Nitrogen Fixation Efficiency of *Azospirillum largimobile* in System of Rice Intensification: SRI

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Introduction

Biological nitrogen fixation is an important process which contributes N to the plants. *Azospirillum largimobile*, an aerobic free living microorganism, has been reported to fix considerable amount of N in many crops. However, under conventional rice growing system its N fixation may be limited by oxygen availability. The system of rice intensification (SRI) is based on careful soil and water management. This system facilitates the oxygen translocation into the soil which may enhance soil microbial activities including N fixation. The objective of this study was to determine the effect of *A. largimobile* inoculation and rice systems on bacterial population, nitrogen fixation efficiency and rice yield.

Materials and Methods

Experimental site and design

Two pot experiments were conducted at Suranaree University of Technology, Nakhon Ratchasima, Thailand.

In the first experiment, 3 methods of *A. largimobile* inoculation to rice plants: 1) seed inoculation 2) seedling inoculation and 3) soil inoculation, were tested in a CRD with 4 replications. The population of *A. largimobile* was determined from the rhizophere by Most Probable Number (MPN) method.

In the second experiment, the effects of rice growing systems and *A. largimobile* soil inoculation on nitrogen fixation efficiency, rice yield and yield components were studied. The experimental design was 2x2 factorial in RCBD with 4 replications. Factor 1 was systems of rice cultivation: 1) SRI and 2) conventional system. Factor 2 was *A. largimobile* inoculation: 1) control and 2) A. largimobile inoculation. In SRI, 15 days old seedlings were transplanted with a single plant/hill while in CS 30 days old seedlings were transplanted with 3 plants/hill. Water level under CS was maintained at 30 cm through out the growing period, while under SRI the soil surface was not continuous flooded during tillering to flowering stage. The population of *A. largimobile* from the soil was measured. Nitrogen fixation efficiency of N-fixing bacteria around the roots was determined at tillering and flowering stage by Acetylene Reduction Assay (ARA) method. Yield and components of yield were determined at 120 days after planting.

Results and Discussion

Experiment 1

The results showed that the method of soil inoculation had the highest population of *A. largimobile* in the rhizophere (Table 1).

Table 1 Effect of inoculation method on *A. largimobile* population in rhizophere of rice.

	A. largimobile (cell g^{-1})	
Treatment	5 DAP	30 DAP
Seed inoculation	$8.5x10^6$ b	$2.9x10^4$ b
Seedling inoculation	$8.5x10^6$ b	$5.8x10^3$ b
Soil inoculation	$2.9x10^8$ a	$1.5x10^7$ a
Control	$1.5x10^2$ c	$1.9x10^2$ c
CV%	8.79	18.79

*Means in a column followed by the same letters are not significantly different at 5% level by DMRT

Experiment 2

N fixation efficiency

There was a significant interaction between rice growing system and *A. largimobile* inoculation on N fixation efficiency. In general, SRI enhanced N fixation efficiency and *A. largimobile* inoculation resulted in higher N fixation efficiency than un-inoculation at seedling to flowering stage (Table 2). However, the magnitude of the differences between inoculation and un-inoculation was greater under SRI than under CS.

Table 2 Effect of rice growing systems and *A. largimobile* inoculation on nitrogen fixation efficiency.

Treatment		Nitrogenase activity (µmole C_2H_4 $plant^{-1}$ day^{-1})	
		Tillering	Flowering
CS	Control	4 b	16 b
	A.. largimobile	147 b	34 a
SRI	Control	21 b	8 b
	A.. largimobile	910 a	44 a
	CV %	3.18	4.50

Means in a column followed by the same letters are not significantly different at 5% level by DMRT

Fig. 1 System of rice intensification (SRI)

Fig. 2 Conventional system (CS)

Microbial population

There was a significant interaction between *A. largimobile* inoculation and growing systems on the population of *A. largimobile* (Table 3). In general SRI promoted the bacterial growth and its population increased when the inoculants were added. However, the magnitude of the increase was greater under SRI than under conventional system.

The results indicated that SRI is suitable for free living N-fixing bacteria such as *A. largimobile* growth and activities. *A. largimobile* is a heterotrophic aerobic rhizobacteria. Its growth and activities are dependent on oxygen availability (Kanungo et al, 1997). Under CS, oxygen contents around the roots may not be sufficient for the respiration of roots and rhizobacteria. The water management under SRI which maintains low water level and leave the soil surface expose to the air in some periods could facilitate the oxygen diffusion into the soil and enhance the bacteria growth and its activities including N fixation.

Table 3 Effect of rice growing systems and *A. largimobile* inoculation on *A. largimobile* population in the soil.

Treatment		*A. largimobile* (cell g^{-1})	
		Tillering	Flowering
CS	Control	$3.1x10^2$ b	$1.7x10^1$ b
	A.. largimobile	$3.9x10^4$ b	$2.6x10^4$ b
SRI	Control	$1.3x10^4$ b	$3.1x10^3$ b
	A.. largimobile	$1.3x10^9$ a	$3.1x10^8$ a
	CV %	26.3	35.58

Means in a column followed by the same letters are not significantly different at 5% level by DMRT

Yield and components of yield

Panicle number, % good grains and grain weight in SRI were significantly higher than those in CS, but they were not significantly different between *A. largimobile* inoculation and un-inoculation in both systems (Table 4). Grain yield was significantly greater under SRI than under CS. *A. largimobile* inoculation tended to produce more yield than control but not statistically significant.

This result is in agreement with Stoop, et al. (2002) who found that SRI increased yield up to 5-10 t ha^{-1} when compared with CS. Higher yield in SRI than in CS may be partly due to the effect of microbial activities such as decomposition and N fixation. However, *A. largimobile* inoculation which enhanced N fixation in this experiment had little effect on rice yield. This could be due to high amount of organic fertilizer was applied to the soil, therefore N was not limited.

Table 4 Effect of rice growing systems and *A. largimobile* inoculation on yield and yield components.

Treatment		yield components				grain yield (g/pot)
		panicle /hill	grains/ panicle	Good grains (%)	100 grains weight (g.)	
SRI	Control	13 a	160 a	90 a	2.8 ab	301 a
	A.largimobile	14 a	132 ab	87 a	3.0 a	314 a
CS	Control	7 c	157 a	77 b	2.6 b	169 b
	A.largimobile	11 b	121 b	81 b	2.6 b	188 b
	CV %	12.5	15.6	14.3	8.5	25.2

Means in a column followed by the same letters are not significantly different at 5% level by DMRT

Conclusion

The method of soil inoculation was the best method for *A. largimobile* inoculation for rice plants. The rice growing systems affected the microbial population, nitrogen fixation, yield and components of yield. Bacterial population and nitrogen fixation efficiency increased with *A. largimobile* inoculation but the magnitude of the increase was greater under SRI than under CS. Yield and components of yield in the SRI were significantly higher than those in the CS. However, they were not significantly different between *A. largimobile* inoculation and un-inoculation in both systems.

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