Studies on the impact of combined N application and inoculation on BNF, growth and yield of cowpea (*Vigna unguiculata* (L.) Walp)

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Abstract

The impact of combined N application and inoculation on BNF, growth, yield, residual N content of plant material and pod N yield of cowpea were studied, using two varieties of cowpea in two separate experiments. In the 1st experiment, local brady-rhizobium multi strain was used for the inoculation with five levels of fertilizer N (40, 80, 120, 160, and 200 Kg N ha^{-1}) applied in split doses. A control treatment was also included without N application as well as without inoculation. There was another treatment that excluded N fertilizer but inoculated with local brady-rhizobium multi strain. In the second experiment same treatments were used but inoculated with two introduced braby-rhizobium strains i.e. THA-291 and THA 230. Both experiments were arranged in a Randomized complete block design with 4 replicates. Nodule fresh weight, stover dry weight at physiological maturity, pod yield, pod N yield at harvesting and percent ^{15}N atom excess and total N in experiment 2 were determined at physiological maturity. Applied fertilizer N inhibited nodulation significantly but contributed positively to stover dry matter production, pod yield and plant N yield. Seed inoculation enhanced nodulation in MI 35 but did not affect nodulation of Bombay. In 2nd experiment, two introduced inoculums i.e., THA-291 and THA-230 were used with same levels of fertilizer N, with a reference crop of *Setaria italica.* The %Ndfa was significantly higher in variety, Bombay compared to variety, MI-35 and increased application of fertilizer N significantly reduced %Ndfa in both varieties. N fixation was significantly higher in Bombay compared to MI-35.
Key words: -*Vigna unguiculata* (L.) Walp, BNF, *Setaria italica*, inoculation, ^{15}N methodology, bradyrhizobia, THA-291 and THA- 30

Introduction

In the case of key plant nutrients, the economic and environmental cost of using higher dosage of chemical N fertilizer has seriously restricted their use in intensive agriculture, which significantly reduced the food production, especially in developing countries. On the other hand, N fixed by legumes is considered to be very important as cost effective and environmentally acceptable source of nitrogen. Legumes are therefore being increasingly used to supplement N requirement of the associated crop. Hence, an increasing attention is being paid to improve N_2 fixation and N residual effect of legumes aimed at developing sustainable and low input cropping systems.
Cowpea (*Vigna ungiculata* (L.) Walp) is one of the most important grain legume crops in the tropics and subtropics, often constituting the main plant protein source in some agricultural regions (Fery 1985). Cowpea, being the most widely cultivated grain legumes in Sri Lanka. Studies were conducted to enhance BNF and yield of cowpea, which has specific relevance to the country. Applied nitrogen has in general an inhibitory effect on nodule initiation, nodule development and nitrogen fixation in legumes (Dart and Wildon 1970). Basically, the potential N benefit from grain legumes is governed by the magnitude of N_2 fixation and the level of N in non- harvested residue (Hanzell and Vallis 1977). Thus, studies on the impact of available N in the soil and other factors, which enhance Biological Nitrogen Fixation (BNF) of legumes, would contribute positively to the efficient use of legumes in multiple cropping systems. Therefore, experiments reported here were conducted with following objectives.

i. Assessment of the response of BNF parameters, plant dry matter production and yield of cowpea to applied N.
ii. Assessment of the genotypic variability of some cowpea varieties in traits associated with BNF, growth and yield.
iii. Determination of the effectiveness of local brady-rhizobial strains in meeting the N-requirements of cowpea.
iv. Assessment of the introduced bradyrhizobial strains in N fixing capability and meeting the N-requirements of cowpea.

Materials and methods

Two separate field experiments were conducted at Bata-atha (6^0 15' N and 80^0 54' E, 5M amsl) in Hambantota district in the dry zone of Sri Lanka, to address above objectives. In the 1st experiment, two varieties of cowpea; Bombay and MI-35 were used with seven treatments. A control treatment was included without N fertilizer and without inoculation. Another treatment without N application but inoculated with local brady-rhizobium multi strain was also used. The other five treatments included different rates of N in split doses at different times as shown in Table 1. All N applied treatments were inoculated with same brady-rhizobium multi strain.

Table 1. Treatment combinations of the experiment

Treatment No.	Treatment combinations				
T_0	Control, (No fertilizer N, No inoculation),				
T_1	Inoculated with local bradyrhizobial multi strain				
	At planting	10 DAP	25 DAP	35 DAP	55 DAP
T_{40}	40 kg Nha^{-1}	20kg	20kg	-	-
T_{80}	80 kg Nha^{-1}	20kg	30kg	30kg	-
T_{120}	120 kg Nha^{-1}	20kg	50kg	50kg	-
T_{160}	160 kg Nha^{-1}	20kg	40kg	50kg	50kg
T_{200}	200 kg Nha^{-1}	20kg	60kg	60kg	60kg

Treatments were laid out in the field according to randomized complete block design with four replications. Following observations i.e. nodule fresh weight, stover dry weight at physiological maturity, pod yield and pod N yield at harvesting were recorded. In the second experiment, same cowpea varieties were used with similar treatments but inoculation was done by using introduced brady-rhizobium strains of THA 291 and THA 230, received from the Biological Nitrogen Fixation Regional Center, in Bangkok. In addition, Thanahal (*Setaria italica* L.), which has an N-uptake pattern similar to those of cowpea, was planted as a reference crop, to determine N_2 fixation by the ^{15}N method (Fried and Middleboe 1977). N fertilizer was applied as Ammonium Sulphate as assigned to the different treatments. The micro-plots were also included for all N applied treatments to apply ^{15}N labelled Ammonium Sulphate. At physiological maturity, percent ^{15}N atom excess by mass spectometer and % N in above ground parts of the cowpea by (Bremner and Mulvaney 1982) was calculated.

Results and Discussion

Nodulation

Table 2. Nodule fresh weight (g/plant) at physiological maturity

Treatments	Variety-Bombay		Variety-MI 35	
	Mean nodule fresh weight	% changes compared to control	Mean nodule fresh weight	% changes compared to control
T_0	0.54	-	0.27	-
T_1	0.53	-11	0.33	+22
T_{40}	0.28	-48	0.11	-59
T_{80}	0.23	-57	0.09	-66
T_{120}	0.15	-74	0.05	-81
T_{160}	0.06	-90	0.11	-60
T_{200}	0.10	-83	0.04	-85

Cowpea variety Bombay had a higher nodule fresh weight compared to variety MI 35 and the inoculation effect has not contributed to the nodulation prominently in variety Bombay, but variety MI 35 had positive response (22% increase over control); probably the variety Bombay may have formed effective

nodulation with available rhizobia in the soil while variety MI 35 requires introduced strains for effective inoculation.
Application of nitrogen fertilizer drastically inhibited nodule fresh weight in both varieties; the effect was severe on cv. MI 35. In both varieties, the nodulation was significantly restricted at the face of high availability of N. Infact, this finding confirmed the earlier observation (Dart and Wildon 1970) even the lowest rate of N application used (40 kgN ha^{-1}) resulted in a significant inhibition of nodulation (by 48% in Bombay and 59% in MI. 35).

Dry matter production
The stover dry matter production showed prominent response to inoculation as well as N application (Table 3) in both varieties, inoculation resulted in an increased stover dry matter production of 59% in variety Bombay and 70% in variety MI 35. The magnitude of dry matter production in each treatment was higher in variety Bombay compared to variety MI 35. Stover dry matter production responded positively to increase application of fertilizes N, but the pattern of response was different in the two varieties.

Table 3. Effect of different N levels on dry matter production

Treatment	Variety-Bombay		Variety-MI 35	
	Stover DM (g/plant)	% increase over to control	Stover DM (g/plant)	% increase over to control
T_0	3.20	-	2.80	-
T_I	5.09	59.0	4.76	70.0
T_{40}	4.92	53.8	4.68	67.4
T_{80}	5.97	86.6	5.01	78.9
T_{120}	6.34	98.1	5.97	113.2
T_{160}	6.80	112.5	5.37	91.8
T_{200}	7.62	138.1	6.07	116.8

Following effects were pronounced in N- response and in inoculation.

i. Variety Bombay was superior in dry matter production to MI 35.
ii. Inoculation resulted considerable dry matter production in both cowpea varieties
iii. Variety Bombay consistently increased dry matter production while dry matter production of variety MI 35 increased only up to 120 Kg N ha^{-1}, and N levels above 160 kg N ha^{-1} imparted an inhibitory effect.

Pod yield
The variety MI 35 showed a significantly higher response to fertilizes N in increasing the pod yield; a rapid increase was noted up to 80 kg N ha^{-1}, then slowly increased up to 160 kg N ha^{-1} and dropped drastically in response to further increase of N. But in variety Bombay the response was very mild up to 160 kg N ha^{-1} and then increased rapidly up to 200 kg N ha^{-1}. Thus, it was clear that, there is a considerably high degree of genetic variability in response to fertilizer N between two varieties. Inoculation resulted in a marked yield increment in both varieties; by 92% in variety Bombay and by 151% in variety MI 35.This very clearly indicated that, the availability of the appropriate type of rhizobia influenced the expression of the full yield potential and thus, it was a limiting factor. Further, it was clear from the results, that the variety Bombay had a higher yield potential but to achieve this yield level, N-requirement of the plant has to be supplied either by increased application of fertilizer N, or by enhanced BNF using appropriate rhizobial strain. Therefore, further studies would be required in screening genetical yield potential of superior varieties (Figure 1).

Pod N yield and stover N yield
Both these parameters are extremely important since cowpea is a major food legume, widely cultivated as a crop component in multiple cropping systems, for its beneficial residual N effect (Frey 1985).
Both these parameters have significantly contributed to the total N yield of the pod as well as stover in both varieties. Pod N yield increased steadily with increasing levels of fertilizer application and at 200 kg N ha^{-1}, the highest pod N level has been reached (0.21 g/plant), an increase of about 128% over to the

control. In variety MI -35, the pod N content was lower in all treatments (0.05-0.17g/plant) compared to variety Bombay (0.09-0.21 g/plant).

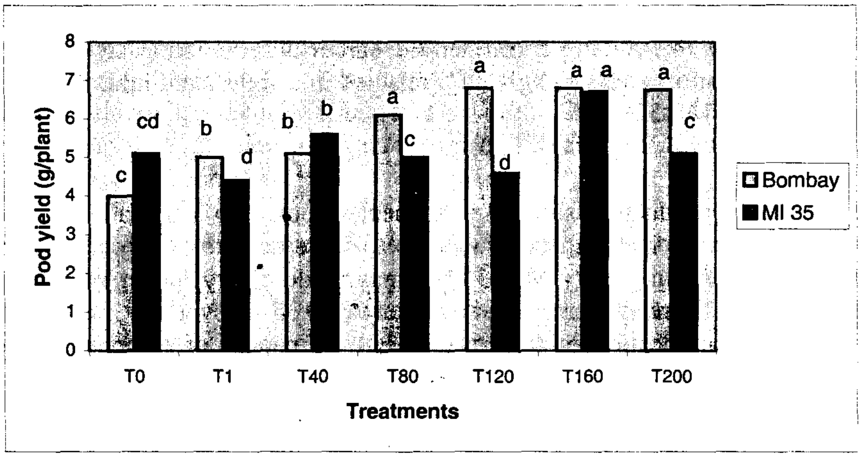

Figure 1. Response of cowpea pod yield to different N regimes and to inoculation
Means with the same letter on the bar are not significantly different at P≤0.05.

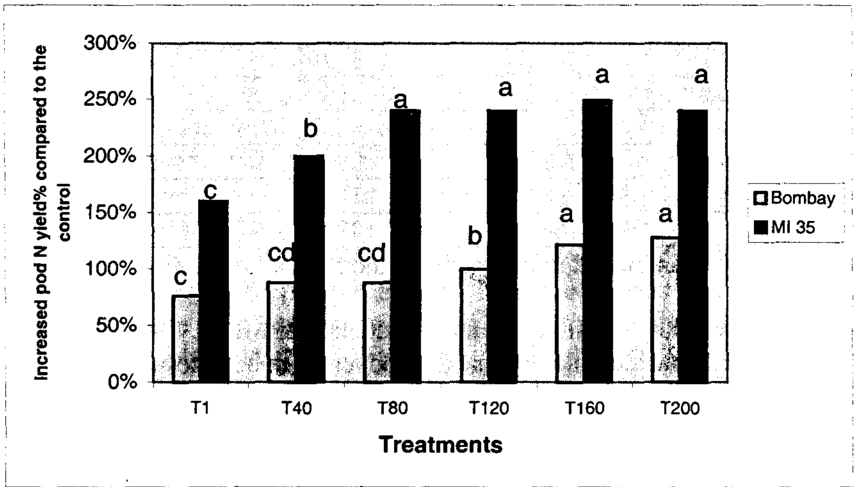

Figure 2. Pod N yield of two cowpea varieties with different N regimes and with inoculation
Means with the same letter on the bar are not significantly different at P≤0.05.

In both varieties, inoculation significantly contributed to the pod N yield with 76% and 116% increase over the control in varieties Bombay and MI 35 respectively. In variety MI 35 corresponding increase in pod N yield in response to fertilizes N was extremely high, which has reached the peak at 160 kg N ha^{-1} where an increase of 249% compared to control was reported. As N level increased further, upto 200 kg Nha^{-1} it has dropped. But in variety Bombay, the highest pod N yield as well as stover N content was achieved at 200kg N ha^{-1}. These observations confirm that DM production and yield parameters, in the variety Bombay depends on meeting its nutritional requirements (mainly N) and availability of appropriate rhizobial strain for an efficient symbiotic relationship.

Biological Nitrogen Fixation

Results of the experiment 2 also showed that inoculation significantly increased the nodule number per plant in cv MI 35 while it showed no effect in cv. Bombay, probably indicating the high affinity of introduced rhizobium strains to cv. MI 35.

In both cvs, increased application of fertilizer N significantly reduced the nodule number per plant and the nodule fresh weight per plant. This probably confirmed the fact that, added fertilizer N drastically reduced the nodulation of cowpea. Shoot dry weight showed no significant response to inoculation or to added N fertilizer.

At physiological maturity, pod yield shows no significant response to inoculation, addition of N fertilizers or to the varietal difference. Shoot dry weight has responded significantly to the addition of N fertilizer in cv MI 35, but shows no response to inoculation. In contray cv. Bombay shows consistently higher shoot weight compared to cv. MI 35 and significantly higher response to inoculation. But, with increasing rates of fertilizer N, shoot dry weight decreased significantly. Total biomass production also responded significantly to the treatment. The cv Bombay showed significantly higher biomass production and a higher response to inoculation, compared to cv MI 35. But, increased levels of fertilizer-N not contributed significantly to increase total biomass.

Table 4. % Ndfa and fixed N (g/plant) of cowpea at Physiological maturity

Treatments	% Ndfa		Fixed N (g/plant)	
	MI 35	Bombay	MI 35	Bombay
T_0	37.4[a]	35.55[c]	0.064[a]	0.081[c]
T_I	27.00[b]	48.32[a]	0.041[b]	0.167[a]
T_{40}	26.78[b]	39.09[b]	0.064[a]	0.112[b]
T_{80}	15.99[c]	33.73[c]	0.033[c]	0.101[b]
T_{120}	13.16[c]	34.15[c].	0.023[d]	0.101[b]
T_{160}	12.33[c]	29.08[d]	0.021[d]	0.097[b]
T_{200}	8.01[d]	18.07[e]	0.017[d]	0.057[a]

The % Ndfa was significantly higher in Bombay compared to cv MI 35. Further, increased application of fertilizer nitrogen significantly reduced % Ndfa in both cvs. The varietal difference to the effect of N fixed was significantly higher in cv Bombay.

Conclusions

The following conclusions can be drawn from the results.

i. Fertilizer N inhibited nodulation and thus BNF of cowpea.
ii. Increased application of N fertilizer contributed significantly towards total dry matter production, pod yield and plant N yield.
iii. The response of cowpea to applied N in all the traits pertaining to BNF, growth, yield and plant N content showed a similar trend, but the magnitude varied according to the genotype.
iv. BNF, growth and yield parameters of cowpea could be further enhanced by seed inoculation with appropriate brady-rhizobial strains.
v. Inoculation with introduced brady-rhizobium strains increased nodulation of both tested cvs, MI 35 and Bombay.
vi. Addition of fertilizer N reduced nodulation and the amount of fixed N.
vii. There were varietal differences in the affinity to added rhizobial strains.

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