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## The Effects of Green Manure (*Sesbania rostrata*) on the Growth and Yield of Rice

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Green manuring with an N<sub>2</sub>-fixing legume crop can meet a substantial portion of rice N requirement and provide organic matter to wetland rice soils to maintain soil fertility. Organic-N fertilizers in the form of flood-tolerant, leguminous, stem-nodulating *Sesbania rostrata* can be useful alternatives to resource-poor rice farmers if applied as green manure. Therefore, the accumulation of fixed N by this green manure, their effect on the performance and yield of rice was examined. Firstly, root and/or stem nodulation, nitrogen fixation (acetylene-reducing activity), growth and N accumulation of *S. rostrata* as affected by inoculation site were studied in a pot experiment. The growth and N accumulation of *S. rostrata* in stem inoculation treatment was the highest. Therefore, stem inoculation method was used for growing *S. rostrata* to incorporate into the rice soils for the second experiment. In the second experiment, the effects of *S. rostrata* green manure application on the growth and yield of rice were studied. Green manure treatments (2 and 4 plants pot<sup>-1</sup>) were compared to urea treatments (40 and 80 kg N ha<sup>-1</sup>) and no application treatment. Green manure treatments increased dry matter weight and grain weight of rice significantly over urea and no application treatments. All green manure treatments increased N uptake of the plant. It can be concluded that incorporation of green manure legumes can add large amount of biologically fixed N to rice soils. The effective utilization of biomass produced by the stem-nodulating *S. rostrata* could be an economically viable component of integrated nutrient management for increasing the yield components and yield of rice per unit area.

### INTRODUCTION

For optimum yield, crops require a supply of mineral nutrients, the most important of which is nitrogen. The economic and environmental costs of the heavy use of chemical N fertilizers in agriculture are a global concern. Sustainability considerations mandate that alternatives to N fertilizers must be urgently sought. Biological nitrogen fixation (BNF), a microbiological process which converts atmospheric nitrogen into a plant-usable form, offers this alternative. Nitrogen-fixing systems provide an economically attractive and ecologically sound means of reducing external inputs and improving internal resources (Bohlool *et al.*, 1992).

In recent years, conventional chemical farming practices, which substantially increased crop yields, have created numerous problems for mankind. Ground water pollution is considered a result of chemical farming practices. Adverse human and animal health effects are caused by residual and cumulative agricultural chemicals used for crop protection (Shrestha and Ladha, 1998). Excessive soil erosion caused by conventional farming systems has seriously reduced soil fertility and crop productivity. Also, higher cost of agricultural chemicals will significantly lower the net cash return to farmers. Renewed interest has recently been given to leguminous

green manure (LGM) to sustain lowland rice productivity, reduce farmers' dependence on mineral N fertilizers, and lower farmers' high cash input. BNF by short duration LGM crops has the potential to cover the substantial portion of the N required by rice (Ladha *et al.*, 1988). Green manuring with nitrogen fixing legume crop can improve soil physical, chemical, and biological properties and consequently crop yields. Green manure has an advantage over other organic manures because it can be grown directly in the field and can be incorporated during land preparation or regular weeding operations. Furthermore, potential benefits of green manuring are reduced nitrate (NO<sub>3</sub><sup>-</sup>) leaching risk and lower fertilizer N requirements for succeeding crops. As a green manure, 40–60 day old *Sesbania rostrata* can substitute for about 50–70 kg N ha<sup>-1</sup> from N fertilizer for lowland rice (Kolar *et al.*, 1993; Manguiat *et al.*, 1992; Meelu *et al.*, 1992). Well nodulated *Sesbania* plants may derive up to 90% N from fixation (Pareek *et al.*, 1990) and therefore contribute N to rice. *S. rostrata* is a tropical legume of West Africa that can form nodules in both roots and stems (Rinaudo *et al.*, 1988). It is recommended as a green manure in both Asia and Africa and is currently grown on about 500,000 hectares in the Delta and the Central Plain of Myanmar (Mar *et al.*, 1995).

Because of its rapid growth, high N<sub>2</sub> fixation potential, and tolerance for flooding, *S. rostrata* is a promising LGM species for lowland rice-farming systems (Pareek *et al.*, 1990). However, little information is available on the potential of *S. rostrata* as green manure compared to the common farming system in terms of rice productivity. Numerous studies have examined growth and nitrogen fixation of legumes and the effects of green

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manure on soils and on the succeeding rice crop (Becker *et al.*, 1990; Buresh and De Datta, 1991; Ladha *et al.*, 1992; Singh *et al.*, 1991), and have concluded that incorporation of green manure legumes can add large quantities of biologically fixed N to lowland rice cropping systems and improve the soil productivity. However, the adoption of sustainable green manure technology in tropical lowland rice farming systems has been limited in the past, and their use is currently declining. The use of LGM in rice cropping systems in Myanmar is very recent and information on optimal green manure management practices is lacking. This paper reports the results of studies on N<sub>2</sub> fixation (acetylene–reduction activity), growth and N accumulation of *S. rostrata* as affected by inoculation site. The effects of *S. rostrata* as a green manure on the growth and yield of rice plants were also studied.

## MATERIALS AND METHODS

### Experiment I

#### *Azorhizobium caulinodans* culture and inoculant preparation

*A. caulinodans* ORS571T, stem nodulating rizobium was provided by Dr. Dreyfus, LSTM, France and was used as inoculants. The strain was cultured in TGYE broth medium (10 g Difcotryptone, 5 g glucose, 5 g yeast extract, 0.9 g CaCl<sub>2</sub> · 2H<sub>2</sub>O and 1 L distilled water; Ladha *et al.*, 1989) at 30 °C for 3 days on a rotary shaker to a cell density of 10<sup>9</sup> cells mL<sup>-1</sup>. For inoculating seeds and stems, the cell suspension was prepared by diluting 2 mL of the broth culture to 200 mL of 0.9% NaCl solution.

#### *Plant culture and experimental treatments*

The experiment was conducted in the phytotron (30 °C, 70% RH) under natural daylight condition at Kyushu University, Japan from March to April in 2008. The pots were filled with 3 kg of soil taken from a paddy field of Kyushu University Farm. The soil was sieved through a 2 mm sieve, thoroughly mixed, and placed in pots which were kept flooded on 3 days before sowing.

*S. rostrata* seeds were scarified with concentrated H<sub>2</sub>SO<sub>4</sub> for 30 minutes, then washed thoroughly with sterile water and germinated on moist filter paper in Petri dishes at 30 °C for 2 days. Three seeds were sown per pot and seedlings were thinned to one on 5 days after sowing (DAS). Four treatments were included in this experiment: (1) Seed inoculation; (2) Stem inoculation; (3) Seed and stem inoculation; and (4) No inoculation. For seed inoculation treatment, seeds were inoculated by applying 5 mL cell suspension on each seed at sowing. At the same time, each seed was applied by 5 mL of NaCl solution for stem and no inoculation treatments. Stems will be inoculated by spraying cell suspension (cell density: 10<sup>7</sup> cells mL<sup>-1</sup>) on the aerial portion of each plant on 30 DAS. Pots in each experiment were arranged in a completely randomized block design with three replications. The BNF ability was estimated by using acetylene reduction assay.

#### *Determination of acetylene–reducing activity (ARA), dry weight, nodule number and nitrogen accumulation*

Sixty days after sowing, the plant of each treatment was uprooted and carefully washed off the soil. The plant roots were removed at about 2–3 cm above the crown and the aerial stem was cut into 4 pieces (about 15 cm each). The root and stem portions of plant from each pot were separately placed in 500 mL glass bottles into which 50 mL of acetylene gas was changed. Gas samples of 1 mL were withdrawn after 5 and 64 minutes from the exposure to acetylene and the amount of ethylene was determined using a gas chromatograph (GC-14A, Shimadzu, Kyoto, Japan) according to the method of Sarr *et al.* (2009).

After measuring acetylene–reducing activity, the numbers of nodules were counted and the leaf, stem, root and nodule dry weight were measured for each plant after oven–drying at 70 °C for 48 h. The leaf, stem, root and nodule samples were separately ground to fine powder by using grinding mill (Cyclotec 1093, Foss Analytical AB, Sweden). And then the digestion of the ground samples was done by salicylic–H<sub>2</sub>SO<sub>4</sub>–H<sub>2</sub>O<sub>2</sub> digestion method (modified Kjeldahl digestion method) stated by Ohyama *et al.* (1991). The digests were determined for total nitrogen by using indophenols method as described in Cataldo *et al.* (1974), for total phosphorus by ascorbic acid method (Murphy and Riley, 1962) and for total potassium by atomic absorption spectrophotometer (Z-5300, Hitachi, Japan).

### Experiment II

#### **Green manure production**

##### *Soil and plant material*

Pot experiment was conducted in the phytotron (25 °C, 70% RH) under natural daylight condition at Kyushu University, Japan from May to June 2008. The soil used for this experiment had the background history of fertilization with organic manure (cow and poultry manure) in the previous years. *S. rostrata* was used as a green manure in the experiment. Seeds were scarified for 30 minutes in concentrated H<sub>2</sub>SO<sub>4</sub>, then washed thoroughly with sterile water and germinated on moist filter paper in Petri dishes at 30 °C for 2 days and transplanted in the pots mentioned above.

##### *Green manure culture and incorporation*

Four treatments were included in the study; (1) CGM-1: *Sesbania* level I (2 plants grown in soil applied with cow manure in the previous year); (2) CGM-2: *Sesbania* level II (4 plants grown in soil applied with cow manure in the previous year); (3) PGM-1: *Sesbania* level I (2 plants grown in soil applied with poultry manure in the previous year); and (4) PGM-2: *Sesbania* level II (4 plants grown in soil applied with poultry manure in the previous year). The pots of all treatments were arranged in a completely randomized block design with three replications. For green manure level I or II treatments, twelve or six scarified seeds of *S. rostrata* were sown, and the plants were thinned to two or four per

pot, respectively on 5 days after planting. No chemical fertilizer was applied to the green manure treatments for evaluating the actual effects of the green manure on the growth and yield of rice. *A. caulinodans* culture and inoculant preparation were done as described in experiment I. Two times of stem inoculation were done by spraying cell suspension (cell density:  $10^7$  cells mL<sup>-1</sup>) on the aerial portion of each plant at 20 and 30 DAS. Sixty days after sowing, the subsamples were taken from each treatment to determine the plant dry weight, N, P and K accumulation and the remaining plant parts were cut into small pieces and mixed thoroughly with the roots. And then the shoot and root pieces were divided into three portions, weighed equally and incorporated into each replicated pot for the next rice experiment on 5 days before transplanting rice.

## Rice production

### Treatment design and cultivation

Pot experiment was conducted in a net-house under natural environmental condition during 2008 (from July to November). Nine treatments were laid out in a completely randomized block design with three replications. (1) C-cont.: Previous cow manure soil with no application; (2) CGM-I: Previous cow manure soil incorporated with 2 plants of *Sesbania* (level I); (3) CGM-II: Previous cow manure soil incorporated with 4 plants of *Sesbania* (level II); (4) P-cont.: Previous poultry manure soil with no application; (5) PGM-I: Previous poultry manure soil incorporated with 2 plants of *Sesbania* (level I); (6) PGM-II: Previous poultry manure soil incorporated with 4 plants of *Sesbania* (level II); (7) Low-U: Low rate of urea (40 kg N ha<sup>-1</sup>) with previous low chemical fertilizer

application; (8) High-U: High rate of urea (80 kg N ha<sup>-1</sup>) with previous complete fertilizer application; and (9) NA: No application.

One 21-day old seedling of rice was transplanted into each pot at 5 days after incorporation of green manure. Rice (*Oryza sativa* L.) variety Manawthukha (Myanmar high yielding variety) was used in the study. In urea treatments, three equal splits were done at basal, tillering and panicle initiation. Total N, P and K amount from applied *S. rostrata* and urea were shown in Table 1. Tap water was used for irrigation. The water level in the pot was kept at 3–5 cm depth above the soil surface until 2 weeks before harvest. Pesticides were applied as needed. Excluding 21 days in the seedbed, the rice plants were grown for an average of 135 days.

### Plant sampling and analysis

During the cultivation period, the plant growth characteristics; plant height, tiller number and SPAD value were measured weekly until panicle initiation. At physiological maturity, the rice plants were cut at 2–3 cm above ground level. Dry weight of straw and grain, and yield components were determined. Grain yield was determined at 14% moisture content after air-dried. Straw dry weight was analyzed after oven-drying to get constant weight at 70 °C for 2 days. Dried grain and straw samples were ground and digested by H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> digestion method (modified Kjeldahl digestion method) stated by Ohyama *et al.* (1991). The total N, P and K were determined by the methods as described above.

### Statistical analysis

For statistical analysis, one-way ANOVA for the

**Table 1.** Amount of total N, P and K from applied *S. rostrata* and urea

Treatment	<i>Sesbania</i>		Urea	Total applied amount from <i>Sesbania</i> and urea		
	Level	Fresh wt. (g pot <sup>-1</sup> )	(g pot <sup>-1</sup> )	N	P (mg pot <sup>-1</sup> )	K
C-cont.	–	–	–	–	–	–
CGM-I	2 plants	57.7	–	587	34.8	479
CGM-II	4 plants	61.6	–	590	32.8	511
P-cont.	–	–	–	–	–	–
PGM-I	2 plants	50.9	–	475	32.8	473
PGM-II	4 plants	64.3	–	741	47.3	663
High-U	–	–	0.350	160	–	–
Low-U	–	–	0.175	80	–	–
NA	–	–	–	–	–	–

C-cont.: Previous cow manure soil, CGM-I: Previous cow manure soil with *Sesbania* level I, CGM-II: Previous cow manure soil with *Sesbania* level II, P-cont.: Previous poultry manure soil, PGM-I: Previous poultry manure soil with *Sesbania* level I, PGM-II: Previous poultry manure soil with *Sesbania* level II, Low-U: Low rate of urea (40 kg N ha<sup>-1</sup>) with previous low chemical fertilizer application, High-U: High rate of urea (80 kg N ha<sup>-1</sup>) with previous complete fertilizer application, NA: No application. *Sesbania* were incorporated in fresh weight basis. Urea fertilizer was applied three times of split application, at basal, tillering and panicle initiation stage.

analysis of variance to test the statistical significance and Tukey–Kramer HSD (Honestly Significant Difference) test to calculate the Least Significant Difference (LSD) for comparing means at 5% probability level were conducted using software JMP (JMP IN, Version 5.1.2., SAS Institute Inc., Cary, NC, USA).

## RESULTS AND DISCUSSIONS

### Effect on Plant biomass, nodulation and nitrogen fixation of different inoculation treatments

Biomass production and N accumulation are often taken into account when predicting the usefulness of a legume for green manuring (Yoshida and Kitou, 1995). Goel *et al.* (1999) reported that the inoculation with certain plant growth-promoting rhizobacteria (PGPR) may enhance crop productivity by making the other nutrients available. The influence of inoculation site on plant dry weight and N accumulation of *S. rostrata* was shown in Table 2 and 3. Plant dry weight and nitrogenase activity showed similar trend. Stem inoculation had a highly significant effect on N accumulation and a significant influence on plant dry weight compared to other treatments. Stem inoculation treatment also accumulated significantly higher N compared to other treatments. It can be seen that dry matter production and N accumulation by *S. rostrata* were significantly correlated with the quantity of ARA. The results suggested that stem inoculation with *A. caulinodans* is able to increase N accumulation, nitrogen fixation and growth of legumes. It is evident from these results that stem inoculation of *S. rostrata* is needed in order to produce a high biomass and accumulate a large amount of N.

Data on nodule numbers and dry weights are shown in Table 2. The means of the total number of root and stem nodules were 95, 141, 81 and 76 nodules plant<sup>-1</sup> for seed inoculation only, stem inoculation only, both seed and stem inoculation and no inoculation treatments, respectively. Although the differences of nodule number and nodule dry weight in all treatments were not statistically significant, the mean values of nodule number and nodule dry weight in the seed inoculation treatment were generally high. As that reason, it is thought that seed

**Table 3.** Effects of *A. caulinodans* inoculation on N, P and K accumulation of *S. rostrata* on 60 DAS

Treatment	N (mg plant <sup>-1</sup> )	P (mg plant <sup>-1</sup> )	K (mg plant <sup>-1</sup> )
Seed inoculation	140.96 ab	16.36 a	131.45 ab
Stem inoculation	187.92 a	20.61 a	163.68 a
Seed and stem inoculation	97.51 b	10.42 a	89.32 b
No inoculation	78.64 b	10.78 a	82.14 b

The same letters within columns are not significantly different using Tukey–Kramer HSD test ( $P < 0.05$ ).

inoculation was effective for *A. caulinodans* to initiate the nodulation on root of *S. rostrata*, forming a large number of nodules in early time, increasing the size of nodules. However, in this situation, an abundant N was required for the formation of nodule and furthermore, the initial growth of host plant was depress and the dry matter accumulation was decreased as described below.

It was found that nitrogenase activity (ARA) increased significantly in stem inoculation treatment. Stem inoculation is generally recommended to optimize N<sub>2</sub> fixation in *S. rostrata*. The high nitrogen fixing potential of *S. rostrata* is probably directly associated with the profuse stem nodulation (Dreyfus, 1982). *S. rostrata* developed nitrogen fixing nodules on the stem after spraying the plants with the bacterial culture of *A. caulinodans* strain ORS571T. Root nodules were also observed, and they were very small and might be low-effective for contributing N<sub>2</sub> fixation. It was observed that only stem inoculation treatment increased nitrogenase activity (ARA) significantly although nodule number and nodule dry weight were not significantly different in all treatments. Although seed inoculation, and both seed and stem inoculation treatments resulted in greater N<sub>2</sub> fixation than no inoculation treatment, the difference of N<sub>2</sub> fixation was no significant statistically. It was surprisingly observed that root and stem nodules were found in no inoculation treatment. It might be due to the presence of indigenous *Azorhizobium* species in the soil.

**Table 2.** Effects of *A. caulinodans* inoculation on nodulation, total plant dry weight and ARA of *S. rostrata* on 60 DAS

Treatment	Root nodules plant <sup>-1</sup>	Stem nodules plant <sup>-1</sup>	Total nodules number plant <sup>-1</sup>	Dry weight of nodules (g plant <sup>-1</sup> )	Total plant dry weight (g plant <sup>-1</sup> )	ARA ( $\mu\text{mol C}_2\text{H}_4 \text{ g}^{-1}$ nodule dry weight hr <sup>-1</sup> )
Seed inoculation	73 a	22 a	95 a	0.25 a	4.79 ab	12.74 ab
Stem inoculation	98 a	43 a	141 a	0.36 a	6.39 a	20.52 a
Seed and stem inoculation	63 a	18 a	81 a	0.19 a	3.31 b	12.64 b
No inoculation	51 a	25 a	76 a	0.22 a	3.24 b	4.35 b

The same letters within columns are not significantly different using Tukey–Kramer HSD test ( $P < 0.05$ ).



### Nutrient application from incorporated green manure

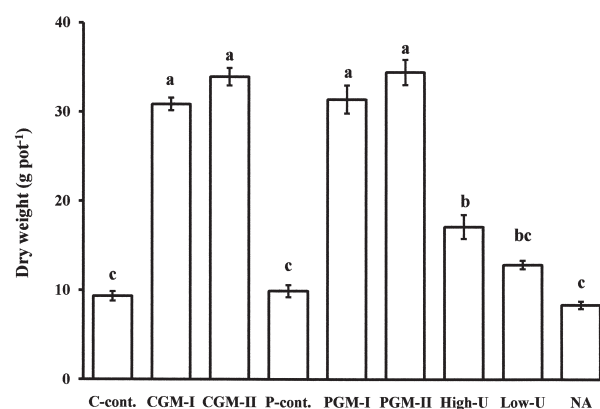
Symbiotic N<sub>2</sub> fixation by leguminous green manure crops can provide a substantial quantity of fresh biomass which may be effectively used for organic manuring. N, P and K amount estimated from the fresh weight of incorporated *S. rostrata* was shown in Table 1. The incorporation of 57.7, 61.6, 50.9 and 64.3 g pot<sup>-1</sup> (fresh weight) of *S. rostrata* provided 586.68, 589.77, 474.46 and 740.80 mg N pot<sup>-1</sup> to the soil for treatment CGM-I, CGM-II, PGM-I and PGM-II, respectively. The use of *S. rostrata* as green manure provided a substantial portion of N requirements for rice. Nutrient accumulation in *Sesbania* was primarily a function of the growing period available for biomass production. Conceivably, increasing plant density will increase N accumulation in the plant population (Herrera *et al.*, 1997). In this experiment, it was found that the harvested amount from green manure was not significantly different even if 2 or 4 plants were grown. It might probably be due to certain limitation of root volume in pot experiment and no limitation of light for photosynthesis. However, it was found that nutrient accumulation was significantly different although the harvested amount from green manure was not different (Table 1).

### Dry matter accumulation and grain yield of rice

The response of rice to green manure and urea application is shown in Table 4. In all green manure treatments, plant height and tiller number of Manawthukha were significantly higher than those in the urea and no application treatments. Such improvement (tiller number, number of panicles and yield) due to the beneficial effect of green manure addition has also been reported by Singh *et al.* (1990). Maximum dry matter weight (34.45 g pot<sup>-1</sup>) was observed in PGM-II which was statistically similar to CGM-II, PGM-I and CGM-I (33.93, 31.41 and 30.87 g pot<sup>-1</sup>). The increase in dry matter weight by *S. rostrata* application has also been reported by Azam, (1990), Rees *et al.* (1993), and Manguiat *et al.* (1997).

Lowest dry matter weight (8.33 g pot<sup>-1</sup>) was noted in NA. It was observed that all maximum dry matter weights were from green manure treatments and the differences between levels (2 and 4 plants pot<sup>-1</sup>) of application were not significant. Because, their growth might be limited by the restricted supply of essential nutrients under pot experiment. Incorporation of *S. rostrata* enhanced the dry matter weight significantly as compared to urea application and NA treatments (Fig. 1). In green manure and urea treatments, the dry matter yield was proportional to the level of N applied (Fig. 2).

Similar trend as dry matter weight was observed in grain yield. All green manure treatments (CGM-II, PGM-I, CGM-I and PGM-II) produced 17.04, 15.41, 14.86 and 13.68 g grain yield pot<sup>-1</sup>, respectively and all green manure treatments were not significant with each other statistically. Green manures contribute significantly to the N nutrition of rice. The increase in grain yield with the use of *S. rostrata* had also been observed by Beri and Meelu (1981) and Mian and Stewart (1985).



**Fig. 1.** Effects of *S. rostrata* and urea application on dry matter yield of rice.

The abbreviations of the treatments (C-cont., CGM-I, CGM-II, P-cont., PGM-I, PGM-II, High-U, Low-U and NA) in Fig. 1, 2, 3, 4 and 5 are the same as described in under Table 1. The same letters indicate not significantly different using Tukey-Kramer HSD (Honestly Significant Difference) test ( $P < 0.05$ ) in Fig. 1, 3, 4 and 5.

**Table 4.** Yield components, grain yield and nutrient accumulation of rice as affected by incorporation of *S. rostrata* and urea

Treatment	Plant height (cm)	Panicles (no. plant <sup>-1</sup> )	Total dry weight (g plant <sup>-1</sup> )	Grain weight (g plant <sup>-1</sup> )	Total accumulation (mg plant <sup>-1</sup> )		
					N	P	K
C-cont.	51	8	9.31 c	2.66 b	71.26 d	22.33 d	151.15 c
CGM-I	67	21	30.87 a	14.86 a	281.26 b	64.81 abcd	383.01 b
CGM-II	68	23	33.93 a	17.04 a	288.73 b	87.08 ab	538.97 a
P-cont.	52	8	9.88 c	2.40 b	80.71 cd	19.60 d	159.56 c
PGM-I	69	22	31.42 a	15.41 a	314.95 ab	102.16 a	561.90 a
PGM-II	69	23	34.45 a	13.68 a	356.03 a	75.47 abc	605.43 a
High-U	56	13	17.1 b	5.32 b	134.21 c	36.40 bcd	230.46 c
Low-U	56	11	12.85 bc	6.09 b	124.95 cd	32.23 cd	182.88 c
NA	54	8	8.33 c	3.56 b	86.4 cd	21.97 d	131.17 c

The same letters indicate not significantly different using Tukey-Kramer HSD (Honestly Significant Difference) test ( $P < 0.05$ ).

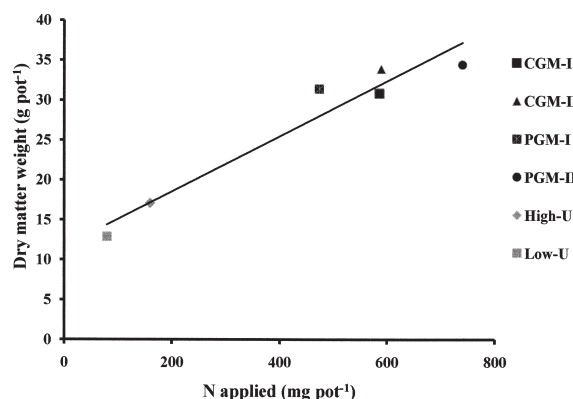


Fig. 2. Relationship between N applied and dry matter yield.

### N, P and K accumulation in rice plants

The highest accumulation of total N was observed in green manure treatments. CGM-I and CGM-II increased rice plant's N uptake by 295% and 305% as compared to C-cont. and PGM-I and PGM-II increased 290% and 341% as compared to P-cont., respectively, in previous cow manure and poultry manure soil. Amounts of N accumulation were 71.26, 281.26 and 288.73 mg N plant<sup>-1</sup> for C-cont., CGM-I and CGM-II, and 80.71, 314.95 and 356.03 mg N plant<sup>-1</sup> for P-cont., PGM-I and PGM-II, respectively. As compared to NA treatment, green

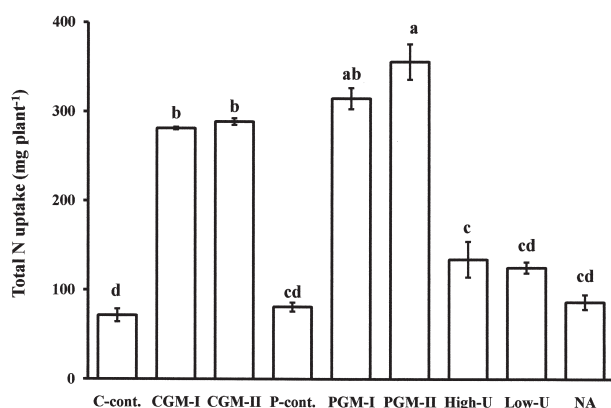


Fig. 3. Effects of *S. rostrata* and urea application on nitrogen accumulation in rice plant.

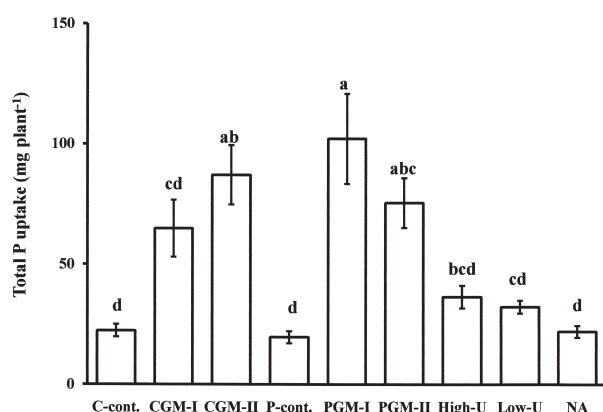


Fig. 4. Effects of *S. rostrata* and urea application on phosphorus accumulation in rice plant.

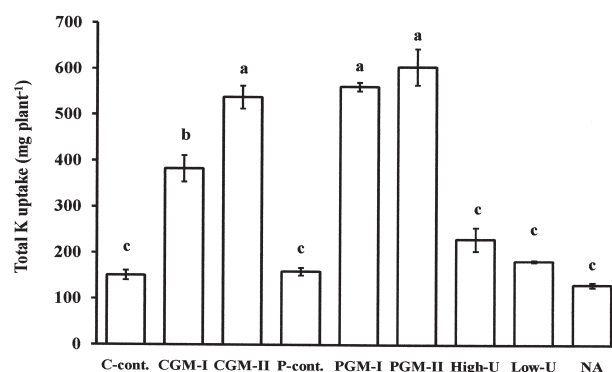


Fig. 5. Effects of *S. rostrata* and urea application on potassium accumulation in rice plant.

manure level 1 and 2 treatments increased rice plant's N uptake by 226% and 234% for CGM-I and CGM-II, and 265% and 312% for PGM-I and PGM-II, respectively (Fig. 3). The increasing of N accumulation in green manure treatments was because of the increase of added N, but those in the urea application and NA treatments did not show an increasing tendency. It might be due to the fact that the application of N in green manure treatments was higher than that of urea (Table 1).

It was observed that phosphorus accumulation of rice plants in green manure treatments remained non-significant with urea application and NA treatments statistically (Fig. 4). The pattern of K accumulation was relatively similar to that of N (Fig. 3 and 5). The results in Fig. 5 showed that the accumulation of K in rice plants was significantly increased by incorporation of green manure. Amounts of K accumulation were 605.43, 561.9, 538.97, 383.01 and 131.17 mg K plant<sup>-1</sup> for PGM-II, PGM-I, CGM-II, CGM-I and NA, respectively. It can be suggested that the total K accumulation in rice plant was increased in proportion to the increase in total N accumulation due to the high nitrogen fixation activity. The results indicated that the incorporation of green manure increased the N and K accumulation of rice plants and enhanced their yield.

### CONCLUSION

As a conclusion from the present study results, stem inoculation method is supposed to be the best for growing *S. rostrata* to incorporate into rice soils. It can be stated that green manure treatments recorded maximum N uptake, and increased the yield components and yield of rice plants as compared to urea application and NA treatments due to its high application of N to the succeeding rice crops. Although green manure treated plants showed significant effects on yield parameters, dry matter weight, grain yield and nutrient accumulation, the differences between two levels (2 plants and 4 plants pot<sup>-1</sup>) were not statistically significant. Therefore, field experiment for further investigation of effectiveness of *S. rostrata* on rice production is needed to know the appropriate level of application.

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