

EFFET OF INOCULATION WITH *AZOSPIRILLUM* *BRAILENSE* NO40 ISOLATED FROM EGYPTIAN SOILS ON RICE GROWTH IN CHINA

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Abstract

A field experiment was conducted to investigate the effect of inoculation with *Azospirillum brasilense* NO40 isolated from Egypt on rice growth and yields under different nitrogen fertilizer levels at Dongyan county of China. Results showed that irrespective of the nitrogen fertilizer level and rice age counts of colonies developed on nitrogen-free medium isolated from rice rhizosphere and root were increased by inoculation with *Azospirillum brasilense* NO40 compared to the control. Plant height, chlorophyll content of the newly developed rice leaves were significantly increased due to inoculation with *Azospirillum brasilense* NO40.

INTRODUCTION

Members of the genus *Azospirillum* are soil-born-nitrogen-fixingbacteria, living in association with roots of plants, were investigated to establish their effects on plant growth and yield (Tyler *et al.*, 1979 and Mertens and Hess, 1984). *Azospirillum* is very common in the rice rhizosphere (Khammas *et al.*, 1989 and Omar *et al.*, 1989b) and strains have also been isolated from maize and wheat roots (Fages and Mulard, 1988). Many studies indicated the importance of *Azospirillum* in fixing nitrogen for cereals (O'Hara *et al.*, 1984; Mertens and Hess 1984; Omar *et al.*, 1989a and Zhang *et al.*, 1990). These bacteria proved to be able to produce auxins and other growth substance in plant rhizosphere (Tien *et al.*, 1979; Inbal and Feldman, 1982; Jain and Partiquin, 1985 Kapulnik *et al.*, 1985). The concentration of nitrogen in the plant aerial tissue increased by *Azospirillum* inoculation (Kapulnik *et al.*, 1981 and Sarig *et al.*, 1984). This might indicate that *Azospirillum* inoculation affected the nitrogen assimilation of host plant.

In Egypt, rice is intensely cultivated in the Nile Delta, and inoculation by *Azos-*

pirillum brasilense NO40 has already shown positive effects on yield in five trials conducted between 1985 and 1990 at the experimental station of Sakha (Omar *et al.*, 1992).

The present study aimed at investigating the beneficial effect of inoculation with *Azospirillum brasilense* NO40 on the growth of rice in temperate agricultural ecosystem in China.

MATERIALS AND METHODS

1. Treatments.

A split plot design of a field experiment with 3 replicates was carried out, on the paddy soil at Dongyan county of Zhejiang province of China in 1998, to test the effect of inoculation with *Azospirillum brasilense* NO40, isolated from Nile Delta soil in Egypt, on rice growth. The isolation of this nitrogen-fixing bacterium was performed using the spermosphere model technique (Omar *et al.*, 1989b). Three doses of nitrogen fertilizer (Urea N 46%) 0, 75, 150 N kg/ha represent the mainplots and two treatments of inoculation, with and without *Azospirillum brasilense* NO40, represent the subplots (3.5 x 4 m). Inoculation was performed by the dipping of germinated rice seeds (Jiayue 280) in bacterial culture which were grown on Watanabe medium for 72 hours (Omar *et al.*, 1989b), thereafter seeds were left for drying before sowing. This treatment provided a final density of ca. 10^6 cells per seed. Control treatments were treated, as inoculated, by dipping in culture but without bacteria. The treated seeds were sown in the field.

2. Test methods.

The rice agronomic and physiological properties of each subplot determined include plant height measured at tillering and milking stages, rice nitrogen status detected by using Chlorophyll Meter (SPAD 502) which is a light-weight, hand-held meter for quick, nondestructive and on site measurement of leaf chlorophyll content developed by the Soil-Plant Analysis Development (SPAD) Section of Minlat camera; ear number counted and estimated before harvesting; grain number and weight of 1000 grains recorded after harvesting and rice grain yield.

3. Bacterial count.

The plate count method was used to enumerate the numbers of bacteria grown on N-free medium (Watanabe) isolated from rice rhizosphere, root and soil (Omar, *et*

al., 1989b). The plate count procedure was as follows: 5 g rice root with adhered soil, 5 g washed and milled rice root as well as 5 g soil were taken from every treatment for counting the number of nitrogen-fixing bacteria on rhizosphere, rice root and soil, respectively. Each sample was put into a 150 ml flask with 45 ml physiological saline, after 30 min shaking, a series of 10^{-1} to 10^{-7} dilutions were prepared. The 10^{-3} to 10^{-8} dilutions were used for plate counting.

RESULTS AND DISCUSSION

1. Variation in N_2 -fixing populations in rice rhizosphere, root and soil as affected by inoculation.

From Figures 1 and 2, it is clear that bacterial populations on nitrogen-free medium isolated from rice rhizosphere and root were apparently increased by inoculation with *Azospirillum brasilense* NO40 compared to control, irrespective of nitrogen fertilizer level or growing stages. However, there was no difference between the two inoculating treatments in the bulk soil at both the tillering and milking stages. At milking stage, population of bacteria grown on nitrogen-free medium of non-inoculated treatment were increased compared to those at tillering stage. These results imply that an associative relationship had been found between the introduced diazotroph and the host plant.

2. Effect of inoculation on rice growth

2.1. Effect on rice leaf chlorophyll content

It is well known that the rice leaf chlorophyll content is an index of its nitrogen nutrition status (Tao *et al.*, 1990 and Peng *et al.*, 1993 & 1996). To detect rice nitrogen nutrition status at tillering and milking stages, the SPAD values (chlorophyll meter reading) of newly developed rice leaves were taken. The results of multiple comparison after ANOVA showed that the SPAD values were significantly affected not only by the nitrogen fertilizer levels but by the NO40 inoculation treatments as well and the significant levels of their effects reached 1% and 5% at tillering and milking stages, respectively. About 2 grades of SPAD value at tillering stage and 3 grades at milking stage were increased by inoculation in contrast to control (Table 1). This evidence may be recognized as the positive effect of inoculation with *Azospirillum* on rice nitrogen nutrition.

2.2. Effect on rice plant height

The plant heights were recorded at tillering and milking stages (Table 1). It was found by ANOVA that both nitrogen fertilizer level and inoculation significantly affected rice plant heights at the two growing stages. It is evident that as nitrogen fertilizer level elevated, the rice plant heights increased, which suggests that nitrogen supply is a limiting factor determining rice growth. The plant heights of inoculated treatments were about 3 and 6 cm higher than those of control at tillering and milking stages, respectively. This finding showed another evidence of the positive effect of inoculation on rice nitrogen nutrition.

3. Effect of inoculation on rice yield

The data of ear numbers, grain numbers per ear and the weight of 1000 grains as well as the estimated rice grain yield are illustrated in Table 2. It could be seen that as nitrogen fertilizer level elevated the ear numbers and the grain numbers per ear as well as the estimated yield increased but the weights of 1000 grains did not change. The positive effect of inoculation with *Azospirillum* on the estimated rice yield could be found at low nitrogen fertilizer levels. However, under high nitrogen fertilizer level there was no positive effect.

Conclusion

From the aforementioned results, it could be concluded that the nitrogen-fixing bacteria *Azospirillum brasilense* NO40 isolated from Nile Delta of Egypt could not only establish the associative relationships with rice in temperate agricultural ecosystem in China, but also show positive effects on rice growth which implies the possible use of this nitrogen-fixing bacterial strain as inoculant strain in rice fields in China. However, to enhance the positive effects of inoculation with this strain on rice growth, the way about how to improve the inoculation method should be further studied.

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Table 1. Multiple comparison of SPAD values and plant heights (cm).

Growing stage	Traits	N-fertilizer levels (kg/ha)			Inoculation treatments	
		0	75	150	Control	Inoculation
Tillering	SPAD value	27.8B	30.0A	35.6A	29.2B	33.1A
	Plant height	55.8b	59.4b	65.8a	58.1b	62.5a
Milking	SPAD value	31.3c	33.9b	35.8a	32.8b	34.6a
	Plant height	73.2b	78.1b	83.9a	75.0b	81.2a

* The significant level is 1% between different capital letters and 5% between different small letters.

Table 2. Yield components of the different treatments.

Treatment N fertilizer (Kg/ha)	Bacteria	Ear number (10^6 ha^{-1})	Grain number (per ear)	Weight of	
				1000 grains (g)	Grain yield (ton ha^{-1})
0	Control	2.84	53.2	27.5	4.15
	Inoculation	2.82	72.2	26.9	5.48
75	Control	2.98	68.9	27.4	5.62
	Inoculation	3.32	63.2	27.2	5.71
150	Control	3.06	87.2	26.4	7.04
	Inoculation	3.43	75.9	26.8	6.91

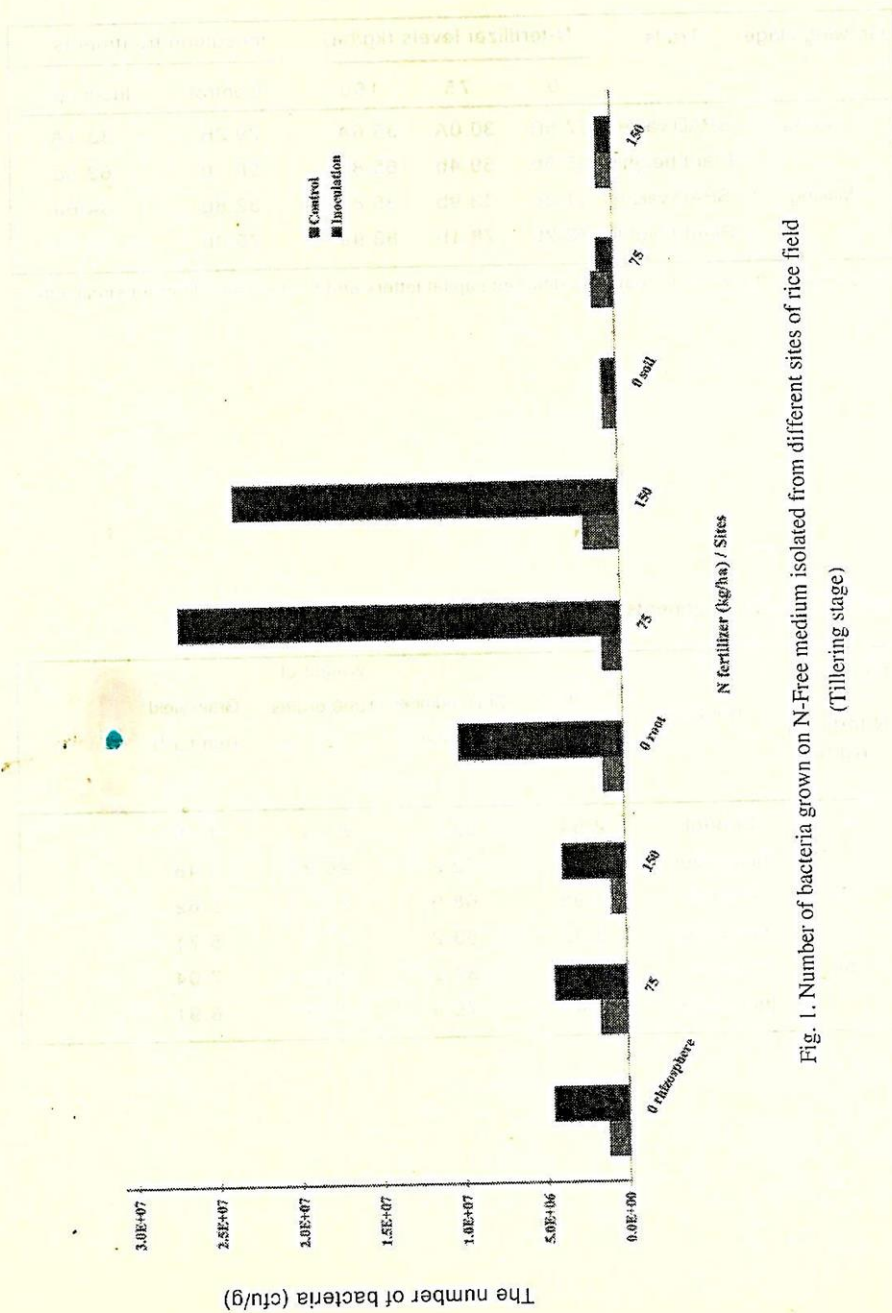


Fig. 1. Number of bacteria grown on N-Free medium isolated from different sites of rice field (Tillering stage)

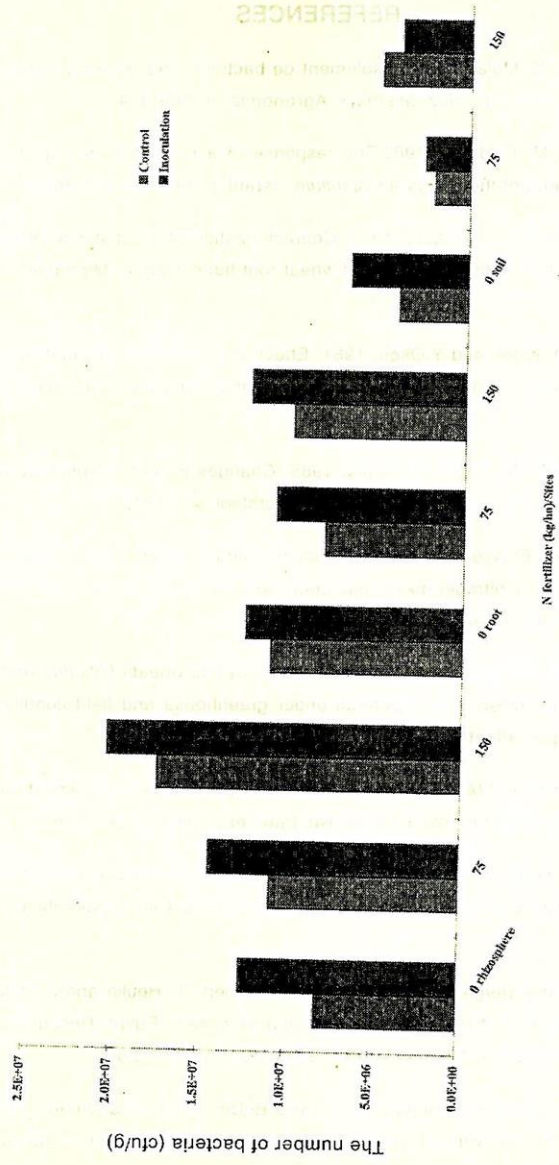


Fig. 2. Number of bacteria grown on N-Free medium isolated from different sites of rice field (Milking stage)

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تأثير التلقيح بالازوسبيريلا برازيلينز سلالة NO40 المعزولة من الأراضي المصرية علي محصول الارز في جمهورية الصين

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اقيمت تجريره حقلية لدراسة تأثير التلقيح البكتيري باستخدام سلالة الازوسبيريلا برازيلينز سلالة NO40 والمعزولة من الأراضي المصرية علي نمو محصول الارز تحت مستويات تسميد ازوتي مختلفة في مقاطعة دونجيان بالصين واطهرت النتائج ان الاعداد البكتيرية التي نمت علي بيئته خالية من النيتروجين والمعزولة من منطقة جذور الارز والريزو سفير الملحقه زادت بالمقارنة عن الاعداد الاخرى المعزولة من المعاملات الغير ملقحة ووجد ان فترات النمو واطوال النباتات والمحتوي الكلورفيلي لورقة الارز كانت معنوية نتيجة التلقيح البكتيري بالازوسبيريلا.