when it was grown with 4 or 6 g N m^{-2}.

Long bean provided BNF input of 0.2-1.0 g N m^{-2} in 2010 and 0.5-1.1 g N m^{-2} in 2011. Regardless of the N fertilizer levels applied in rice crops, removal of N in long bean was 3.6-5.3 g N m^{-2} in 2010 and 3.3-4.8 g N m^{-2} in 2011 (Table 2). Mung bean provided BNF input of 0.3-1.1 g N m^{-2} in 2010 and 0.5-1.2 g N m^{-2} in 2011. Regardless of the N fertilizer levels applied in rice crops, removal of N in mung bean was 3.6-5.4 g N m^{-2} in 2010 and 3.4-4.9 g N m^{-2} in 2011 (Table 2). Nitrogen fixed from both mung bean and long bean was appreciably higher when fertilizer was not applied. Legume contributions from BNF were lowest in treatments with the highest level of N fertilizer applied to the preceding rice crop. Our observations suggest that legumes incorporated into rice cropping sequence contribute not only to increased productivity but also to the maintenance and improvement of soil fertility by virtue of their capacity to fix substantial amounts of atmospheric N. Legumes can play a positive role in boosting soil N fertility (Sullivan, 2003). However, they must leave behind more N from N_2 fixation than the amount of soil N they remove (Ladha et al., 1996). A large number of plant characteristics contribute to BNF, including biomass yield, legume N demand, capacity to fix N_2, and adaptability to specific environments (Ladha et al., 1996).

Biomass, grain yield and harvest indices of rice

Higher biomass accumulation was credited by higher levels (8 or 12 g N m^{-2}) of N application in all cases. Lower biomass was recorded when rice was grown with zero N fertilizer. Both years, the incorporation of legume residue and N fertilizer amended soil significantly increased grain yield of rice (Table 3). In 2010 and 2011, rice after long bean with 8 and 12 g N m^{-2}, produced significantly higher rice grain yields (538-570 gm^{-2}). The minimum yield was obtained in rice after corn (293-349 g m^{-2}) and rice after fallow (319-371 g m^{-2}) and when no N fertilizer was applied in rice crops. Rice after mung bean with 8 g N m^{-2} and 12 g N m^{-2} gave similar yields (489-521 g m^{-2}) corresponds to rice after long bean in 2010. In 2011, slightly lower yield was obtained in rice after long bean but similar trend was observed. Rice after corn with 8 g N m^{-2} and 12 g N m^{-2} showed similar and comparatively poor yield than other counterparts. No appreciable difference was observed on rice after fallow with 8 g N m^{-2} and 12 g N m^{-2} (Table 3). In both years, rice yield showed that mung bean and long bean was effective even in 2011 when no fertilizer was applied in rice crop. In the second year of the experiment, incorporation of legume residues slightly decreased grain yield of rice in the zero-N control but higher than rice after fallow with 4 g N $m^{-2}$and very close with 8 g N m^{-2}. There was an insignificant increase or even a decrease in grain yield associated with residue incorporation, especially without application of fertilizer N (Thuy et al., 2008). A multi-location research project on the management of crop residue for sustainable production concluded that residue incorporation did not lead to higher grain yields (IAEA, 2003). Bijay et al., (2008) summarized 51 data sets from rice-rice-cropping system experiments and reported that statistically significant increases in grain yield associated with residue incorporation were found in seven experiments. In most cases, legume residue retention does not reduce rice yield in N-fertilized plots. In some cases, it may increase or decrease yield but in general has positive effect on yield. Where N fertilizer is not applied, legume residues often increase both yield and N uptake. Clearly, the effect of residues on grain yield depends on soil characteristics, incorporation method, amount of residue returned to soil (Motior et al., 2011), and timing and rate of N-fertilizer application (Ponnamperuma, 1984).

Table 3. Biomass accumulation, grain yield and harvest index (HI) of rice as affected by N fertilizer and legume residue

N fert. ($g\ m^{-2}$)	Biomass ($g\ m^{-2}$)		Grain yield ($g\ m^{-2}$)		Harvest index	
	2010	2011	2010	2011	2010	2011
Rice after mung bean						
0	953 c	912 c	407 c	396 c	43 c	43 c
4	1066 b	987 b	495 b	449 b	46 b	46 b
8	1129 a	1072 a	540 a	489 a	48 a	46 b
12	1132 a	1079 a	565 a	521 a	49 a	48 a
Rice after long bean						
0	949 b	929 c	417 c	404 c	43 c	43 b
4	1069 a	1038 b	502 b	472 b	47 b	46 a
8	1136 a	1083 a	538 a	495 ab	47 b	46 a
12	1138 a	1103 a	570 a	538 a	50 a	49 a
Rice after corn						
0	906 b	873 b	349 d	293 c	38 c	34 c
4	932 b	906 b	407 c	358 b	44 b	40 b
8	1026 a	961 a	472 a	407 a	46 ab	42 ab
12	1059 a	1007 a	508 a	440 a	48 a	44 a
Rice after fallow						
0	941 c	906 c	371 c	319 c	35 c	35 c
4	1065 b	994 b	428 b	375 b	38 b	38 b
8	1137 a	1042 a	521 a	440 a	42 a	42 a
12	1127 a	1059 a	554 a	456 a	43 a	43 a

Means followed by the same letters are not significantly different at the 5% level

Harvest indices were also affected significantly by incorporation of legume residue and N fertilizer application for both years (Table 3). In 2010 and 2011, rice after long bean with 4, 8 g N m^{-2}, obtained identical HI while 12 g N m^{-2} obtained slightly HI. The lowest HI was recorded in rice after fallow and rice after corn when no N fertilizer was applied in rice crops. Rice after mung bean with 8 or 12 g N m^{-2} in 2010 and 4, 8 or 12 g N m^{-2} gave superior HI, respectively. Rice after corn with 8 g N m^{-2} and 12 g N m^{-2} showed similar HI for both years. No appreciable difference was observed on rice after fallow with 8 or 12 g N m^{-2} (Table 3).

Conclusions

The N difference methods employed in this study could show that N derived from long bean and mung bean is readily available and can be used efficiently by rice crop. Long bean and mung bean is capable of producing a large quantity of dry matter and accumulating significant quantities of nitrogen and can fix substantial amount of N for rice crop. The combined application of mung bean or long bean along with N fertilizer at the rate of 4 g m^{-2} can be an alternative N fertilizer management method to reduce N losses from N fertilizer applied to rice crop. Mung bean and long bean residues incorporated into the soil supplied N to rice crop and produced benefits comparable with that of 4 g fertilizer N m^{-2}. Such kinds of tropical legumes that improve annual productivity of rice might be attractive to farmers who are generally resource-poor farmers. Thus, long bean and mung bean has the potential to substitute or supplement for chemical/inorganic N fertilizer.

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