RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2015.41.305

ScienceAsia 41 (2015): **305–314**

Application of blue-green algae and mineral fertilizers to direct seeding lowland rice

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Received 4 Jun 2014
Accepted 16 Sep 2015

ABSTRACT: Fertilizer treatments consisting of cyanobacteria blue-green algae (BGA, *Anabaena* sp.) combined with nitrogen, phosphorus, and potassium were compared with untreated control rice variety Chainat 1 in greenhouse and field experiments from December 2011 to March 2012. In the greenhouse experiment, application of BGA resulted in significantly higher phosphorus and potassium in seeds compared with the untreated control. BGA significantly increased seed weight, and the difference was 16% compared to 47% between application of NPK 16-16-8 + NPK 46-0-0 (125 kg/ha) of chemical fertilizer and untreated control. In field experiments, the differences between BGA and untreated control for nitrogen, phosphorus, and potassium in seeds and straws were not significant, and application of NPK 16-16-8 + NPK 46-0-0 of chemical fertilizer was highest for nitrogen, phosphorus, and potassium in seeds and straws. Application of NPK 16-16-8 + NPK 46-0-0 of chemical fertilizer gave the highest straw dry weight, total dry weight, and seed weight, but the treatments gave similar 1000-seed weight. Application of BGA clearly increased growth and yield of rice in a greenhouse, but the effects were not clear in the field. This information is useful for organic rice production.

KEYWORDS: chemical fertilizers, cyanobacteria, nutrient uptake, seed weight

INTRODUCTION

Nitrogen-fixing blue-green algae (BGA, *Anabaena* sp.) grow abundantly in tropical regions and are particularly common in paddy fields[1]. Cyanobacteria or blue green algae are a diverse group of prokaryotes that often form complex associations with bacteria and green algae in structures known as cyanobacterial mats. They are the major N fixers in freshwater and marine systems[2]. The most vigorous nitrogen-fixing BGA have been assessed for use as green fertilizer in rice fields and favourable effects have been reported in India[3] and other countries[4,5]. Application of BGA in the rice paddy fields promotes rice growth and yield[6,7]. The BGA inoculum with N:P:K (30:20:20 kg/ha) was found to be the most effective treatment for rice productivity[8].

A pretreatment of rice seedlings with a blue-green algal culture and/or inoculation of the field with a composite inoculum of nitrogen-fixing BGA however does not significantly increase rice yield[4]. Although the use of BGA in rice production is a low-cost technology, it has many constrains that prevent its success. Factors such as phosphate deficiency, presence of high concentrations of nitrogen in flood water, low pH, and arthropod grazer population limit the growth and nitrogen fixing activities of cyanobacteria in rice fields[9].

The use of BGA for rice production is a promising strategy to reduce the dependence on expensive chemical fertilizers. Integrated management of rice production reduces production cost, and transplanting is currently replaced by direct seedling. The efficacy of BGA in promoting rice growth and yield under field conditions of direct seedling rice is still unsolved, and detailed studies on the efficacy of BGA in promoting rice growth and yield are necessary. This study therefore reports the effects of the BGA in rice cultivar Chainat 1.

The objectives of this study were to investigate

Table 1 Chemical and physical properties of soils at three locations in the experiments.

Locations	pH	EC (dS/m)	CEC (cmol/kg)	O.M. (%)	Total N (%)	P (mg/kg)*	K (mg/kg)†	Ca (mg/kg)‡
Greenhouse	5.47	0.056	27.67	1.29	0.048	5.36	104.0	2490
Maha Sarakham	5.82	0.137	8.45	0.87	0.031	8.16	55.7	620
Kalasin	4.75	0.086	14.59	3.04	0.117	9.95	105.5	1234
		Sand (%)	Silt (%)	Clay (%)		Classification		
Greenhouse		65.9	32.0	2.1		Sandy loam		
Maha Sarakham		89.9	8.0	2.1		Sand		
Kalasin		67.9	23.8	8.3		Sandy loam		

* Available P; † exchangeable K; ‡ exchangeable Ca.

the effects of blue green algae on nutrient uptake of rice, to determine the effects of BGA on the growth and yield of rice under greenhouse and field conditions and to compare biofertilizer of BGA with chemical fertilizers. The information will be useful for the application of BGA for rice production.

MATERIALS AND METHODS

Two experiments were conducted to investigate the effects of blue green algae on growth, yield and nutrient uptake of rice Chainat 1 during December 2011 to March 2012. A greenhouse experiment was conducted at the Faculty of agricultural technology, Rajabhat Maha Sarakham University in the Northeast of Thailand, and field experiments were conducted at two locations in Maha Sarakham and Kalasin provinces.

Greenhouse experiment

Soil was collected from the rice field and the soil samples were analysed for physical and chemical properties before planting and after harvesting. The soil samples were air-dried under shade, crushed into powder, and sieved through the screen of 80 mesh. The soil samples were analysed for physical and chemical properties. Soil particles were analysed using a hydrometer method[10]. Total nitrogen was analysed using Kjeldahl method[11]. Available phosphorus was analysed using Bray II method[10]. Exchangeable potassium and exchangeable calcium were analysed using NH_4OAc and atomic absorption spectrophotometry[12]. Soil pH was analysed using standard glass electrode[13]. Cation exchange was analysed using a method described previously[14] and organic matter was analysed using Walkley and Black method[11]. Electrical conductivity was measured using the method described by Rhoades[15]. Data for soil analysis are presented in Table 1.

Rice variety Chainat 1 used in this study is a high-yielding variety, non-glutinous, photoperiod insensitive, and is suitable for growing in irrigated areas where direct seedling is generally practised. The rice seeds were sowed in large containers of 80 cm in diameter and 50 cm in height. The containers were filled with dry soil, and the seeds were sowed with spacing of 20 × 20 cm at the seed rate of 3–5 seeds per hill. Water was supplied to the containers soon after sowing, and soil moisture was maintained at field capacity. The seedlings were thinned to obtain 1 plant per hill at 7 days after emergence.

Five treatments consisting of no fertilizer control, BGA, BGA + NPK 0-16-8, BGA + NPK 16-16-8, and NPK 16-16-8 + NPK 46-0-0 were arranged in a completely randomized design with six replications. NPK 0-16-8 or NPK 16-16-8 was applied 15 days after emergence (DAE) at the rate equivalent to 187.5 kg/ha, and NPK 46-0-0 was applied 30 days before anthesis at the rate equivalent to 125 kg/ha. The calculation of fertilizers was based on the rates applied in the field.

Sample unit was one container and there were 30 containers totally. BGA is a commercial product in liquid form kindly provided by the Agro-biomate Co., Ltd. The biofertilizer of 1 l was diluted with clean water of 30 l and spayed to the treatments at 15 DAE. Sufficient water was supplied to the experiment, and water level was maintained at 10 cm at tillering until near harvest.

Data for growth and development were recorded for the number of tillers at anthesis and harvest, plant height at 30, 45, 60, 75, and 90 DAE and days to flowering. SPAD (soil plant analysis development) chlorophyll meter reading (SCMR) was also recorded at 30, 45, 60, 75, and 90 DAE. This parameter was used as a surrogate trait for chlorophyll density in leaves.

Data for yield and yield component were recorded at harvest. These included 1000-seed weight, number of panicles per hill, number of seeds per panicle, number of filled seeds, number of unfilled seeds, seed weight, and total dry weight (without root). Harvest index was also calculated as the ratio of seed weight to total dry weight.

Nitrogen, phosphorus, and potassium in straws and seeds were analysed at days to anthesis 75% and harvest. Samples in each plot were analysed for nitrogen by micro-Kjeldahl method and the concentrations of nitrogen were determined by automated indophenol method [16]. Phosphorus was analysed by wet oxidation using perchloric acid and HNO_3 and vanadomolybdate colour was measured by a spectrophotometer at wavelength of 420 nm. Potassium was analysed by wet oxidation, and the concentrations were measured using atomic absorption spectrophotometer [12]. Nutrient uptake for each sample was expressed as g/m^2.

Field experiment

Soil samples were taken from the farmer's fields before rice planting and after rice harvesting at the depth between 0 and 15 cm. The soil was air-dried and sieved. The methods for soil preparation and analysis for soil physical and chemical properties were the same as in greenhouse experiment. The soil data are presented in Table 1.

The experiment was conducted at farmer's fields in Maha Sarakham and Kalasin provinces. Rice variety Chainat 1 was used in this study. Treatment consisting of no fertilizer control, BGA, BGA + NPK 0-19-8, BGA + NPK 16-16-8 and NPK 16-16-8 + NPK 46-0-0 were arranged in a completely randomized design with four replications for the two locations. NPK 0-16-8 was applied at 15 DAE at the rate of 187.5 kg/ha. NPK 16-16-8 was applied at 15 DAE at the rate of 187.5 kg/ha, and NPK 46-0-0 was applied 30 days before anthesis at the rate of 125 kg/ha. Blue-green algae was applied to the crop as mentioned in the greenhouse experiment.

The plants were grown in plots of 10×10 m for each plot with the alleys of 1 m which were separated by two rice bunds. Conventional soil preparation was practised. The soil was ploughed for twice and puddled. The seeds were sowed at the rate of 93.75 kg/ha. Weeds were controlled manually and regularly. Irrigation was also available as crop need by using water in farm ponds near the experimental sites. Level of water was maintained at 10–15 cm at tillering until near harvest.

Data analysis

ANOVA was performed for greenhouse data according to a completely randomized design and field data according to randomized complete block design, Duncan's multiple range test was used to compare means [17]. All calculations were done using MSTAT-C [18]. Unless indicated otherwise, statistically significant means $p \leqslant 0.05$.

RESULTS

Soil properties

Soil types were classified as sandy loam in greenhouse and Kalasin and as sand in Maha Sarakham. The soils in greenhouse and Maha Sarakham were acidic (pH = 5.47, 5.82) whereas that in Kalasin was highly acidic (pH = 4.75) (Table 1). The soils in all locations were not saline as the electrical conductivity values were low, ranging from 0.056–0.137 dS/m. The soil in greenhouse has high cation exchange capacity (CEC) (27.67 cmol/kg), but in Maha Sarakham had low CEC (8.45 cmol/kg), and in Kalasin it had moderate CEC (14.59 cmol/kg).

The soils in greenhouse and Maha Sarakham were low in organic matter (1.3%, 0.87%) and total nitrogen (0.048%, 0.031%), whereas the soil in Kalasin was rather fertile, having moderate organic matter (3%) and total nitrogen (0.117%). All soils in the experiments had insufficient available phosphorus, ranging from 5.36–9.95 mg/kg. The soil in Maha Sarakham was low in exchangeable phosphorus (55.7 mg/kg) and exchangeable calcium (620 mg/kg), whereas the soils in greenhouse and Kalasin were sufficient for exchangeable phosphorus (104.0 and 105.5 mg/kg) and exchangeable calcium (2490 and 1234 mg/kg).

Nutrient uptake

In greenhouse, BGA was greater than control for nitrogen, phosphorus and potassium in seeds. Although they were not significantly different for nitrogen, differences in phosphorus and potassium between control and BGA were significant ($p \leqslant 0.01$) (Table 2). There was no significant difference between control and BGA for nitrogen, phosphorus and potassium in straws. However, nitrogen, phosphorus and potassium in seeds and straws were increased with high application of fertilizers, and NPK 16-16-8 + NPK 46-0-0 was the highest for nitrogen, phosphorus and potassium in seeds and straws.

Although nitrogen concentration (17.69 kg/ha) in seeds of rice variety Chainat 1 grown in the field in Maha Sarakham was much higher than in Kalasin (12.56 kg/ha), the difference was not statistically significant because of high variation in environments as indicated by high C.V. values

Table 2 Means for N, P, and K in seeds and straws of Chainat 1 rice grown in greenhouse.

Treatments†	Seeds			Straw		
	N (g/m^2)	P (g/m^2)	K (g/m^2)	N (g/m^2)	P (g/m^2)	K (g/m^2)
NPK 16-16-8 + NPK 46-0-0	5.04^{a}	1.31^{a}	1.46^{a}	8.11^{a}	0.79^{a}	20.61^{a}
NPK 16-16-8 + BGA	2.15^{b}	1.22^{a}	1.33^{a}	2.83^{b}	0.56^{b}	15.60^{b}
NPK 0-16-8 + BGA	1.11^{c}	0.52^{bc}	0.69^{c}	1.27^{c}	0.34^{c}	8.65^{c}
BGA	1.34^{c}	0.67^{b}	0.89^{b}	1.39^{c}	0.33^{c}	8.25^{c}
Control	0.85^{c}	0.46^{c}	0.60^{c}	1.22^{c}	0.35^{c}	8.37^{c}
C.V. (%)	29.5	16.9	16.1	23.7	20.8	23.9

† In this Table and the following: NPK 0-16-8, NPK 16-16-8, and NPK 46-0-0 are chemical fertilizers of nitrogen, phosphorus, and potassium applied at the rates of 187.5, 187.5, and 125 kg/ha, respectively.
In this Table and the following: Means in the same column followed by the same letter were not significantly different at 0.05 probability level by Duncan's multiple range test.

Table 3 Means for N, P, and K in seeds and straws of Chainat 1 rice grown in the field.

Locations/treatments	Seeds			Straws		
	N (kg/ha)	P (kg/ha)	K (kg/ha)	N (kg/ha)	P (kg/ha)	K (kg/ha)
Maha Sarakham	17.69	4.25	80.87^{a}	10.81	4.37	4.50
Kalasin	12.56	3.00	59.62^{b}	11.06	4.75	4.94
NPK 16-16-8 + NPK 46-0-0	24.69^{a}	5.81^{a}	102.44^{a}	18.75^{a}	6.81^{a}	6.59^{a}
NPK 16-16-8 + BGA	12.56^{b}	3.25^{b}	67.81^{b}	11.31^{b}	4.31^{b}	4.61^{b}
NPK 0-16-8 + BGA	14.37^{b}	3.25^{b}	56.12^{b}	8.37^{b}	3.94^{b}	4.34^{b}
BGA	12.62^{b}	3.00^{b}	61.69^{b}	8.19^{b}	3.87^{b}	4.18^{b}
Control	11.50^{b}	2.87^{b}	63.19^{b}	8.00^{b}	3.81^{b}	4.06^{b}
C.V. (%)	34.1	28.2	19.1	24.3	28.4	25.8

(34%) (Table 3). Maha Sarakham was also higher than Kalasin for phosphorus and potassium in seeds and nitrogen in straws, but it had lower concentrations of phosphorus and potassium in straws. A significant difference between Maha Sarakham and Kalasin was found for the concentrations of potassium in seeds only, and potassium concentration (80.87 kg/ha) in seeds in Maha Sarakham was significantly ($p \leqslant 0.05$) higher than in Kalasin (59.62 kg/ha).

Concentrations of nitrogen phosphorus and potassium in seeds and straws of rice variety Chainat 1 grown in the field followed the same pattern. Generally, the concentrations of nitrogen, phosphorus, and potassium increased with high application of fertilizer although the concentrations of potassium in seeds were slightly confounding as NPK 16-16-8 + BGA had slightly lower concentration compared to BGA and control. However, NPK 16-16-8 + BGA, NPK 0-16-8 + BGA, BGA, and control were not statistically different, but they were significantly ($p \leqslant 0.05$) lower than NPK 16-16-8 + NPK 46-0-0 for nitrogen, phosphorus, and potassium from seeds and straws. Only the application of fertilizers at the highest dose was significantly ($p \leqslant 0.05$) higher than other doses.

Plant height

Plant height was measured at 30, 45, 60, 75, and 90 DAE in field experiment and greenhouse experiment. There were two locations in Maha Sarakham and Kalasin in field experiment, and there was only one greenhouse in greenhouse experiment. Except for location difference, the treatments were the same in field experiment and greenhouse experiment, and the results of the experiments were compared directly. The rice grown in Kalasin was significantly ($p \leqslant 0.05$) higher than Maha Sarakham for plant height at 30, 45, 60, and 75 DAE, but the difference was not statistically significant at 90 DAE (Table 4).

In field experiment and in greenhouse experiment, the plants treated with BGA were higher than those untreated control at 30, 45, 60, 75, and 90 DAE although most differences were not significant except in greenhouse experiment at 75

Table 4 Means for plant height (cm) of Chainat 1 rice.

Locations/treatments	Field experiment (days)					Greenhouse experiment (days)				
	30	45	60	75	90	30	45	60	75	90
Maha Sarakham	38.6^{b}	48.6^{b}	59.1^{b}	65.0^{b}	83.8					
Kalasin	45.2^{a}	55.6^{a}	63.6^{a}	76.1^{a}	80.7					
NPK 16-16-8 + NPK 46-0-0	44.9^{a}	55.1^{a}	63.5^{ab}	76.4^{a}	90.2^{a}	50.3^{a}	67.0^{b}	70.4^{b}	86.8^{b}	110.3^{a}
NPK 16-16-8 + BGA	44.8^{a}	55.0^{a}	65.7^{a}	73.3^{ab}	82.9^{b}	52.1^{a}	70.7^{a}	75.9^{a}	91.1^{a}	104.8^{b}
NPK 0-16-8 + BGA	39.3^{b}	49.5^{b}	58.5^{b}	68.0^{b}	80.4^{b}	44.0^{b}	53.8^{cd}	57.0^{c}	70.1^{cd}	83.6^{c}
BGA	41.5^{ab}	51.7^{ab}	61.5^{ab}	68.6^{b}	79.6^{b}	46.9^{b}	55.2^{cd}	59.1^{c}	72.3^{c}	82.4^{c}
Control	39.0^{b}	49.2^{b}	57.6^{b}	66.7^{b}	78.1^{b}	44.0^{b}	52.6^{d}	56.3^{c}	68.3^{d}	82.0^{c}
C.V. (%)	7.6	6.1	6.8	6.1	12.9	4.9	3.4	3.6	3.0	3.9

Table 5 Means for SPAD chlorophyll meter reading of Chainat 1 rice.

Locations/treatments	Field experiment (days)					Greenhouse experiment (days)				
	30	45	60	75	90	30	45	60	75	90
Maha Sarakham	24.8^{a}	25.3^{a}	25.8^{a}	30.2^{a}	32.1^{a}					
Kalasin	21.7^{b}	23.0^{b}	23.4^{b}	28.7^{b}	26.4^{b}					
NPK 16-16-8 + NPK 46-0-0	24.1	25.0	27.0^{a}	36.5^{a}	34.2^{a}	37.1	30.4	32.3	35.3^{a}	34.0^{a}
NPK 16-16-8 + BGA	23.7	24.6	23.4^{b}	27.2^{b}	27.9^{b}	38.2	31.5	32.5	29.1^{b}	28.3^{b}
NPK 0-16-8 + BGA	23.3	24.2	24.1^{b}	27.3^{b}	28.9^{b}	35.3	30.8	32.1	28.6^{bc}	27.4^{b}
BGA	22.6	23.5	23.9^{b}	27.7^{b}	26.8^{b}	34.4	30.8	31.2	27.9^{c}	27.4^{b}
Control	22.6	23.4	24.5^{b}	28.4^{b}	28.5^{b}	32.6	31.9	32.0	27.9^{c}	27.1^{b}
C.V. (%)	6.6	6.4	6.2	4.3	7.5	13.5	5.1	5.7	3.1	3.2

DAE in which the plants treated with BGA were significantly ($p \leqslant 0.05$) higher than those untreated control. The results indicated that application of BGA tended to increase plant height. In general, the plants treated with NPK 0-16-8 + BGA were not significantly different from those treated with BGA and untreated control for plant height but, in general, they were significantly ($p \leqslant 0.05$) lower than the plants treated with NPK 16-16-8+BGA and NPK 16-16-8+NPK 46-0-0 except for NPK 16-16-8+BGA at 75 and 90 DAE. The plants treated with NPK 16-16-8 + BGA and the plants treated with NPK 16-16-8 + 46-0-0 were very similar for plant height except for some evaluation times. For example, the plants treated with NPK 16-16-8 + BGA were significantly ($p \leqslant 0.05$) higher than the plants treated with NPK 16-16-8 + NPK 46-0-0 in greenhouse experiment at 45 and 75 DAE and, in both experiments at 90 DAE, the plants treated with NPK 16-16-8 + NPK 46-0-0 were significantly ($p \leqslant 0.05$) higher than those treated with NPK 16-16-8 + BGA.

SPAD chlorophyll meter reading (SCMR)

The plants grown in Maha Sarakham had significantly ($p \leqslant 0.05$) higher SCMR than those grown in Kalasin at 30, 45, 60, 75, and 90 DAE (Table 5). There was no significant difference among the treatments for SCMR at 30 and 45 DAE in field experiment and at 30, 45, and 60 DAE in greenhouse experiment. However, significant ($p \leqslant 0.05$) differences among treatments were observed at 60, 75, and 90 DAE in field experiment and at 75 and 90 DAE in greenhouse experiment. In field experiment, untreated control, BGA, NPK 0-16-8+BGA and NPK 16-16-8 + BGA at 60, 75, and 90 DAE were not significantly different, but they were significantly ($p \leqslant 0.05$) lower than NPK 16-16-8 + NPK 46-0-0. In greenhouse experiment, most differences among untreated control, BGA, NPK 0-16-8+BGA, and NPK 16-16-8+BGA at 75 and 90 DAE were not significant except for untreated control and BGA, which were significantly ($p \leqslant 0.05$) lower than NPK 16-16-8 + BGA. Untreated control, BGA, NPK 0-16-8 + BGA, and NPK 16-16-8 + BGA were significantly lower than NPK 16-16-8 + NPK 46-0-0 in field experiment at 60, 75, and 90 DAE and greenhouse experiment at 75 and 90 DAE. In general, an application of fertilizers increased SCMR, but differences among treatments were observed at the highest dose and late evaluation times.

Table 6 Means for straw dry weight (SDW), total dry weight (TDW), number of tillers/plant (NT/P), number of panicle/plant (NP/P), and number of seeds/panicle (NS/P) of Chainat 1 rice.

Locations/treatments	Field experiment					Greenhouse experiment				
	SDW†	TDW†	NT/P	(NP/P)	NS/P	SDW‡	TDW‡	NT/P	NP/P	NS/P
Maha Sarakham	3998[a]	6030[a]	5.7[a]	6.8[a]	83.2[a]					
Kalasin	2894[b]	5049[b]	2.3[b]	3.3[b]	42.8[b]					
NPK 16-16-8 + NPK 46-0-0	5255[a]	8088[a]	4.7	5.8	68.5	1161.7[a]	1766.7[a]	17.5[a]	18.1[a]	144.8
NPK 16-16-8 + BGA	3260[b]	5450[b]	4.1	5.4	65.2	831.3[b]	1372.9[b]	11.2[b]	11.3[b]	127.7
NPK 0-16-8 + BGA	3000[b]	4855[b]	3.8	4.9	64.5	467.5[c]	753.3[c]	8.2[c]	8.5[cd]	126.3
BGA	2904[b]	4731[b]	3.9	4.8	62.1	445.0[c]	767.5[c]	9.0[c]	9.0[c]	103.3
Control	2813[b]	4578[b]	3.5	4.5	54.8	451.3[c]	669.2[c]	6.8[d]	7.3[d]	121.1
C.V. (%)	16.7	14.0	20.8	17.8	16.7	16.1	14.7	9.2	9.2	14.8

† (kg/ha), ‡ (g/m^2).

Straw dry weight, total dry weight, number of tillers/plant, number of panicles/plant, and number of seeds/panicle

Straw dry weight, total dry weight, number of tillers/plant, number of panicles/plant, and number of seeds/panicle were significantly higher in Maha Sarakham than in Kalasin (Table 6). In field experiment, significant differences among treatments were observed for straw dry weight and total dry weight, whereas the differences among treatments for number of tillers/plant, number of panicles/-plant and number of seeds/panicle were not statistically significant. Untreated control, BGA, NPK 0-16-8 + BGA, and NPK 16-16-8 + BGA were not significantly different, but they were significantly lower than NPK 16-16-8 + NPK 46-0-0 for straw dry weight and total dry weight. Although differences among treatments were not significant for some traits, all traits showed increasing trend towards high application of fertilizers. Control treatment was lowest and NPK 16-16-8 + NPK 46-0-0 was highest for these traits.

In greenhouse experiment, significant differences among the treatments were observed for straw dry weight, total dry weight and number of tillers/plant and number of panicles/plant, whereas the differences were not significant for number of seeds/panicle. Straw dry weight, total dry weight, number of tillers/plant, number of panicles/plant and number of seeds/panicle were increased with application of high fertilizers. In general, untreated control, BGA and NPK 0-16-8 + BGA were significantly lower than NPK 16-16-8 + BGA and NPK 16-16-8 + NPK 46-0-0 for straw dry weight, total dry weight and number of tillers/plant, and NPK 16-16-8 + BGA was lower than NPK 16-16-8 + NPK 46-0-0 for these traits. BGA was significantly higher than untreated control for number of tillers/plant and number of panicles/plant.

Seed weight, 1000-seed weight, filled seeds, unfilled seeds, and harvest index

Seed weight was higher in plants from Kalasin than those from Maha Sarakham although the difference was not significant (Table 7). However, significantly higher 1000-seed weight, unfilled seeds, and harvest index were found in Kalasin, filled seeds were significantly higher in Maha Sarakham.

In field experiment, significant differences among treatments were observed for seed weight and 1000-seed weight, but the differences among treatments for filled seeds, unfilled seeds and harvest index were not significant. Application of high rates of fertilizers resulted in the increase in seed weight as indicated by the highest seed weight of NPK 16-16-8 + NPK 46-0-0 (2833 kg/ha) and the lowest seed weight of untreated control (1765 kg/ha). NPK 16-16-8+NPK 46-0-0 was significantly higher than other treatments for seed weight and 1000-seed weight. However, the differences among treatments for 1000-seed weight did not show a clear pattern.

In greenhouse experiment, significant differences among treatments were observed for seed weight, 1000-seed weight and harvest index, but the differences among treatments for filled seeds and unfilled seeds were not significant. Seed weight and 1000-seed weight increased with application of high fertilizer rates, but differences in harvest index did not show a clear pattern although BGA was highest (0.42) for harvest index and the untreated control was lowest (0.32). BGA was significantly higher than untreated control for seed weight, and the difference was 16%. NPK 16-16-8 + NPK 46-0-0 was

Table 7 Means for seed weight (SW), 1000-seed weight (TSW), filled seeds (FS), unfilled seeds (US), and harvest index (HI) of Chainat 1 rice.

Locations/treatments	Field experiment					Greenhouse experiment				
	SW†	TSW‡	FS (%)	US (%)	HI	SW#	TSW‡	FS (%)	US (%)	HI
Maha Sarakham	2032	18.5[b]	85.8[b]	14.2[a]	0.34[b]					
Kalasin	2156	28.9[a]	93.2[a]	6.8[b]	0.42[a]					
NPK 16-16-8 + NPK 46-0-0	2833[a]	25.6[a]	90.5	9.5	0.36	605.0[a] (47%)*	30.4[a]	83.5	16.5	0.34[bc]
NPK 16-16-8 + BGA	2190[b]	23.8[ab]	90.1	9.9	0.40	541.7[a] (43%)	27.8[ab]	84.7	15.3	0.39[ab]
NPK 0-16-8 + BGA	1855[b]	22.4[b]	87.8	12.2	0.38	285.8[bc] (13%)	24.5[bc]	81.1	18.8	0.38[ab]
BGA	1827[b]	23.8[ab]	89.3	10.7	0.38	322.5[b] (16%)	22.2[cd]	84.2	15.7	0.42[a]
Control	1765[b]	22.9[ab]	89.9	10.1	0.38	217.9[c]	20.5[d]	74.0	25.9	0.32[c]
C.V. (%)	15.2	8.5	3.0	26.3	8.4	20.1	11.3	8.9	29.6	13.2

† kg/ha; ‡ g; # g/m^2. * Numbers in parenthesis indicate percent increase from the control.

highest for seed weight, and the difference between NPK 16-16-8+NPK 46-0-0 and the untreated control was 47%. NPK 16-16-8 + NPK 46-0-0 was also highest (30.4 g) for 1000-seed weight, whereas the untreated control was also lowest (20.5 g), and the difference between NPK 16-16-8 + NPK 46-0-0 and the untreated control was 9.7 g.

DISCUSSION

Nutrient uptake

Soils in greenhouse and Maha Sarakham were acidic and soils in Kalasin was highly acidic. Soil pH could affect the growth of BGA and nutrient uptake of rice. In general, nutrient uptake of the soil in Maha Sarakham was better than that in Kalasin, especially for potassium. Higher nutrient uptake in Maha Sarakham soil would possibly be due to higher soil fertility and higher soil pH. Soil pH in the range of 6.5–7.5 promotes availability and uptake of nitrogen, phosphorus, and potassium. Soil pH lower than 6.0 reduces availability of these nutrients and increases availability and uptake of iron that can be toxic to some plants[1]. In previous investigation, the use of BGA in high pH soil was not successful[4]. The pH for BGA growth in culture media ranges from 7.5 to 10, and lower pH limits growth[19]. Soil pH in this study was much lower than that for optimum growth of BGA. The results indicated that soil pH is an important factor determining the success in application of BGA in rice production. Application of lime as soil amendment could increase soil pH and soil fertility[20].

Comparing the nutrient uptake among the treatments, it would be expected that nitrogen uptake in the plants treated with BGA would be higher than those of untreated control. Fixed nitrogen from BGA should provide a significant amount of nitrogen to the plants and the application of chemical fertilizers would result in greater uptake of plant nutrients than the BGA treatment or untreated control. In this study, however, BGA had significantly higher nitrogen uptake than the untreated control in greenhouse experiment, but the difference was not significant for field experiment, although BGA tended to have higher nitrogen uptake than the untreated control. Application of chemical fertilizers at the highest rate (NPK 16-16-8 + NPK 46-0-0) had the highest uptake of nitrogen, phosphorus, and potassium in both greenhouse and field experiments.

The results of this study supported theoretical expectation for application of chemical fertilizers at high rates in both greenhouse and field experiments. However, for the application of BGA compared to the untreated control, nitrogen uptake of BGA was significantly higher than the untreated control for greenhouse experiment only, whereas the results for nitrogen uptake of BGA compared to untreated control in field experiment was rather confounding. The contribution of biological nitrogen fixation of BGA to the rice ecosystems has been well documented. However, pH lower than 6.9 limits the success in application of BGA in agriculture[21].

In previous investigations, rice yield was closely correlated with plant nutrient uptake[22]. Increase nutrient uptake was associated with the increases in biomass, grain yield, and nutrients in grains and straws[23]. In this study, the authors expected that nutrient uptake could provide useful information on the performance of rice crop treated with single and combinations of BGA and chemical fertilizers at different rates.

Other soil parameters such as total nitrogen, available phosphorus and exchangeable potassium and exchangeable calcium could affect the differences in rice performance between locations, and these parameters were differences between locations. However, this study could not be able to separate each effect of these soil nutrients and considered as location effects. In contrast, soil pH values were rather similar among experimental sites and greenhouse. Low soil pH limits the uptake of most soil nutrients, but it promotes the release of iron, manganese, boron, copper and zinc that can be toxic to both rice and BGA.

Plant height and chlorophyll content

Plant height is a parameter indicating growth of many crops. In previous investigations in rice, plant height was a good parameter to monitor growth of plants affected by drought stress[24] or nutrient deficiency[25]. In this study, the rice plants treated with BGA had a height that did not differ significantly from untreated control from 30 DAE to 90 DAE in both greenhouse and field experiments. High rates of chemical fertilizer application (NPK 16-16-8 + NPK 46-0-0 and NPK 16-16-8 + BGA) gave taller plants than did the low rates of fertilizer application (NPK 0-16-8 + BGA, BGA and control). Plant height is thus a poor parameter to detect small differences among fertilizer treatments.

SCMR that measures chlorophyll in the leaves is an indicator of plant health. SCMR has been used for screening of drought tolerance in peanut[26,27] and to determine nitrogen status in maize[28], potato[29], and rice[30]. In this study, BGA was not different from untreated control in both greenhouse and field experiments. SCMR values were very similar among untreated control, BGA, NPK 0-16-8 + BGA and NPK 16-16-8 + BGA, but they were significantly lower than NPK 16-16-8 + NPK 46-0-0, which was the highest rate at 60, 75, and 90 DAE in greenhouse and 75 and 90 DAE in the field. SCMR had lower discrimination power than plant height as it could not separate lower rates of fertilizer application. In addition, SRM was less sensitive to fertilizer application than plant height.

Chainat 1 is a new HYV variety with short stems and is suitable for all seasons in irrigated areas. This variety responded poorly for plant height and SCMR, and the differences in plant height and SCMR among treatments were not clears neither in greenhouse nor in field experiments.

Crop productivity

Blue green algae (BGA), NPK 0-16-8 + BGA, and NPK 16-16-8 + BGA, NPK 16-16-8 + NPK 46-0-0, and control were evaluated for traits related to crop productivity such as straw dry weight, total dry weight, number of tillers/plant, number of panicles/plant and number of seeds/panicle, seed weight, 1000-seed weight, filled seeds, unfilled seeds, and harvest index. In greenhouse experiment, the treatments were significantly different for straw dry weight, total dry weight, number of tillers/plant, number of panicles/plant, seed weight, 1000-seed weight and harvest index, and, in field experiment, the treatments were significantly different for straw dry weight, total dry weight, seed weight, 1000-seed weight and harvest index.

It was clear from the greenhouse experiment that higher seed weight of BGA-treated plants was associated with higher number of tillers/plant, number of panicles/plant, and harvest index. In field experiment, however, BGA-treated plants did not show clear differences from the control for these traits. The differences between the results in greenhouse and in the fields might be due largely to high environmental fluctuation in the fields and difference in population densities (direct sowing for fields and one plant per hill for greenhouse).

In a previous investigation in Khon Kaen, which is adjacent to Maha Sarakham, 5 strains of BGA were tested in greenhouse and field experiments on rice variety RD 23, and *Tolypothrix* sp. was the strain with higher grain yield than the untreated control in greenhouse experiment in Bangkok[31]. In general, the authors found that the strains of BGA could increase grain yield of about 12–26% under greenhouse conditions. Under field conditions, however, the application of fertilizers at the highest rate gave the highest grain yield in the field in Khon Kaen, and no statistical difference was observed among BGA strains, biofertilizer, and the control. The discrepancy of the results between greenhouse and field experiments are perhaps due to higher environmental variation under field conditions.

Biological nitrogen fixation is important in agriculture to replace expensive chemical fertilizers. In rice based agro-ecosystems, many legumes have been used successfully to replenish organic matter and fixed nitrogen to the soils in the forms of cash crops such as peanut[32] and green fertilizers such as *Sesbania rostrata*[33] and sunhemp (*Crotalaria juncea*)[34]. In this study, BGA could improve rice grain yield in greenhouse but using BGA in the field was not successful. Although BGA has been widely used as biofertilizer in rice fields, its success is dependent on soil properties especially for low pH soil that limits the success of BGA application.

Raising soil pH is important for the success in application of BGA in rice fields, and the application of BGA in low pH soils is not recommended. This information is useful for biofertilizer business to develop BGA fertilizers that tolerate low pH soils and to agricultural extension to give recommendation to the farmers and researchers to develop the application of other types of biological nitrogen fixation in rice fields with acidic soils.

In this study, the persistence of BGA in soil was not evaluated. Making conclusion with confidence is therefore not possible. However, the consistency of the results in greenhouse experiment and field experiment gave useful information to farmers and scientists who want to adopt this technology for organic rice production.

Acknowledgements: The research was funded by the Agro-biomate Co., Ltd. The authors also acknowledge the Faculty of Agricultural Technology, Rajabhat Maha Sarakham University and the Faculty of agriculture, Khon Kaen University for providing research facilities.

REFERENCES

1. Marschner H (1995) *Mineral Nutrition of Higher Plants*, 2nd edn, Academic Press, London.
2. Shridhar BS (2012) Review: Nitrogen fixing microorganisms. *Int J Microbiol Res* **3**, 46–52.
3. Pabby A, Prasanna R, Singh PK (2003) *Azolla-Anabaena* symbiosis—From traditional agriculture to biotechnology. *Indian J Biotechnol* **2**, 26–37.
4. Reddy PM, Roger PA, Ventura W, Watanabe I (1986) Blue-green algal treatment and inoculation had no significant effect on rice yield in an acidic wetland soil. *Philippine Agr Sci* **69**, 629–32.
5. Asuming-Brempong S (2014) Sustainable growth of rice in Ghana: The role of biofertilizers (phosphate solubilizing microorganisms and *Azolla Anabaena*) to rice improvement. *Global Adv Res J Agr Sci* **3**, 1–7.
6. Ghosh TK, Saha KC (1993) Effects of inoculation with N_2-fixing cyanobacteria on the nitrogenase activity in soil and rhizosphere of wetland rice (*Oryza sativa* L.). *Biol Fertil Soils* **16**, 16–20.
7. Begum ZNT, Mandal R, Islam S (2011) Effect of cyanobacterial biofertilizer on the growth and yield components of two HYV of rice. *J Algal Biomass Util* **2**, 1–9.
8. Paudel YP, Pradhan S, Pant B, Prasad BN (2012) Role of blue green algae in rice productivity. *Agr Biol J N Am* **3**, 332–5.
9. Roy M, Srivastava RC (2013) Assembling BNF system in rice plant: frontier areas of research. *Curr Sci* **104**, 326–34.
10. Drilon JR (1980) *Standard Methods of Analysis for Soil, Plant, Water and Fertilizer*, Los Baños, Laguna, Philippines.
11. Bremner JM (1996) Nitrogen-total. In: Sparks DL, Page AL, Helmke PA, Loeppert RH (eds) *Methods of Soil Analysis, Part 3: Chemical Methods*, Soil Science Society of America, Madison, WI, pp 1085–121.
12. Cottenie A (1980) *Soil and Plant Testing as a Basis of Fertilizer Recommendation*, FAO, Rome.
13. McLean EO (1982) Soil pH and lime requirement. In: Page AL (ed) *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties*, 2nd edn, American Society of Agronomy, Madison, WI, pp 199–224.
14. Peech M (1965) Hydrogen-ion activity. In: Black CA (ed) *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties*, American Society of Agronomy, Madison, WI, pp 914–26.
15. Rhoades JD (1996) Salinity: electrical conductivity and total dissolved solids. In: Sparks DL, Page AL, Helmke PA, Loeppert RH (eds) *Methods of Soil Analysis, Part 3: Chemical Methods*, Soil Science Society of America, Madison, WI, pp 417–35.
16. Schuman GE, Stanley MA, Knudsen D (1973) Automated total nitrogen analysis of soil and plant samples. *Soil Sci Soc Am J* **37**, 480–1.
17. Gomez KA, Gomez AA (1984) *Statistical Procedures for Agricultural Research*. Wiley, New York.
18. MSTAT Development Team (1994) *MSTAT-C Version 2.1: A Microcomputer Program for the Design, Management, and Analysis of Agronomic Research Experiments*, Michigan State Univ, East Lansing, MI.
19. Panda H (2013) *Integrated Organic Farming Handbook*. Vedams eBooks (P) Ltd, New Delhi, India.
20. Anetor MO, Akinrinde EA (2007) Lime effectiveness of some fertilizers in a tropical acid alfisol. *J Cent Eur Agr* **8**, 17–24.
21. Nayak S, Prasanna R (2007) Soil pH and its role in cyanobacterial abundance and diversity in rice field soils. *Appl Ecol Environ Res* **5**, 103–13.
22. Begum S, Rahman MM, Main MJA, Islam MR, Uddin M (2001) Effect of nitrogen supplied from manure and fertilizer on growth, yield and nutrient uptake of rice. *J Biol Sci* **1**, 708–10.
23. Brohi AR, Karaman MR, Topbaş MT, Aktaş A, Savaşli E (2000) Effect of potassium and magnesium fertilization on yield and nutrient content of rice crop grown on artificial siltation soil. *Turk J Agr Forest* **24**, 429–35.
24. Bunnag S, Pongthai P (2013) Selection of rice (*Oryza sativa* L.) cultivars tolerant to drought stress at the vegetative stage under field conditions. *Am J Plant Sci* **4**, 1701–8.
25. Azhiri-Sigari T, Gines HC, Casimero MC, Sebastian LS (2003) Soil fertility status of PhilRice CES ricefields in Maligaya, Nueva Ecija by soil analysis & minus-one-element technique (MOET). *Philippine J Crop Sci* **28**, 31–47.
26. Songsri P, Jogloy S, Kesmala T, Vorasoot N, Akkasaeng

C, Patanothai A, Holbrook CC (2008) Heritability of drought resistance traits and correlation of drought resistance and agronomic traits in peanut. *Crop Sci* **48**, 2245–53.
27. Jongrungklang N, Toomsan B, Vorasoot N, Jogloy S, Kesmala T, Patanothai A (2008) Identification of peanut genotypes with high water use efficiency under drought stress conditions from peanut germplasm of diverse origins. *Asian J Plant Sci* **7**, 628–38.
28. Yang Y, Timlin DJ, Fleisher DH, Lokhande SB, Chun JA, Kim SH, Staver K, Reddy VR (2012) Nitrogen concentration and dry-matter accumulation in maize crop: Assessing maize nitrogen status with an allometric function and a chlorophyll meter. *Comm Soil Sci Plant Anal* **43**, 1563–75.
29. Shukla AK, Khan MA, Upadhayay NC, Singh BP, Lal SS, Pandey SK (2007) Chlorophyll meter: A tool for assessing dynamic nitrogen supply in potato crop under organic *vis-á-vis* inorganic nutrient management options. *Potato J* **34**, 95–6.
30. Turner FT, Jund MF (1991) Chlorophyll meter to predict nitrogen topdress requirement for semidwarf rice. *Agron J* **8**, 926–8.
31. Choonluchanon S, Swatdee P, Tangcham B, Tanweenukoon J, Sudhishurnark P (1991) The effect of five strains of blue green algae on growth and yield of RD 23 rice. *Thai Agr Res J* **9**, 11–6, [in Thai].
32. Toomsan B, Cadisch G, Srichantawong M, Thongsodsaeng C, Giller KE, Limpinuntana V (2000) Biological N_2 fixation and residual N benefit of pre-rice leguminous crops and green manures. *Neth J Agr Sci* **48**, 19–29.
33. Becker M, Diekmann KH, Ladha JK, De Datta SK, Ottow JCG (1991) Effect of NPK on growth and nitrogen fixation of *Sesbania rostrata* as a green manure for lowland rice (*Oryza sativa* L.). *Plant Soil* **132**, 149–58.
34. Choudhury ATMA, Kennedy IR (2004) Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. *Biol Fertil Soils* **39**, 219–27.