

DOI: 10.14720/aas.2015.105.2.07

Agrovoc descriptors: rice, oryza, nitrogen fertilizers, rhizobacteria, azotobacter, *Azospirillum*, plant growth stimulants, growth, crop yield

Agris category code: f01, fo4, f62

## Grain, milling, and head rice yields as affected by nitrogen rate and bio-fertilizer application

Saeed FIROUZI<sup>2</sup>

Received May 29, 2014; accepted August 22, 2015.

Delo je prispelo 29. maja 2014, sprejeto 22. avgusta 2015.

### ABSTRACT

To evaluate the effects of nitrogen rate and bio-fertilizer application on grain, milling, and head rice yields, a field experiment was conducted at Rice Research Station of Tonekabon, Iran, in 2013. The experimental design was a factorial treatment arrangement in a randomized complete block with three replicates. Factors were three N rates (0, 75, and 150 kg ha<sup>-1</sup>) and two bio-fertilizer applications (inoculation and uninoculation with Nitroxin, a liquid bio-fertilizer containing *Azospirillum* spp. and *Azotobacter* spp. bacteria). Analysis of variance showed that rice grain yield, panicle number per m<sup>2</sup>, grain number per panicle, flag leaves area, biological yield, grains N concentration and uptake, grain protein concentration, and head rice yield were significantly affected by N rate, while bio-fertilizer application had significant effect on rice grain yield, grain number per panicle, flag leaves area, biological yield, harvest index, grains N concentration and uptake, and grain protein concentration. Results showed that regardless of bio-fertilizer application, rice grain and biological yields were significantly increased as N application rate increased from 0 to 75 kg ha<sup>-1</sup>, but did not significantly increase at the higher N rate (150 kg ha<sup>-1</sup>). Grain yield was significantly increased following bio-fertilizer application when averaged across N rates. Grains N concentration and uptake were significantly increased as N rate increased up to 75 kg ha<sup>-1</sup>, but further increases in N rate had no significant effect on these traits. Bio-fertilizer application increased significantly grains N concentration and uptake, when averaged across N rates. Regardless of bio-fertilizer application, head rice yield was significantly increased from 56 % to 60 % when N rate increased from 0 to 150 kg ha<sup>-1</sup>. Therefore, this experiment illustrated that rice grain and head yields increased with increasing N rate, while bio-fertilizer application increased only rice grain yield.

**Key words:** nitrogen fertilizer, plant growth-promoting rhizobacteria, rice yield and yield components

### IZVLEČEK

#### VPLIV GNOJENJA Z RAZLIČNIMI ODMERKI DUŠIKOVIH GNOJIL IN BIO-GNOJIL NA PRIDELEK ZRNJA IN MLEVSKE LASTNOST RIŽA

Z namenom vrednotenja učinkov gnojenja z različnimi odmerki dušika in uporabe bio-gnojil na pridelek riža in njegove mlevske lastnosti je bil izveden poljski poskus na Rice Research Station of Tonekabon, Iran, v letu 2013. Načrt poskusa je bil faktorski naključni bločni poskus s tremi ponovitvami. Preučevani dejavniki v poskusih so bili tri gnojenja z različnimi odmerki dušika (0, 75, in 150 kg ha<sup>-1</sup>) in uporaba dveh bio-gnojil (z ali brez inokulacije z bio-gnojilom Nitroxin, tekoče bio-gnojilo, ki vsebuje bakterije iz rodov *Azospirillum* spp. in *Azotobacter* spp.). Analiza variance je pokazala, da je imelo gnojenje z dušikom značilen učinek na pridelek zrnja riža, število latov na m<sup>2</sup>, število zrn na lat, površino najvišjega lista (zastavarja), biološki pridelek, privzem in vsebnost N v zrnju, vsebnost beljakovin v zrnju in pridelek oluščenega riža, uporaba bio-gnojil pa je imela značilen vpliv le na pridelek zrna riža, število zrn na lat, površino najvišjega lista, biološki pridelek, žetveni indeks, privzem in vsebnost N v zrnju in vsebnost beljakovin v zrnju. Rezultati raziskave so pokazali, da sta se pridelek zrnja riža in njegov biološki pridelek povečala ne glede na uporabo bio-gnojil, ko se je gnojenje z dušikom povečalo z 0 na 75 kg ha<sup>-1</sup>, vendar gnojenje z večjimi odmerki N (150 kg ha<sup>-1</sup>) ni imelo značilnega vpliva na povečanje teh dveh parametrov. Pridelek zrnja se je značilno povečal pri uporabi bio-gnojil pri vseh odmerkih dušika. Vsebnost in privzem N v zrnju sta se značilno povečala pri povečanju gnojenja z dušikom iz 0 na 75 kg ha<sup>-1</sup>, vendar nadaljna povečanja gnojenja z N niso imela značilnega vpliva na ta parametra. Uporaba bio-gnojil je značilno povečala vsebnost in privzem N v zrnju pri vseh odmerkih dušika. Ne glede na uporabo bio-gnojil, se je pridelek oluščenega riža značilno povečal iz 56 % na 60 %, ko se je gnojenje z N povečalo z 0 na 150 kg ha<sup>-1</sup>. Izsledki te raziskave kažejo, da se je pridelek zrnja riža in oluščenega riža povečal z večjim gnojenjem z dušikom, uporaba bio-gnojil pa je povečala le pridelek zrnja.

**Ključne besede:** dušikova gnojila, rast stimulirajoče rizobakterije, pridelek riža in njegove komponente

<sup>1</sup> Department of Agronomy and Plant Breeding, Rasht Branch, Islamic Azad University, Rasht, Iran; P.o.Box: 41335-3516; E-mail: firoozi@iaurasht.ac.ir

## 1 INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food crop for about 3 billion world's population. In many countries, rice provides more than 70 % of human caloric intake. In Iran, much of the population consumes rice at least once a day (Kanawapee et al., 2011). Iran's total rice production stands at 2.2 million tonnes per annum, while its annual consumption is about three million tonnes (Rezazadeh et al., 2013). So, Iran has to import about 800 thousand tonnes of rice from some rice producing countries. Consequently, increasing the rice production in the country is an essential.

Plant nutrients, which come primarily from chemical fertilizer, are essential for crop production. In agriculture, nitrogen is an essential element for crop growth and development. Nitrogen is a basic constituent of chlorophyll, proteins and all enzymes are involved in photosynthesis, especially Rubisco which alone accounts for more than 75 % of the total leaf nitrogen (Hak et al., 1993). Li et al. (2012) and Manzoor et al. (2006) declared that rice grain yield was significantly increased with increasing nitrogen fertilizer application rate. At the same time, nitrogen has been known as an important factor influencing rice milling quality (Perez et al., 1996). Dilday (1988) reported that the head rice yield decreased 7 to 22 % and 2 to 6 % in 'Lemont' and 'Newbonnet' rice cultivars,

respectively, when no nitrogen applying compared to applying 200 kg N ha<sup>-1</sup>. Perez et al. (1996) indicated that the head rice yield was significantly increased as N rate increased from 0 to 225 kg ha<sup>-1</sup>.

A lot of studies noted that the nitrogen use efficiency is relatively low in paddy fields. This indicates that a major portion of applied nitrogen is wasted in paddy fields. Nitrogen losses occur through denitrification, volatilization, and leaching which may cause the air and water pollutions (Brown et al., 2012). Therefore, reducing the chemical nitrogen rate by applying the bio-fertilizers may be a solution. Bio-fertilizers are substances which comprise of living microorganisms that stimulate the plant growth by increasing the supply or availability of primary nutrients to the plant and the synthesis of growth-promoting substances. Hence, bio-fertilizers can be expected to reduce the use of chemical fertilizers. Plant growth-promoting rhizobacteria like as *Azospirillum* and *Azotobacter* can supply nutrients for crops through nitrogen fixation and produce phytohormones like auxins, cytokinins, gibberellins and ethylene (Keyeo et al., 2011). Therefore, this experiment was conducted to evaluate the effects of nitrogen rate and bio-fertilizer application on rice grain yield, yield components and head rice yield.

## 2 MATERIALS AND METHODS

### 2.1 Experimental site, design, and plant growth conditions

A field experiment was conducted at Rice Research Station of Tonekabon, Iran, in 2013. The soil texture was loamy with 1.5 % organic matter content, pH 7.7, total N 0.9 g kg<sup>-1</sup>, available P 5.0 mg kg<sup>-1</sup>, and available K 140.0 mg kg<sup>-1</sup>. Three N rates (0, 75, and 150 kg ha<sup>-1</sup>) and two bio-fertilizer applications (inoculation and uninoculation with Nitroxin, a commercial liquid bio-fertilizer containing *Azospirillum* spp. and *Azotobacter* spp. bacteria), organized into a randomized complete block design with a 3 × 2 factorial treatment arrangement and three blocks, were applied to plot area. The colony forming unit (cfu) of *Azotobacter* and *Azospirillum* were

10<sup>9</sup> cells per gram of carrier material. Bio-fertilizer (Nitroxin) was applied at two stages according to the manufacture recommendation, i.e. seed dipping and seedling root dipping methods. Plot size was 3 m by 4 m. Consistent with the lowland paddy field practices in north of Iran, rice seeds ('Taron') were sown in a nursery seedbed on 18 April and seedlings from these seeds were transplanted on 14 May at a hill spacing of 0.20 by 0.20 m, with three seedlings per hill. Fertilizer N (as urea) was split-applied, with 34 % just before final land preparation, 33 % at panicle initiation, and 33 % at flowering stage. Also, all plots received 100 kg P ha<sup>-1</sup> as triple superphosphate and 75 kg K ha<sup>-1</sup> as potassium sulfate just before final land preparation. Additionally, all weeds were

removed manually whenever necessary. Plots were harvested on 21 August.

## 2.2 Plant sampling

At the soft dough stage, five randomly chosen plants were removed from each plot and the flag leaves area (cm<sup>2</sup>) was determined with an area meter (Model LI-3100, LI-COR, Lincoln, NE, USA). At maturity stage, rice grain yield (based on 14 % humidity) was determined from 2.5 m<sup>2</sup> per plot. Moisture content of grains was measured using a digital grain moisture meter (Model GMK-303R5-Korea) and grain yield per pot was calculated as ((100 - moisture content of the sample) × fresh grain weight)/86 to convert the sample to 14 % moisture content. Yield components, that is, number of panicles per square meter, number of filled grains and 1000-grain weight, were determined from 12 plants (excluding the border ones) sampled randomly from each plot. To determine aboveground biomass, a 1 m<sup>2</sup> sample from each plot was randomly chosen, clipped at the ground level, threshed, dried at 70°C for three days, weighed, and expressed as the dry weight of above-ground plant per hectare. Harvest index was the proportion (percentage) of filled grain weight to total above-ground dry biomass. For measuring grain N concentration, rice grains were grounded to pass through a 1-mm sieve. N concentration was determined using micro-Kjeldahl method as described by Pregl (1945) and was expressed as the percent of grain dry weight. N uptake in grain was calculated by multiplying grain dry weight by grain N concentration. Grain protein concentration was calculated as 6.25 × nitrogen content measured by the micro Kjeldahl technique.

Paddy samples for milling quality evaluation were harvested from the 2.5 m<sup>2</sup> per plot, threshed by a simple motorized thresher and dried up to 8 %, wet basis (w.b.) using the laboratory dryer (Memmert Model 600, Germany) set at 45 °C. Paddy moisture content was determined using the digital grain moisture meter (Model GMK-303R5-Korea). After drying process, 200 g of dried paddy from each treatment were dehulled by a laboratory rubber roll huller (SATAKE Co. Ltd, Japan) and then was milled using a laboratory rice whitener (McGill Miller, USA). Mass of each milled rice sample was weighed by a laboratory scale with an accuracy of 0.01 g, separated into broken and head rice kernels by sizing with a laboratory rotary sieve (SATAKE TRG058, Japan) and then broken and whole kernel fractions were weighed. Head rice yield was determined as the mass of head milled rice after milling, divided by the mass of dried paddy prior to milling process (Zhao and Fitzgerald, 2013). Milling yield was also measured as the mass ratio of total milled rice to the un-hulled dried paddy prior to milling process.

## 2.3 Data analysis

Data were analyzed by analysis of variance (ANOVA) procedure using SAS program (SAS Inst. 2004) and means were compared using Fisher's Protected LSD at the 0.05 level of significance. Pearson correlation coefficients were calculated using correlation analysis to assess the interrelationships between the different measured parameters.

# 3 RESULTS AND DISCUSSION

## 3.1 Yield and yield components

The main effects of nitrogen rate and bio-fertilizer application were significant, but there was no significant interaction of nitrogen rate and bio-fertilizer application for grain yield (Table 1). Regardless of bio-fertilizer application, rice grain yield was significantly increased from 4251.4±346.5 to 5016.2±349.3 kg ha<sup>-1</sup> (18 %) as N application rate increased from 0 to 75 kg ha<sup>-1</sup>, but did not significantly increase at the higher N rate (150 kg ha<sup>-1</sup>) (Table 2). Grain yield was

significantly increased by bio-fertilizer application when averaged across N rates (Table 2). Pedraza et al. (2009) reported that inoculation of rice plants with *Azospirillum* significantly increased grain yield. Mukhopadhyay et al. (2013) also reported that the highest grain yield for rice was obtained when bio-fertilizer was applied with 60 % of recommended N rate. Induction of longer roots with increased number of root hairs and root laterals is a growth response attributed to IAA production by *Azospirillum* and *Azotobacter*, which increase grain yield in inoculated plants

(Keyeo et al., 2011). Panicle number per m<sup>2</sup> was significantly affected only by nitrogen rate, while grain number per panicle was significantly influenced by both nitrogen rate and bio-fertilizer application. Regardless of bio-fertilizer application, the highest (323±25.9 panicle) and the lowest (248.2±18.1 panicle) panicle number per m<sup>2</sup> was observed in plots receiving 0 and 150 kg N ha<sup>-1</sup>, respectively (Table 2). Averaged across bio-fertilizer applications, grain number per panicle was significantly increased from 96.4±3.7 to 107.6±4.3 when nitrogen application rate increased from 0 to 150 kg ha<sup>-1</sup> (Table 2). These results indicated that nitrogen application increased sink size by increasing both panicle number per m<sup>2</sup> and grain number per panicle, while bio-fertilizer inoculation increased sink size only by increasing grain number per panicle. Similarly to these results, Weerakoon et al. (2005) also reported that panicle number per m<sup>2</sup> and grain number per panicle increased with increases in N rate. On the other hand, 1000-grain weight was significantly affected neither by nitrogen rate nor by bio-fertilizer application (Table 1 & 2). Contrary to this result, Weerakoon

et al. (2005) reported that 1000-grain weight increased with increasing in N rate. Regardless of N rate, grain number per panicle was significantly increased by 11 % when bio-fertilizer was applied. Similar result was reported by others (Mukhopadhyay et al., 2013; Isawa et al., 2010). Bio-fertilizer application increased panicle number per m<sup>2</sup> and 1000-grain weight by 17 % and 4 %, respectively, but the increases were not statistically significant. In contrast, Mukhopadhyay et al. (2013) and Isawa et al. (2010) found that panicle number per m<sup>2</sup> and 1000-grain weight for rice were significantly increased following bio-fertilizer application. The ANOVA also showed that the interaction between nitrogen rate and bio-fertilizer application were not significant for all of yield components (Table 1). This indicates that all yield components had similar responses to N rate with or without bio-fertilizer application. Rice grain yield was positively correlated with panicle number per m<sup>2</sup>, grain number per panicle, 1000-grain weight, flag leaves area, biological yield, harvest index, grain N concentration, and grain N uptake at  $P < 0.01$  level (Table 3).

**Table 1:** Mean squares of ANOVA for grain yield (Y), panicle number per m<sup>2</sup> (PN), grain number per panicle (GN), 1000-grain weight (ThGW), flag leaves area (FLA), biological yield (BY), harvest index (HI), grain N concentration (GNC), grain N uptake (GNU), grain protein concentration (GPC), milling yield (MY), and head rice yield (HRY) as affected by nitrogen rate and bio-fertilizer application

S.O.V	Df	Y	PN	GN	ThGW	FLA	BY	HI	GNC (%)	GNU	GPC	MY	HRY
R	2	43778 <sup>ns</sup>	23.4 <sup>ns</sup>	128*	1.39 <sup>ns</sup>	7647157 <sup>ns</sup>	589981 <sup>ns</sup>	1.4 <sup>ns</sup>	0.002 <sup>ns</sup>	29 <sup>ns</sup>	0.01 <sup>ns</sup>	9.6 <sup>ns</sup>	12.1**
Nitrogen (N)	2	1885091**	37.4*	191**	11.14 <sup>ns</sup>	54259227**	7098144**	7.2 <sup>ns</sup>	0.21**	1729**	8.3**	1.9 <sup>ns</sup>	19.8**
Bio-fertilizer (B)	1	5221834**	37.9 <sup>ns</sup>	638**	3.65 <sup>ns</sup>	20723922*	17608156**	25.6*	0.41**	4402**	16.1**	0.2 <sup>ns</sup>	0.05 <sup>ns</sup>
N×B	2	349822 <sup>ns</sup>	1.2 <sup>ns</sup>	19 <sup>ns</sup>	0.02 <sup>ns</sup>	2085236 <sup>ns</sup>	1007564 <sup>ns</sup>	3.2 <sup>ns</sup>	0.017 <sup>ns</sup>	188 <sup>ns</sup>	1.1 <sup>ns</sup>	1.6 <sup>ns</sup>	0.28 <sup>ns</sup>
Error	10	294163	9.4	30	10.60	4583681	886630	4.2	0.02	156	0.8	0.9	1.49
CV (%)	-	11	16	5	13	22	7	5	10	15	10	2	2

\*, \*\* represent significance at 0.05 and 0.01 probability level, respectively  
ns represents no significant difference

### 3.2 Biological yield and harvest index

Both nitrogen rate and bio-fertilizer application had significant effects ( $P < 0.01$ ) on biological

yield, while harvest index was significantly influenced only by bio-fertilizer application (Table 1). Moreover, no significant interactions between nitrogen rate and bio-fertilizer application were

found for both biological yield and harvest index (Table 1). Regardless of bio-fertilizer application, biological yield increased from  $10653.3 \pm 648.6$  to  $12159.1 \pm 637.3$   $\text{kg ha}^{-1}$  (14.1 %) when N rate increases from 0 to  $75 \text{ kg ha}^{-1}$ , with no further increase in biological yield as N rate increased from 75 to  $150 \text{ kg ha}^{-1}$  (Table 2). Harvest indices

were similar for all N rates when averaged across bio-fertilizer applications (Table 2). Thus, the increase in grain yield was due to an increase in total biomass production rather than harvest index. Mukhopadhyay et al. (2013) reported that harvest index increased following bio-fertilizer application.

**Table 2:** Grain yield (Y), panicle number per  $\text{m}^2$  (PN), grain number per panicle (GN), 1000-grain weight (ThGW), flag leaves area (FLA), biological yield (BY), harvest index (HI), grain N concentration (GNC), grain N uptake (GNU), grain protein concentration (GPC), milling yield (MY), and head rice yield (HRY) response to nitrogen rate and bio-fertilizer application

Factors \ Traits	Y ( $\text{kg ha}^{-1}$ )	PN (No. $\text{m}^2$ )	GN (No. panicle)	ThGW (g)	FLA ( $\text{cm}^2$ )	BY ( $\text{kg ha}^{-1}$ )	HI (%)	GNC (%)	GNU ( $\text{kg ha}^{-1}$ )	GPC ( $\text{kg ha}^{-1}$ )	MY (g 100g)	HRY (%)
<b>Nitrogen rates</b> ( $\text{kg ha}^{-1}$ )												
0	$4251.4 \pm 346.5^b$	$248.2 \pm 18.1^b$	$96.4 \pm 3.7^b$	$22.7 \pm 1.0^a$	$6216.1 \pm 224.9^b$	$10653.3 \pm 648.6^b$	$39.6 \pm 0.9^a$	$1.29 \pm 0.1^b$	$55.6 \pm 6.9^b$	$8.0 \pm 0.4^b$	$69.8 \pm 0.6^a$	$56.8 \pm 0.7^a$
75	$5016.2 \pm 349.3^a$	$306.8 \pm 23.7^{ab}$	$100.3 \pm 2.9^b$	$23.5 \pm 0.9^a$	$9937.4 \pm 1132.0^a$	$12159.1 \pm 637.3^a$	$40.9 \pm 1.0^a$	$1.59 \pm 0.0^a$	$81.6 \pm 11.3^a$	$9.9 \pm 0.7^a$	$69.9 \pm 0.7^a$	$58.5 \pm 0.7^b$
150	$5342.8 \pm 237.3^a$	$323.0 \pm 25.9^a$	$107.6 \pm 4.3^a$	$25.4 \pm 1.4^a$	$12168.2 \pm 1239.2^a$	$12753.4 \pm 402.7^a$	$41.8 \pm 0.7^a$	$1.63 \pm 0.1^a$	$87.5 \pm 6.3^a$	$10.2 \pm 0.5^a$	$70.8 \pm 0.5^a$	$60.5 \pm 0.6^c$
LSD (0.05)	697.03	61.9	7.1	4.1	2754.03	1210.7	2.6	0.19	16.4	1.2	1.2	1.5
<b>Bio-fertilizer application</b>												
application	$5409.0 \pm 192.6^a$	$315.0 \pm 20.5^a$	$107.0 \pm 2.8^a$	$24.3 \pm 1.0^a$	$10513.2 \pm 1286.8^a$	$12855.9 \pm 345.9^a$	$42.0 \pm 0.6^a$	$1.65 \pm 0.1^a$	$90.0 \pm 7.1^a$	$10.3 \pm 0.3^a$	$70.3 \pm 0.5^a$	$58.6 \pm 0.8^a$
No application	$4332.3 \pm 258.9^b$	$269.3 \pm 19.0^a$	$95.0 \pm 2.4^b$	$23.4 \pm 0.9^a$	$8366.9 \pm 870.7^a$	$10878.1 \pm 495.0^b$	$39.6 \pm 0.8^b$	$1.35 \pm 0.0^b$	$59.0 \pm 5.0^b$	$8.4 \pm 0.5^b$	$70.1 \pm 0.5^a$	$58.9 \pm 0.6^a$
LSD (0.05)	568.8	51.0	6.02	3.4	2140.0	989.0	2.1	0.15	13.4	1.0	0.9	1.3

### 3.3 Flag leaves area

There were significant effects of nitrogen rate and bio-fertilizer application on flag leaves area, while the interaction effect of nitrogen rate and bio-fertilizer application was not significant (Table 1). Regardless of bio-fertilizer application, flag leaves area was significantly increased from  $6216.1 \pm 224.9$  to  $12168.2 \pm 1239.2$   $\text{cm}^2$  when N rate increased from 0 to  $150 \text{ kg ha}^{-1}$  (Table 2). Averaged across N rates, flag leaves area was significantly increased following bio-fertilizer application by 25 % (Table 2). Lemaire et al. (2008) declared that N shortage reduced leaf expansion in  $C_3$  cereals plants. Insufficient N

availability during rice growth stage declined both flag leaf size and number and, therefore, reduced total net photosynthesis (Zong and Shangguan, 2014), which in turn decreased rice grain yield. Besides, Toth et al. (2002) suggested that lower rates of photosynthesis under low N supply are often attributed to reduction in chlorophyll content and Rubisco activity. Flag leaves area was positively correlated with grain and biological yields, yield components height, grain N concentration and uptake, and head rice yield at  $P < 0.01$  level, but not correlated with HI, and milling yield (Table 3).

**Table 3:** Correlation coefficients for measurements of rice as influenced by N rate and bio-fertilizer application

	Y	PN	GN	ThGW	FL	BY	HI	GNC	GNU	GPC	MY
PN	0.51*	1									
GN	0.55**	0.49*	1								
ThGW	0.54**	0.01 <sup>ns</sup>	0.22 <sup>ns</sup>	1							
FL	0.57**	0.51*	0.60**	0.47 <sup>ns</sup>	1						
BY	0.97**	0.52**	0.58**	0.50*	0.56**	1					
HI	0.86**	0.42 <sup>ns</sup>	0.35 <sup>ns</sup>	0.52*	0.44 <sup>ns</sup>	0.73**	1				
GNC	0.75**	0.65**	0.70**	0.42 <sup>ns</sup>	0.65**	0.74**	0.62**	1			
GNU	0.92**	0.60**	0.67**	0.52*	0.66**	0.89**	0.78**	0.94**	1		
GPC	0.75**	0.65**	0.70**	0.42 <sup>ns</sup>	0.65**	0.74**	0.62**	0.99**	0.94**	1	
MY	0.01 <sup>ns</sup>	-0.14 <sup>ns</sup>	0.21 <sup>ns</sup>	-0.24 <sup>ns</sup>	0.07 <sup>ns</sup>	0.08 <sup>ns</sup>	-0.09 <sup>ns</sup>	0.01 <sup>ns</sup>	0.01 <sup>ns</sup>	0.01 <sup>ns</sup>	1
HRY	0.51*	0.15 <sup>ns</sup>	0.26 <sup>ns</sup>	0.22 <sup>ns</sup>	0.64*	0.42 <sup>ns</sup>	0.29 <sup>ns</sup>	0.69**	0.68**	0.69**	0.54**

\*, \*\* represent significance at 0.05 and 0.01 probability level, respectively

ns represents no significant difference

Y, Grain yield; PN, panicle number per m<sup>2</sup>; GN, grain number per panicle; ThGW, 1000-grain weight; FLA, Flag leaves area; BY, biological yield; HI, harvest index; GNC, grain N concentration; GNU, grain N uptake; MY, milling yield; and HRY, head rice yield

### 3.4 Grain N concentration, grain N uptake, and grain protein concentration

The main effects of nitrogen rate and bio-fertilizer application were significant ( $P < 0.01$ ) for grain N concentration, grain N uptake, and grain protein concentration, but there were no significant interactions of nitrogen rate  $\times$  bio-fertilizer application for all of them (Table 1). Regardless of bio-fertilizer application, grain N concentration, grain N uptake, and grain protein concentration had similar responses to N rate. Grain N concentration, grain N uptake, and grain protein concentration were significantly increased as N rate increased up to 75 kg ha<sup>-1</sup>, but further increases in N rate had no significant effect on these traits (Table 2). Regardless of N rate, grain N concentration, grain N uptake, and grain protein concentration were significantly increased when bio-fertilizer was applied (Table 2). The higher grain N concentration resulting from inoculation may be attributed to increased N uptake by a larger root surface area associated with additional root hairs and lateral root development and/or to biological nitrogen fixation (Biswas et al., 2000). Cong et al. (2009) and Pedraza et al. (2009) also reported that rice grain N uptake was significantly increased with applying bio-fertilizer.

### 3.5 Milling yield and head rice yield

The ANOVA results showed that nitrogen rate and bio-fertilizer application as well as the interaction between them had no significant effects on milling yield (Table 1). Head rice yield was significantly affected by nitrogen rate, while bio-fertilizer application and the interaction between nitrogen rate and bio-fertilizer application were not significant (Table 1). Averaged across bio-fertilizer applications, head rice yield was significantly increased from 56.8 $\pm$ 0.7 % to 60.5 $\pm$ 0.6 % when N rate increased from 0 to 150 kg ha<sup>-1</sup> (Table 2). The positive influence of nitrogen on head rice yield is contributed to this fact that N fertilization increases the packing of protein matrix between endosperm starches in rice grains and grain protein makes it more resistant to cracking and breakage during milling process (Blumenthal et al., 2008). Leesawatwong et al. (2005) also noted that the head rice yield was positively correlated with relative abundance of the storage protein in the lateral section of the endosperm of rice kernels. The positive effect of nitrogen on head rice yield also was reported by Perez et al. 1996, and Dilday (1988). Head rice yield was positively correlated with grain yield, flag leaves area, grain N concentration and uptake, grain protein concentration, and milling yield, but not correlated with yield components, biological yield, and harvest index (Table 3).

#### 4 CONCLUSIONS

This experiment illustrated that rice grain and head yields increased with increasing N rate, while bio-fertilizer increased only rice grain yield. Regardless of bio-fertilizer application, rice grain yield was significantly increased from  $4251.4 \pm 346.5$  to  $5016.2 \pm 349.3$  kg ha<sup>-1</sup> as N application rate increased from 0 to 75 kg ha<sup>-1</sup>, but did not significantly increase at the higher N rate

(150 kg ha<sup>-1</sup>). Grain yield was significantly increased by bio-fertilizer application when averaged across N rates. Regardless of bio-fertilizer application, head rice yield was significantly increased from  $56.8 \pm 0.7$  % to  $60.5 \pm 0.6$  % when N rate increased from 0 to 150 kg ha<sup>-1</sup>.

#### 5 ACKNOWLEDGEMENTS

The author is grateful for the funding supplied by the Rasht branch, Islamic Azad University and

Rice research institute of Iran for providing research facilities.

#### 6 REFERENCES

- Biswas J.C., Ladha J.K., Dazzo F.B., Yanni Y.G., Rolfe B.G. 2000. Rhizobial inoculation influences seedling vigor and yield of rice. *Agron. J.* 92: 880–886, doi: 10.2134/agronj2000.925880x
- Blumenthal J.M., Baltensperger D.D., Cassman K.G., Mason C.S., Pavlista A.D. 2008. Importance and effect of nitrogen on crop quality and health. Published in *Nitrogen in the Environment: Sources, Problems, and Management*, Second edition, edited by J. L. Hatfield and R. F. Follett (Amsterdam: Elsevier, 2008). Copyright © 2008 Elsevier, doi: 10.1016/b978-0-12-374347-3.00003-2
- Brown J.R., Blankinship J.C., Niboyet A., van Groenigen K.J., Dijkstra P., LeRoux X. 2012. Effects of multiple global change treatments on soil N<sub>2</sub>O fluxes. *Biogeochemistry* 109, 85–100, doi: 10.1007/s10533-011-9655-2
- Cong P.T., Dung T.D., Hien T.M., Hien N.T., Choudhury A.T.M.A., Kecskés M.L., Kennedy I.R. 2009. Inoculant plant growth-promoting microorganisms enhance utilisation of urea-N and grain yield of paddy rice in southern Vietnam. *Eur. J. Soil Biol.* 45, 52–61, doi: 10.1016/j.ejsobi.2008.06.006
- Dilday R.H. 1988. Effect of nitrogen fertilizer on milling quality of rice (*Oryza Sativa*). *Proceedings Arkansas Academy of Science.* 42:26-27
- Hak R., Rinderle-Zimmer U., Lichtenthaler H.K., Natr L. 1993. Chlorophyll a fluorescence signatures of nitrogen deficient barley leaves. *Photosynthetica* 28: 151–159
- Isawa T., Yasuda M., Awazaki H., Minamisawa K., Shinozaki S., Nakashita H. 2010. Azospirillum sp. strain B510 enhances rice growth and yield. *Microbes Environ.* 1: 58–61, doi: 10.1264/jsme2.ME09174
- Kanawapee N., Sanitchon J., Srihaban P., Theerakulpisut P. 2011. Genetic diversity analysis of rice cultivars (*Oryza sativa* L.) differing in salinity tolerance based on RAPD and SSR markers. *Electronic Journal of Biotechnology*, 14(6): No. 4, pp: 17
- Keyeo F., Ai'shah O.N., Amir H.G. 2011. The effects of nitrogen fixation activity and phytohormone production of diazotroph in promoting growth of rice seedlings. *Biotech.* 10: 267-273, doi: 10.3923/biotech.2011.267.273
- Leesawatwong M., Jamjod S., Kuo J., Dell B., Rerkasem B. 2005. Nitrogen fertilizer increases seed protein and milling quality of rice. *Cereal Chemistry* 82: 588–593, doi: 10.1094/CC-82-0588
- Lemaire G., van Oosterom E., Jeuffroy M.H., Gastal F., Massignam A. 2008. Crop species present different qualitative types of response to N deficiency during their vegetative growth. *Field Crops Res.* 105, 253–265, doi: 10.1016/j.fcr.2007.10.009
- Li Y., Chen X., Shamsi I.H., Fang P., Lin Y. 2012. Effects of Irrigation Patterns and Nitrogen Fertilization on Rice Yield and Microbial Community Structure in Paddy Soil. *Pedosphere*, 22 (5): 661–672, doi: 10.1016/S1002-0160(12)60051-4

- Manzoor Z., Awan T.H., Zahid M.A., Faiz F.A. 2006. Respons of rice crop (SUPER BASMATI) to different nitrogen levels. *J. Anim. Pl. Sci.* 16(1-2): 52-55
- Mukhopadhyay M., Datta J.K., Garai T.K. 2013. Steps toward alternative farming system in rice. *Europ. J. Agronomy* 51:18–24, doi: 10.1016/j.eja.2013.06.005
- Pedraza R.O., Bellone C.H., de Bellone S.C., Sorte P.M.F.B., Teixeira K.R.S. 2009. Azospirillum inoculation and nitrogen fertilization effect on grain yield and on the diversity of endophytic bacteria in the phyllosphere of rice rainfed crop. *Europ. J. Soil Boil.* 45: 36–43, doi: 10.1016/j.ejsobi.2008.09.007
- Perez C.M., Juliano B., Liboon S., Alcantara J.M., Cassman K.G. 1996. Effects of late nitrogen fertilizer application on head rice yield, protein content, and grain quality of rice. *Cereal Chem.* 73: 556–560
- Pregl F. 1945. *Quantitative Organic Microanalysis*. 4<sup>th</sup> ed. J.A. Churchill Ltd. London, p.126-129
- Rezazadeh T., Aghaiypour Kh., Heidari Z. 2013. The significance of food safety in trade and banning the importation of GMO products into Iran. *Croat. J. Food Sci. Technol.* (2013) 5 (2) 92-95.
- SAS 2004. SAS Institute, version 9.1.3. Cary, NC, USA.
- Toth, V.R., Meszkaros I., Veres S., Nagy J. 2002. Effects of the available nitrogen on the photosynthetic activity and xanthophyll cycle pool of maize in field, *J. Plant Physiol.* 159: 627–634, doi: 10.1078/0176-1617-0640
- Weerakoon W.M.W., Ingram K.T., Moss D.N. 2005. Atmospheric CO<sub>2</sub> concentration effects on N partitioning and fertilizer N recovery in field grown rice (*Oryza sativa* L.). *Agric. Ecosyst. Environ.* 108: 342–349, doi: 10.1016/j.agee.2004.12.014
- Zhao X., Fitzgerald M. 2013. Climate Change: Implications for the Yield of Edible Rice. *PLOS ONE*, 8(6): e66218. pp:9
- Zong Y., Shanguan Z. 2014. Nitrogen deficiency limited the improvement of photosynthesis in maize by elevated CO<sub>2</sub> under drought. *J. Integ. Agric.* 13(1): 73-81, doi: 10.1016/S2095-3119(13)60349-4