Maejo Int. J. Sci. Technol. **2013**, *7*(03), 377-384

Maejo International
Journal of Science and Technology

ISSN 1905-7873
Available online at www.mijst.mju.ac.th

Full Paper

Effect of organic fertiliser residues from rice production on nitrogen fixation of soya (*Glycine max* L. Merrill), Chiang Mai 60 variety

Nattida Luangmaka*, Somchai Ongprasert and Jiraporn Inthasan

Division of Soil Resources and Environment, Faculty of Agricultural Production, Maejo University, Chiang Mai 50290, Thailand

*Corresponding author, e-mail: l_pucca31@hotmail.com

Received: 18 February 2013 / Accepted: 23 September 2013/ Published: 23 September 2013

Abstract: A field study was undertaken on the residual effect of organic fertilisers applied to the preceding rice cropping on nitrogen fixation of soya in a rice-soya cropping system. The experiment was conducted on a farmer's lowland paddy in Mae Rim district, Chiang Mai province, Thailand. Organic fertiliser treatments assigned were: 1) control (no fertiliser), 2) animal manure of cattle (AM), 3) compost (CP), 4) azolla (AZ), 5) AM + CP, 6) AM + AZ, 7) CP + AZ and 8) AM + CP + AZ. Soya seeds were planted without rhizobial inoculation in December 2011, four months after the application of organic fertilisers. Nodule weight, total shoot nitrogen accumulation and relative ureide index at various growth stages were recorded as the indices of nitrogen fixation. Results of the study demonstrate that the residues from the application the organic fertilisers of narrow C/N ratios during the land preparation for rice cropping four months before soya cultivation promoted nitrogen fixation by native rhizobia.

Keyword: soya, nitrogen fixation, ureides, organic fertiliser residue

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INTRODUCTION

Soya planted areas in Thailand declined from 480 to 93 thousand hectares, reducing output from 0.63 to 0.15 million tons during 1989-2012 [1]. The domestic production was therefore far below the domestic feed and soya bean oil industry demand. Approximately, one half of soya bean production is produced from paddy rice - soya cropping system. However, the soya bean production from such paddy-based production system has been dramatically waning due to better economic returns from rice-rice and rice-corn cropping systems as a result of the government's price intervention in rice and corn markets. To maximise the yields of rice and corn, farmers have used chemical fertilisers more intensively and this may lead to unsustainability of the systems. On the other hand, organic rice-soya production system in which organic fertilisers are applied during land preparation for rice cropping has been promoted by many parties. The indirect benefit from such practice of enhancing nitrogen fixation in soya cropping is expected besides the direct benefit in giving nutrients for rice. This experiment is aimed at studying the benefit from the residue of organic fertiliser application in paddy rice cropping of enhancing nitrogen fixation of soya grown after rice as there is still no definite conclusion as to the extent of the fixation occurring in this system.

MATERIALS AND METHODS

The on-farm experiment was conducted at lowland paddy fields of a farmer at Mae Rim district, about 35 km north of Chiang Mai city. Soya (var. Chiang Mai 60 from Chiang Mai Field Crop Research Centre, Chiang Mai) was grown without rhizobial inoculation as a second crop after paddy rice during December 2011 - April 2012. Organic fertilisers of narrow C:N ratios were applied: 1) control (no fertiliser), 2) animal manure (AM): 6.25 tons ha^{-1}, 3) compost (CP): 6.25 tons ha^{-1}, 4) azolla (AZ): 12.5 tons ha^{-1}, 5) AM + CP: 3.125 + 3.125 tons ha^{-1}, 6) AM + AZ : 3.125 + 12.5 tons ha^{-1}, 7) CP + AZ : 3.125 + 12.5 tons ha^{-1}, and 8) AM + CP + AZ: 3.125 +3.125 + 12.5 tons ha^{-1}. All organic fertilisers were applied during land preparation for rice transplanting in July 2011. Their compositions were reported as follows: 1.82%N, 0.82%P, 0.43%K, 1.82%Ca and 0.56%Mg (AM); 1.09%N, 0.48%P, 1.2%K, 2.20%Ca and 0.17%Mg (CP); 3.52%N, 0.27%P, 1.19%K, 1.77%Ca and 2.81%Mg (AZ). The experimental design was a randomised complete block with 4 replications. Each experimental unit of soya was grown in a 4 x 4 m plot with 25 x 25 cm plant spacing. Fallow irrigation technique was managed during soya cultivation. Root sap samples were collected (10 plants/sample) by plastic syringe and transferred into a glass tube with 95% ethanol at 1:1 ratio. The stages at which the sap samples were collected were: V4 (vegetative growh stage, fourth node), R2 (full bloom), R4 (full pod) and R6 (full seed). All the xylem sap samples were kept at -4^{o}C until analysis [2]. The samples were analysed for ureide-N, nitrate-N and amino-N by ureide technique and the relative ureide index (%RUI) was calculated from the molar concentration of ureide, amino and nitrate using the following equation [3, 4]:

$$\%\ RUI = \left[\frac{4 \text{ x Ureide}}{4 \text{ x Ureide} + \text{Amino} + \text{Nitrate}}\right] \text{X } 100$$

Nitrogen fixation (Nfix) was calculated from %RUI using the following equations [3, 4]:

a) %Nfix = 7.7+0.64%RUI for V1-V5 stages
b) %Nfix = 4.8+ 0.83%RUI for R1-R2 stages
c) %Nfix = 21.3+ 0.67%RUI for R3-R6 stages

Shoot and nodule samples were also collected at V4, R2, R4 and R6 stages. All plant samples were oven-dried at 70-73^{o}C for 48 hours and weighed. The nitrogen content in shoot was determined by Kjeldahl digestion and distillation method [5]. Total nitrogen accumulations in shoot at various growth stages were then calculated [5]. Nitrogen uptake from soil was the difference between nitrogen accumulated and nitrogen fixed at R6 stage.

RESULTS AND DISCUSSION

Nodule Dry Weight

The nodule dry weight of soya was lowest in the control treatment with 58, 87, 168 and 143 mg plant^{-1} at V4, R2, R4 and R6 respectively (P<0.05) (Table 1). At V4 stage, the CP and AZ applications provided the highest nodule dry weight at 104 mg plant^{-1}. Generally, the AZ treatment gave higher nodule dry weight than other treatments (P<0.05). Interestingly, at the R4 and R6 growth stages, the nodule mass observed in AM+AZ treatment was at 235 and 212 mg plant^{-1} respectively. The effect of organic fertiliser residue in this experiment is comparable to the former results (117-270 mg plant^{-1}) at V5-R6 stages based on nodule dry weight of the same variety [6]. Other experiments, however, reported nodule dry weight as varying with soya cultivar and environmental conditions [7-9]. From the present experiment, addition of AZ in paddy rice field resulted in the highest nodule dry weight of soya compared to other treatments. This is consistent with a report on rice experiment that showed high organic matter with a high-rate application of AZ [10].

Table 1. Soya nodule dry weight (mg plant^{-1}) at different stages of growth

Treatment	V4	R2	R4	R6
Control	58^{d}	87^{b}	168^{c}	143^{d}
AM	82^{c}	121^{a}	169^{c}	145^{d}
CP	62^{d}	120^{a}	169^{c}	146^{d}
AZ	60^{d}	126^{a}	213^{b}	178^{c}
AM+CP	93^{b}	121^{a}	231^{a}	200^{b}
AM+AZ	84^{c}	123^{a}	235^{a}	212^{a}
CP+AZ	104^{a}	124^{a}	234^{a}	211^{a}
AM+CP+AZ	76^{c}	124^{a}	232^{a}	211^{a}
CV (%)	7.0	3.6	2.3	4.0
F-test	0.05	0.01	0.01	0.05

Note: Means with different letters in each column are significantly different.

Relative Ureide Index

The %RUI was affected most by both AZ and CP+AZ applications at R2, R4 and R6 stages. The data in Table 2 shows that the values of %RUI at V4, R2 and R4 stages were highest at 61, 87 and 82% respectively (P<0.05) when CP+AZ was applied. However, the %RUI in CP+AZ treatment was not significantly different from AZ application (86% and 82% at R2 and R4 respectively). At R6 stage, AZ at 12.5 tons ha^{-1} gave the highest %RUI (76%, P<0.01). The control treatment gave the lowest % RUI at all growth stages.

Total Shoot Nitrogen Accumulation

The content of shoot N uptake of soya appeared to be associated with shoot dry weight (Table 3). The lowest shoot N uptake was found in the control at every stage of growth. Application of AM+CP+AZ gave the highest shoot N uptake at V4, R2 and R4 stages while at R6 stage, the highest shoot N uptake (85 kg N ha^{-1}) was recorded on applying AZ at a rate of 12.5 tons ha^{-1}. In

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general, the total shoot N-uptake is substantially increased with the use of various organic fertilisers [11-13].

Table 2. %RUI of soya at different stages of growth

Treatment	V4	R2	R4	R6
Control	38^{d}	77^{b}	61^{c}	44^{d}
AM	56^{ab}	85^{a}	73^{b}	63^{bc}
CP	44^{cd}	86^{a}	78^{ab}	53^{cd}
AZ	45^{cd}	86^{a}	82^{a}	76^{a}
AM+CP	59^{a}	85^{a}	81^{a}	64^{bc}
AM+AZ	56^{ab}	84^{a}	79^{ab}	66^{ab}
CP+AZ	61^{a}	87^{a}	82^{a}	64^{bc}
AM+CP+AZ	49^{bc}	85^{a}	80^{a}	67^{ab}
CV (%)	8.4	1.5	3.4	7.4
F-test	0.01	0.01	0.05	0.05

Note: Means with different letters in each column are significantly different.

Table 3. Nitrogen accumulation (kg ha^{-1}) in soya at different stages of growth

Treatment	V4	R2	R4	R6
Control	23^{d}	29^{c}	36^{e}	48^{d}
AM	35^{c}	42^{b}	51^{d}	80^{a}
CP	35^{c}	42^{b}	50^{d}	63^{bc}
AZ	40^{ab}	46^{a}	55^{bc}	85^{a}
AM+CP	37^{bc}	42^{b}	53^{cd}	66^{b}
AM+AZ	37^{bc}	40^{b}	55^{bc}	60^{c}
CP+AZ	40^{ab}	47^{a}	58^{b}	63^{bc}
AM+CP+AZ	42^{a}	47^{a}	61^{a}	68^{b}
CV (%)	7.4	5.2	4.9	5.8
F-test	0.05	0.05	0.05	0.05

Note: Means with different letters in each column are significantly different.

Nitrogen Fixation and Nitrogen Uptake from Soil

All organic residues from organic paddy rice had a significant effect on nitrogen fixation (Figure 1) which was highest for AZ (53.9 kg N ha^{-1}). Compatible with other data, the control treatment provided the lowest nitrogen fixation at only 17.5 kg N ha^{-1} ($P<0.05$). Soil nitrogen uptake was determined by total plant nitrogen accumulated minus fixed nitrogen. AM gave the highest amount of nitrogen uptake from soil (32.2 kg ha^{-1}) but there was no significant level between control, CP and AM treatments. CP + AZ treatment gave the lowest amount of nitrogen uptake from soil ($P<0.05$).

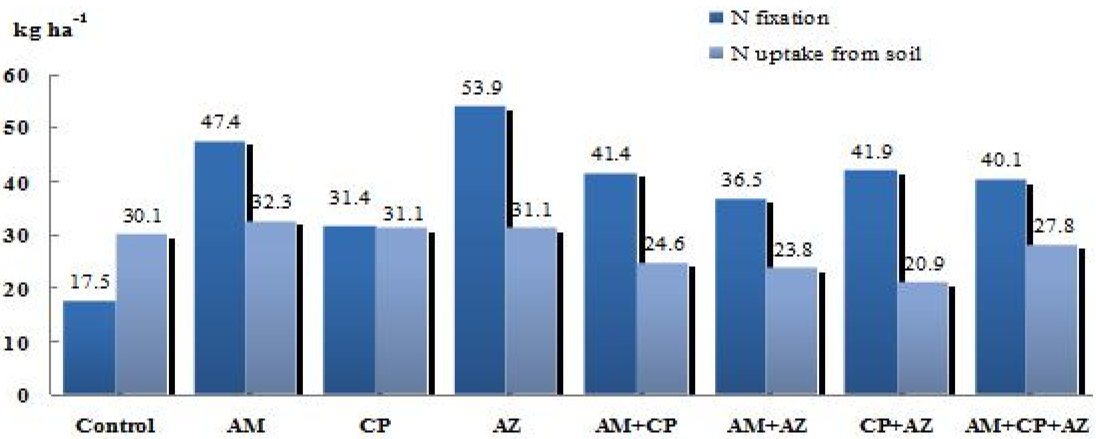

Figure 1. Nitrogen fixation and nitrogen uptake from soil (kg ha^{-1})

The amount of nitrogen uptake appeared to correspond with %RUI, which peaked at R2 stage and then slightly decreased at R4 and R6 stages. Generally, %RUI is high during the stage of flowering to the beginning of seed production [4, 11, 14]. AZ application in paddy rice field was observed to contribute to the highest nitrogen fixation in soya grown after paddy rice. Other organic fertilisers also provided higher nitrogen fixation than did control treatment. This result is supported by research work which reported high levels of nitrogen fixation when soya was grown in conditions without chemical fertilisers or with inoculated rhizobium strains [6, 11-13]. The extent of nitrogen fixation in the present experiment (17.5-53.9 kgN ha^{-1}), however, was less than that obtained in temperate areas (100 kgN ha^{-1}) [15], which may be attributable to the difference in physical and environmental conditions between the temperate and tropical zones.

All of the organic matter used in this experiment had a C:N ratio of less than 24:1, comparable to those used in other studies [16-17]. The C:N ratio can affect the microbial activity in the decomposition of organic matter and mineralisation process [17-18]. Organic fertilisers such as cow manure, hay, compost and azolla were shown to have high concentrations of Cd, Zn, Ni, Fe, Mo and Cr [19-21]. The availability of some elements such as Fe, Mo and Ni could suggest high nitrogen fixation, nodulation forming and seed yield [22-24].

The average efficiency of nitrogen fixation was 57.4% (Table 4). CP +AZ gave the highest efficiency at 67.0%, although this was not significantly different from the AZ treatment. However, all organic fertiliser applications gave an efficiency of at least 50%, which was higher than that obtained in control treatment. The residues from the organic fertilisers could enhance the activity of endophytic bacteria and the rhizobium population around the rhizosphere zone between rice and soya [25]. Table 5 shows soya seed yields subsequently obtained. Apparently, the control treatment provided the lowest yield level and the addition of all organic fertilisers proved to enhance the soya seed yield, with AM+CP+AZ giving the highest yield (1,463 kg ha^{-1}). The average yield was 1,310 kg ha^{-1}, which was coparable to one at 1,656 kg ha^{-1} obtained in a former study performed in the same district in 1999 to increase the efficiency of soya production with chemical fertilisers on the same variety [26].

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Table 4. Nitrogen fixing efficiency of soya (% of total N uptake at R6 stage)

Treatment	N fixing efficiency
Control	36.8 %
AM	59.5 %
CP	50.2 %
AZ	63.5 %
AM+CP	62.7 %
AM+AZ	60.6 %
CP+AZ	67.0 %
AM+CP+AZ	59.0 %

Table 5. Soya seed yield (kg ha^{-1})

Treatment	Yield (kg ha^{-1})
Control	981^{b}
AM	1294^{a}
CP	1225^{ab}
AZ	1331^{a}
AM+CP	1256^{a}
AM+AZ	1238^{ab}
CP+AZ	1369^{a}
AM+CP+AZ	1463^{a}
CV (%)	10.61
F-test	0.01

Note: Means with different letters in each column are significantly different.

CONCLUSIONS

The application of organic fertilisers before paddy rice land preparation significantly enhanced the relative ureide index and nitrogen uptake of the following soya crop at all studied stages. The advantage came from high microbial nitrogen fixation compared to control as shown in the R6 stage. The residues from the application of organic fertilisers of narrow C:N ratios during land preparation for rice cropping four months before growing soya thus clearly promote nitrogen fixation by native rhizobia. Application of organic fertilisers such as animal manure, compost, azolla or their combinations is therefore confirmed and suggested as a good practice of sustainable management of rice-soya cropping system in lowland paddies.

ACKNOWLEDGEMENTS

The financial support for this work from the National Research Council of Thailand (NRCT) is gratefully acknowledged.

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