

RESPONSE OF NITROGEN FERTILIZER AND LEGUMES RESIDUES ON BIOMASS PRODUCTION AND UTILIZATION IN RICE-LEGUMES ROTATION

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Corresponding author email: mmotiorrahman@gmail.com**ABSTRACT**

A cropping sequence including rice after winged bean, rice after bush bean, rice after corn, and rice after fallow with varying rates of nitrogen (N) fertilizer were carried out to investigate the effects of legume residues and N fertilizer to clarify dynamics of soil N supply, N utilization in soil-plant system and grain yield of rice. Nitrogen fertilizer was applied in both legumes at rates of 0, 2, 4 and 6 g m⁻². The first crop cycle for rice and corn were fertilized with N fertilizer at rates of 0, 4, 8 and 12 g m⁻² with no chemical fertilizer applied in the 2nd crop cycle for the legumes, corn and rice. In 2010, bush bean and winged bean accumulated 5.0-5.9 and 6.2-6.8 g N m⁻² of which 10.0-29.4 and 21.7-42.1 % was derived from N₂ fixation. In 2011, bush bean and winged bean accumulated 4.7-5.6 and 5.8-6.9 g N m⁻² of which 13.2-28.8 and 29.5-41.4% was derived from N₂ fixation and estimated by total N difference method. Higher yield was recorded by rice after winged bean with N at rates of 4, 8 and 12 g N m⁻² during both years. This superior performance of rice after winged bean is likely linked to greater N fixation (21.7-42.1%) and N uptake by both legumes. Such tropical legumes that improve productivity of rice might be attractive to farmers who are generally resource-poor farmers. The results revealed that bush bean and winged bean can supply >50% and >95% of N required for rice and can be a viable alternative organic N source to enhance soil fertility and reduce cost.

Key words: Biomass, Crop rotation, Legume residue, Nitrogen

INTRODUCTION

Nitrogen deficiency is one of the most yield-limiting nutrients in rice growing regions world wide (Fageria *et al.* 2003) and it represents one of the major costs in crop production (Stevens *et al.* 2005). 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. Thus, efficient N utilization should be realized in agriculture for environmental and economic reasons (Stevens *et al.* 2005; Delin *et al.* 2008). Coordination between soil N supply and crop N demand is the one of the key to rational N application (Fageria and Baligar, 2005). The nitrogen requirement for plant growth is normally met with fertilizer (Kirda *et al.* 2001). Reducing fertilizer N use in rice production systems while maintaining the native soil N resource and enhancing crop N output is desirable from both environmental and economic perspectives. Inclusion of legume in crop rotation systems may minimize soil N losses and by improved recycling of N through plant residues (Derksen *et al.* 2001). Thus indigenous soil nitrogen management and N derived from legume have the potential to increase the N nutrition of crops and total N output from rice-based cropping system (Ladha and Reddy, 2003).

The extensive use of continuous annual cropping systems and mono crop rotations has generally been economically successful, but the lack of diversification has had negative economic, biological, and environmental consequences (Sulc and Tracy, 2007) including reduction of crop diversity, loss of soil organic matter, degradation of soil physical characteristics (Xing and Zhu, 2000), increased surface and groundwater contamination (Zhu *et al.* 2000). Reducing the incidence of monocultures is touted to improve environmental health and soil quality and to reduce residual nitrate (Gregory *et al.* 2002). Nitrate leaching has been reduced up to 96% by the introduction of annual or perennial legumes into preceding crops (Ladha and Reddy, 2003). Fertilizers are an important component to sustain high yields under mono cropping systems. Malaysia spent RM 9.17 billion to import about 4.16 million tons of mineral fertilizer in 2008 (Ali, 2009). To minimize the dependence on mineral fertilizers, the government 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 (Kumar and Goh, 2000), improve soil structure, water holding capacity (Russell *et al.* 2006), soil organic C and N, mineralizable C and N (Kumar *et al.* 2001; Goh *et al.* 2001), higher grain yield, economic returns, while reducing production risk and increasing long-term sustainability as well as to achieve agronomic and environmental benefits (Delin *et al.* 2008).

Productivity is decreasing as well as environment is threatening 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 uncovered 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 practices 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. Winged bean is an annual or perennial tropical legume which is capable of producing large quantities of biomass and accumulating greater amount of nitrogen and can fix substantial quantities of N for subsequent crops (Motior *et al.* 1999). Forage legumes have been used widely in pasture-crop rotations to upgrade soil N fertility (Peoples and Baldock, 2001) but their introduction to cropping systems has been limited. The impact of these measures on soil fertility is generally known but the particular benefits it brings to the farming scenarios in Malaysia are not well documented. Both winged bean and bush 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. Rice based double cropping system only locally, but their positive impacts to subsequent crops are limited. However in recent years, sustainability or use of natural resources rather than strengthen in the food production has fascinated the attention of many people and there has been a positive attitude towards use of legumes to improve the soil fertility for the subsequent rice crop. The use of winged bean and bush bean alone or combined with chemical N fertilizers offer favorable circumstances to investigate N contribution to rice crop rotation systems in Malaysia. Therefore the present study was carried out to determine the amount of N fixed by winged bean and bush bean using the total N difference method and also

the amount of fertilizer N required to optimize rice yield when both legumes are included in the system.

MATERIALS AND METHODS

The experiments were conducted in a glass house at the University of Malaya, Kuala Lumpur, Malaysia during 2010 and 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 hold 1.75 ± 0.48 % organic C, 0.18 ± 0.04 % total N, NH₄-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 K₂O 14.9 ± 9.06 (mg 100⁻¹ g soil). The texture of soil in experimental pot was clay loam. Soils were thoroughly mixed and sieved through a 2-mm mesh to obtain homogenous soil composites as the growing medium in pots. Soils were air-dried before being used in the experimental pots. Polyethylene pots (height 46 cm x diameter 54 cm = surface area 1 m²) were filled with soil up to about 40 cm height of each pot. The seeds of bush bean, winged bean and corn were sown in soil maintaining saturated moisture until germination. N fertilizer was applied in soil at 7 days after germination. Each treatment was replicated five times and the experiments were laid out under completely randomized design.

In the 2010, bush bean, winged 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 bush bean and winged bean while 4, 8 and 12 g m⁻² was applied in corn and rice crop. In addition, 16 pots filled with soil were placed to fulfill the requirement of rice after fallow crop rotation. 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 bush bean, winged bean and corn was grown in the same soil as third crop in 2nd cycle but no chemical fertilizer was applied. After harvesting of corn and or incorporation of legume residues again rice was grown as 2nd rice crop to see the residual effect of legume residue for the subsequent crop. Nitrogen fertilizer (urea) was applied 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, bush bean, winged bean and corn was planted in pots. After 70 days of each crop age, all crops were harvested. Bush bean and winged bean plant residues were chopped into small pieces 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°C for 48 hours then dry weight into whole plants of dry samples were converted. Out of six plants per pot, two plant samples were taken from each pot for dry matter and N determination for each crop at final harvest.

Rice seedlings (14 days old) were transplanted in pots on July 15 and July 16 during 2010 and 2011, respectively. During 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 dry matter and grain yield of rice were determined from each pot. Plant samples (grain, culm and leaf) were dried to constant weight at 70°C and analyzed for total N by the micro-Kjeldahl method (Bremner and Mulvaney, 1982).

The nitrogen difference method (NDM) was used to estimate biological nitrogen fixation (BNF) to total N accumulation in legumes (Peoples and Herridge, 1990; Peoples *et al.* 2002). Plant samples were dried at 70°C for about 48 h, weighed; milled and N concentration was determined by Kjeldahl digestion (IAEA, 2001). Corn was used as non-fixing control or reference crop. It is well documented that sources of N for non-fixing crops are from the 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 as % 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 N₂ 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)].

Data was analyzed by one-way ANOVA using general linear model. Treatment means were tested at 5% level of significance (SAS, 2003). Further statistical validity of the differences among treatment means was estimated using the least significant differences (LSD) comparison method.

RESULTS AND DISCUSSION

Biomass production and nitrogen accumulation of legume crops: Nitrogen fertilizer had significant influence on above ground biomass and nitrogen accumulation of legume crops (Table 1). During 2010, aboveground biomass yield at harvest was 179.8-192.2 g m⁻² for winged bean and 148.2-170.0 g m⁻² for bush bean with corresponding N uptake of 6.2-6.8 g m⁻² for winged bean and 5.0-5.9 g m⁻² for bush bean, respectively. During 2011, aboveground biomass yield at harvest was 169.4-193.2 g m⁻² for winged bean and 140.1-162.9 g m⁻² for bush bean with corresponding N uptake of 5.8-6.9 g m⁻² for winged bean and 4.7-5.6 g m⁻² for bush bean, respectively (Table 2). During both years, winged bean obtained consistently higher biomass and N accumulation compared to bush bean. The N amount derived from legumes depends on the legumes species grown, biomass production and N content in the plant tissue. Sullivan (2003) found similar results and Motior *et al.* (2011) reported that broad bean produced >10 kg biomass m⁻² at physiological maturity which contained >3.5 g N m⁻². The production of N from legumes is a great benefit of growing grain legume, forage or cover crops as green manures. Evans *et al.* (2001) stated that N accumulations from legume crops varied from 45-225 kg N ha⁻¹. Rochester and Peoples (2005) found that faba bean crop residues contributed about 116-199 kg N ha⁻¹ which was lower than those achieved by green manure vetch (164 to 264 kg ha⁻¹). In this study, the total N inputs from winged bean plant residues were significantly greater than bush bean plant residues because of more dry matter produced.

Soil N and Biological nitrogen fixation by the legumes: The plant N derived from N₂ fixation (% Ndfa) in bush bean was 10-29.4% and winged bean was 21.7-42.1% in 2010 as determined by the total NDM. Irrespective of N fertilizer used in rice crops during 2010 plant N derived from N₂ fixation (% Ndfa) in bush bean was 13.2-28.8 % and winged bean was 29.5-41.4 % of total plant N during 2011 as estimated by the total NDM (Table 2). Peoples and Herridge (1990) reported that estimates of % Ndfa for other forage legumes and *Cajanus cajan* were within the range of 44 to 95 %. Nitrogen fixation by broad bean and hairy vetch was 41 and 78% of total plant nitrogen (Motior *et al.* 2009). Nitrogen recovery efficiency in winged bean and bush bean was significantly higher during 2nd year than 1st year (Table 2). Application of a poorer rate of N fertilizer was collaborated with the highest N₂ fixation and nitrogen recovery efficiency in both winged bean and bush bean.

Winged bean provided BNF input of 1.5-2.6 g N m⁻² during 2010 and 2.0-2.4 g N m⁻² during 2011. Irrespective of the levels of N fertilizer used in rice, N removal in winged bean was 3.6-5.3 g N m⁻² during 2010 and 3.4-4.9 g N m⁻² during 2011 and N removal in bush

bean was 3.6-5.3 g N m⁻² during 2010 (Table 1) and 3.4-4.9 g N m⁻² during 2011, respectively (Table 2). When fertilizer was not used N fixed from both bush bean and winged bean was appreciably higher. Legume contributions through nitrogen fixation were the lowest in treatments with the highest level of N fertilizer applied to the preceding rice crop. Nitrogen fixed by winged bean was remarkably higher than bush bean. It might be due to poor biomass yield by bush bean. The present findings suggested that legumes residues added into rice-legume rotation systems contribute not only to gained productivity but also to the conservation and amelioration of soil fertility by virtue of their capacity to fix substantial quantities of atmospheric nitrogen. Soil N fertility is increased by the contribution of legumes

(Sullivan, 2003). However, they must refrain behind higher N from N₂ fixation than the amount of soil N they discharge. A large number of plant characteristics contribute to BNF, including dry matter yield, legume N demand, capacity to fix N₂, and flexibility to specific environments (Bijay *et al.* 2008). During 2010, N in bush bean in top plant residues was about >5 g N m⁻² and for winged bean was >6 g N m⁻² which was integrated into the soil. During 2011, biomass of winged bean mixed into the soil corresponded to 5.8 g to 6.9 g N m⁻² (Table 2). During both years, winged bean supplied considerably higher N than bush bean through their residues at each season. The higher quantities of N₂ fixed in winged bean resulted from its greater amount of dry matter production.

Table-1: Biomass accumulation (g m⁻²) of bush bean, winged bean, and corn as affected by nitrogen fertilizer

N fertilizer (g m ⁻²)		Bush bean		Winged bean		Corn	
Legume	Corn	2010	2011	2010	2011	2010	2011
0	0	148.2 b	140.1 b	179.8 b	169.4 b	462.5 d	449.5 d
2	4	155.7 ab	149.8 ab	187.3 a	184.7 a	537.5 c	514.7 c
4	8	165.5 ab	156.4 a	190.6 a	187.6 a	579.8 a	560.3 b
6	12	170.0 a	162.9 a	192.2 a	193.2 a	635.2 a	602.6 a

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

Table-2: Nitrogen uptake and nitrogen recovery efficiency of winged bean and bush bean as affected by nitrogen fertilizer and estimates of the proportion of plant N derived from N₂ fixation of winged bean and bush bean determined by N-difference method

Crops and Nitrogen (g m ⁻²)	Nitrogen uptake (g m ⁻²)		Legume N ^a (g m ⁻²)		N ₂ Fixation (%) by N difference method ^b		Nitrogen recovery efficiency (%)	
	2010	2011	2010	2011	2010	2011	2010	2011
Bush bean:								
0	5.0 b	4.7 b	1.5 a	1.4 a	29.4 a	28.8 a	0	0
2	5.3 b	5.1 a	1.0 b	1.1 ab	19.4 b	22.2 b	14.0 b	18.0 a
4	5.7 a	5.4 a	0.9 b	0.9 b	16.5 c	16.1 c	16.3 a	15.3 b
6	5.9 a	5.6 a	0.6 c	0.7	10.0 d	13.2 d	14.8 ab	14.8 b
Winged bean:								
0	6.2 a	5.8 b	2.6 a	2.4 a	42.1 a	41.4 a	0	0
2	6.5 a	6.3 a	2.2 b	2.4 a	33.4 b	37.4 a	15.5 a	29.0 a
4	6.7 a	6.6 a	2.0 b	2.1 ab	29.4 b	31.8 b	14.5 a	20.5 b
6	6.8 a	6.9 a	1.5 c	2.0 b	21.7 c	29.5 b	11.2 b	19.5 c
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

^aData collected from average percentage of total N derived from N₂ fixation (%Ndfa) values derived from columns 2nd and 6th; 3rd and 7th columns of table 2 as N fixed = 1/100 (% Ndfa X total N).

^bN fixed by legumes was calculated based on N-difference method. Corn used as reference plants for estimation of N₂ fixation by N-difference method

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

Rice after bush bean:							
N fertilizer (g m ⁻²)	Biomass (g m ⁻²)		Grain yield (g m ⁻²)		HI		
	2010	2011	2010	2011	2010	2011	
0	987.0 b	921.8 c	423.5 c	358.3 c	43 c	39 c	
4	1058.6 ab	993.5 b	495.1 b	439.7 b	47 b	44 b	
8	1074.9 a	1074.9 a	547.2 a	472.3 ab	50 a	44 b	
12	1123.8 a	1084.0 a	553.7 a	521.2 a	49 a	48 a	
Rice after winged bean:							
N fertilizer (g m ⁻²)	Biomass (g m ⁻²)		Grain yield (g m ⁻²)		HI		
	2010	2011	2010	2011	2010	2011	
0	1154.7 b	1107.5 b	537.5 b	508.1 b	47 b	46 b	
4	1262.2 a	1211.7 a	645.0 a	602.6 a	51 a	50 a	
8	1283.4 a	1244.3 a	667.8 a	625.4 a	52 a	50 a	
12	1289.9 a	1254.1 a	684.0 a	658.0 a	53 a	52 a	
Rice after corn:							
N fertilizer (g m ⁻²)	Biomass (g m ⁻²)		Grain yield (g m ⁻²)		HI		
	2010	2011	2010	2011	2010	2011	
0	905.5 b	873.0 b	348.5 d	293.2 c	38 c	34 c	
4	931.6 b	905.5 b	407.2 c	358.3 b	44 b	40 b	
8	1026.1 a	960.9 a	472.3 a	407.2 a	46 ab	42 ab	
12	1058.6 a	1006.5 a	508.1 a	439.7 a	48 a	44 a	
Rice after fallow:							
N fertilizer (g m ⁻²)	Biomass (g m ⁻²)		Grain yield (g m ⁻²)		HI (%)		
	2010	2011	2010	2011	2010	2011	
0	941.4 c	905.5 c	371.3 c	342.0 c	39 c	38 c	
4	1065.1 b	993.5 b	488.6 b	423.5 b	46 b	43 b	
8	1136.8 a	1042.3 a	534.2 a	472.3 a	47 b	45 b	
12	1127.0 a	1058.6 a	553.7 a	504.9 a	49 a	48 a	

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

Grain yield and harvest indices of rice: Both years, soil amended by legume residues and N fertilizer had significant influence on rice grain yield (Table 3). Higher rice grain yields (645-684 gm⁻²) was recorded from rice after winged bean with 4, 8 and 12 g N m⁻² during 2010 and 2011 while poor yield was produced by rice after fallow and rice after corn with when N fertilizer was not applied in rice crops. Rice after bush bean with 8 g N m⁻² and 12 g N m⁻² gave identical and moderate rice yields. Rice after corn with 8 g N m⁻² and 12 g N m⁻² produced at par rice yield. Rice after fallow with 8 g N m⁻² and 12 g N m⁻² produced identical yield (Table 3). The grain yield of rice proved that winged bean was more effective than N fertilizer applied in rice crop for both years. Similar results were obtained when hairy vetch was grown with 4 or 8 g N m⁻² in producing rice grain yield (Motior *et al.* 2009). Incorporation of legume residues slightly decreased rice grain yield in the zero-N control during 2011 but higher than rice after fallow with 8 or 12 g N m⁻². The present findings differed with the observation of Thuy *et al.* (2008). They reported that rice yield did not increase or even decrease when rice straw residue was added without fertilizer N. Similar results were also

reported by IAEA (2003). The possible reason for this is the use of rice straw residues under upland conditions which were not fully decomposed during the rice growing season. On the other hand Bijay *et al.* (2008) reported that grain yield of rice significantly increased when residue was incorporated into flooded soil. Our observation suggested that legume residue had positive influence on both rice yield and N uptake when fertilizer was not used. Clearly, the effect of legume residues on rice 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.

Harvest indices (HI) were significantly affected by incorporation of legume residue and N fertilizer application for both years (Table 3). During 2010 and 2011, rice after winged bean with 4, 8 and 12 g N m⁻², obtained identical 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 bush bean with 12 g N m⁻² gave superior HI although it differed from other levels of N application. Rice after corn with 8 g N m⁻² and 12 g N m⁻² showed similar HI. 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, HI was higher in rice after winged bean and even zero-N application in 2nd year rice crop.

Conclusions: The N difference methods employed in this study proved that N derived from winged bean and bush bean was readily available and can be used efficiently by rice crop. Winged bean and bush bean were capable of producing large amounts of biomass and accumulating large quantities of N and can fix substantial quantities of N for rice crop. The combined application of bush bean and N fertilizer or winged bean alone can be an alternative N fertilizer management method to reduce N losses from N fertilizer applied to rice crop. Bush bean and winged bean residues incorporated into the soil supplied N to rice crop and produced benefits comparable with that of 4 to 8 g fertilizer N m⁻². Such kinds of tropical legumes those improve productivity of rice might be attractive to farmers who are generally resource-poor farmers. Thus, winged bean and bush bean has the potential to substitute or supplement for inorganic N fertilizer.

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