



The role of Azolla on improving nitrogen efficiency in rice cultivation

Etty Safriyani^{1,3*}, Mery Hasmeda², Munandar², Firdaus Sulaiman², Holidi³, Kartika Kartika¹

1. Graduate School, Universitas Sriwijaya, Palembang 30139, Indonesia
2. College of Agriculture, Universitas Sriwijaya, Inderalaya 30662, Indonesia
3. College of Agriculture, Universitas Musi Rawas, Indonesia

Abstract

Nitrogen is essential for both vegetative and generative stages of plant growth. Nitrogen efficiency is related to plant growth, production cost, and fertilizer residue. Azolla has the ability to increase nitrogen fixation from the atmosphere. Thus, Azolla cultivation in paddy field is one strategy for improving nitrogen fertilizer efficiency and increasing plant vegetative and generative growth. The objective of this study was to evaluate the role of Azolla on improving nitrogen efficiency. This study was conducted from January to April 2018 in irrigated paddy field of Lubuklinggau Selatan II, Lubuklinggau. The study was laid out in a randomized block design arrangement with 4 replications. Treatments were combinations of Azolla and urea which were applied during vegetative and generative stages: 115 kg N ha⁻¹ without Azolla (K1), 115 kg N ha⁻¹ + 1000 kg ha⁻¹ Azolla (K2), 86 kg N ha⁻¹ + 1000 kg ha⁻¹ Azolla (K3), 58 kg N ha⁻¹ + 1000 kg ha⁻¹ Azolla (K4), 29 kg N ha⁻¹ + 1000 kg ha⁻¹ Azolla (K5), and zero N+1000 kg ha⁻¹ azolla (K6). The application of 86 kg N ha⁻¹ and 1000 azolla kg ha⁻¹ (K3) increased 15.54% rice growth, 25.49% yield, and improved the N fertilizer agronomic efficiency (AE), agro-physiological efficiency (APE), utilization efficiency (UE), and N efficiency ratio (NER).

Keywords: Azolla; nitrogen; paddy growth; plant production

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Introduction

Rice is the main source of carbohydrate for most of Indonesian. In 2015, rice harvested area in Indonesia was up to 14.117 million hectares with total production of approximately 75.398 million tons of dried grains and 5.88-ton ha⁻¹ productivity (Indonesian Ministry of Agriculture, 2016). Rice productivity is highly affected by nutrient availability. Fertilizer application is necessary to enhance nutrient availability for rice growth (Kartika et al., 2018a). Nitrogen is one of

the macro-essential nutrients affecting rice productivity. Nitrogen acts as a limiting factor for the rice growth and plays an important role in maintaining and increasing rice productivity (Sainju, 2013; Fageria, 2014). Nitrogen plays an important role in plant metabolism including physiological process and also bio-chemical, main element of chlorophylls, amino acid, and protein synthesis, inhibiting leaf senescence and contributing in the production of chemical components for plant protection (Fageria et al., 2010; Kartika et al., 2018b). Stunt rice is found under nitrogen deficiency environment. Urea is the world's most commonly used fertilizer for

*Corresponding author

E-mail address: ettysafriyani@fpuunmura.ac.id

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nitrogen supply for rice cultivation. In addition, Azolla could be used for increasing N fixation from the atmosphere.

Azolla is an aquatic fern that forms symbiotic relationships with N₂-fixing cyanobacterium *Anabaena azollae* and supplies N to the paddy field (Kollah et al., 2016). Azolla enhances N uptake of plant (Raja et al., 2012; Bhuvaneshwari and Singh, 2015). Previous studies revealed that Azolla application in paddy fields increased plant height, number of effective tiller, leaf area, and plant biomass (Bhuvaneshwari and Singh, 2015), increased growth and yield (Mahalingam et al., 2014). Azolla is a source of biofertilizer and its application is able to increase fertilizer efficiency, especially nitrogen.

Nitrogen uptake is controlled by several internal factors such as plant physiology, plant type, and certain plant needs for N. External factors affecting N uptake are light intensity, air, water status, soil pH, growing season, fertilizing method, and soil characteristics (Sato et al., 2012). Low N uptake ability correlates with reduced plant N utilization efficiency and strongly influences growth and yield. Nitrogen efficiency is concerned with the ratio of nutrient utilized by crop to the amount of nutrient applied. Azolla cultivation in rice field is expected to improve N uptake in order to supply sufficient nutrient for plant growth and yield. Thus, this study focused on evaluating the role of Azolla in improving nitrogen efficiency, rice growth, and yield.

Materials and Methods

This study was conducted on a farmer's irrigated paddy field at Karang Ketuan of Lubuklinggau Selatan II (3°12'42.4"S 102°56'23.3"E), Indonesia. The study was conducted for 4 months from January to April 2018. Study was arranged in 500 m² paddy field characterized with 1.55% organic C, 0.34 % N-total, and C: N ratio 5. Soil tillage was conducted manually 20 days prior to transplanting until 25 cm depth and fertilized with 2.5-ton cow manure. The paddy field was divided into 4 replications consisting of 6 blocks of 2 m x 2 m and laid on a randomized block design. The treatments were 115 kg N ha⁻¹ without Azolla (K1), 115 kg N ha⁻¹ + 1000 kg ha⁻¹ Azolla (K2), 86 kg N ha⁻¹ + 1000 kg ha⁻¹

Azolla (K3), 58 kg N ha⁻¹ + 1000 kg ha⁻¹ Azolla (K4), 29 kg N ha⁻¹ + 1000 kg ha⁻¹ Azolla (K5), and zero N+1000 kg ha⁻¹ Azolla (K6).

Rice used in this study was Inpari 42 variety belonging to *Oryza sativa*. Seeds were soaked in water for 24 hours and germinated for 48 hours. Germinated seeds were sown in 12 m x 4 m nursery bed with the density of 25 g m⁻². Rice seedlings were transplanted to the paddy field 15 days after sowing and the planting distance was 25 cm x 25 cm. Phosphorous and potassium fertilizers were applied 36 kg ha⁻¹ and 60 kg ha⁻¹, respectively in transplanting day. Urea was applied three times during rice growth at transplanting, 30 days after transplanting (DAT), and 60 DAT. Plants were irrigated daily and set to 5 cm depth until 80 DAT. The rice was harvested on 95 DAT.

Azolla was cultivated 20 days prior to transplanting with the application rate of 100 g m⁻². The Azolla used during the study was derived from Azolla propagation carried out at the study site, 2 cm -2.5 cm, sterilized, and harvested on 20 DAT and 34 DAT. 80% of the Azolla was immersed into the ground and 20% was sown on the water surface.

The observed variables were plant height, number of tillers per hill, number of panicles per hill, number of grains per hill, weight of grains per hill, and dry weight of straw. Leaf N content was observed twice at the termination of vegetative stage and senescence stage. Straw and grain N contents were analyzed after harvest and macro Kjeldhal method was used for analyzing N contents of each plant organ, Azolla, and soil.

The data from experiment were subjected to statistical analysis software (SAS 9.0 for Windows) and analyzed by Analyses of Variance (ANOVA). Significant differences between treatments were analyzed by Honestly Significantly Difference test (HSD) at 5% level (Gomez and Gomez, 1984). Nitrogen efficiency was calculated using the formula suggested by Fageria (2014) :

$$a. \text{ Agronomic efficiency (AE), the yield obtained per unit of nutrient applied} = \frac{\text{yield of fertilized crop} - \text{yield of unfertilized crop}}{\text{quantity of fertilizer applied}}$$

b. Physiological efficiency (PE), biological production obtained per unit of nutrient uptake =

$$\frac{\text{dry matter of fertilized crop} - \text{dry matter of unfertilized crop}}{\text{nutrient uptake of fertilized crop} - \text{nutrient uptake of unfertilized crop}}$$

c. Agro-physiological efficiency (APE), yield obtained per unit of nutrient uptake =

$$\frac{\text{yield of fertilized crop} - \text{Yield of unfertilized crop}}{\text{N uptake in grain and straw of fertilized crop} - \text{N uptake of unfertilized crop}}$$

d. Apparent recovery efficiency (ARE), quantity of nutrient taken up per unit of nutrient applied =

$$\frac{\text{nutrient uptake of fertilized crop} - \text{nutrient uptake of unfertilized crop}}{\text{quantity of fertilizer applied}} \times 100\%$$

e. Utilization efficiency (UE) = Physiological efficiency × recovery efficiency

f. Nutrient efficiency ratio (NER), N utilization efficiency =

$$\frac{\text{Yield}}{\text{N in plant tissue}}$$

Results

Growth and yield

Nitrogen application and Azolla effected rice growth as indicated by plant height and shoot dry weight. Plant height was gradually increased until the termination of vegetative stage and was observed biweekly. Nitrogen application and Azolla significantly affected plant height at 14 DAT, 28 DAT, and 42 DAT. Application of 86 kg N ha⁻¹ + Azolla 1000 kg ha⁻¹ (K3) obtained the highest

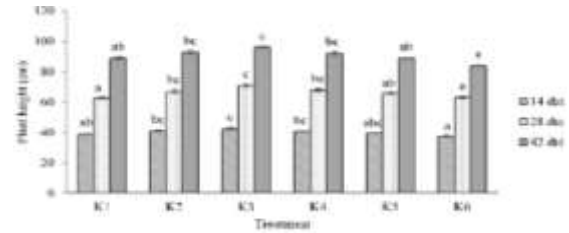


Fig. I. Plant height during vegetative growth of rice treated with different nitrogen application rates and Azolla; bars (means ± standard deviation, n=4) with different letters are significantly different based on hsd 0.05. K1 =115 kg N ha⁻¹ without Azolla; K2 =115 kg N ha⁻¹ + 1000 kg ha⁻¹ Azolla; K3 =86 kg N ha⁻¹ + 1000 kg ha⁻¹ Azolla; K4 =58 kg N ha⁻¹ + 1000 kg ha⁻¹ Azolla; K5 =29 kg N ha⁻¹ + 1000 kg ha⁻¹ Azolla; K6 = zero N + 1000 kg ha⁻¹ Azolla

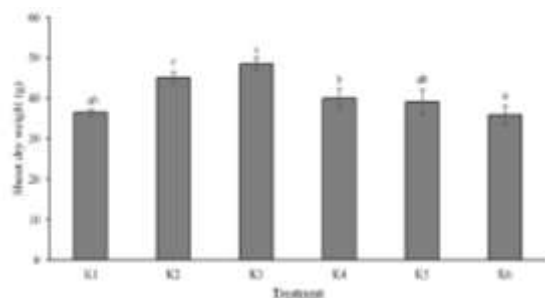


Fig. II. Shoot dry weight at termination of vegetative growth stage of rice treated with different nitrogen application rates an Azolla; bars (means ± standard deviation, n=4) with different letters are significantly different based on hsd 0.05.

height and the plant growth without N application + Azolla 1000 kg ha⁻¹ (K6) showed the lowest height in every observation time (Fig. I). There was a significant difference between shoot dry weight of plants treated with different N and Azolla applications. The highest shoot dry weight was found at K3 (48.53 g) and K2 (45.07 g) while the lowest shoot dry weight was recorded at K6 (35.93 g) (Fig. II).

Regardless of different N application rates, Azolla biomass sharply decreased from 20 DAT to 35 DAT. Different rates of nitrogen application did not show significant effect on biomass of Azolla (Fig. III). Azolla in this study contained 20.12% organic C, 3.07% N, 1.20% P, and 3.44% K (details of Azolla nutrient contribution are shown in Table 1).

Regarding the number of tillers, crops treated with N showed better performance than those not treated with N. Regardless of N

Table 1
Nutrient Azolla contribution

Treatment	Nutrient azolla kg ha ⁻²			
	N-total	P-total	K-total	C-organic
K2	93.60	36.59	104.89	613.459
K3	109.61	42.85	122.82	718.385
K4	96.69	37.79	108.35	633.729
K5	114.91	44.91	128.75	753.066
K6	116.52	45.54	130.561	763.629
Average	106.27	41.54	119.074	696.454

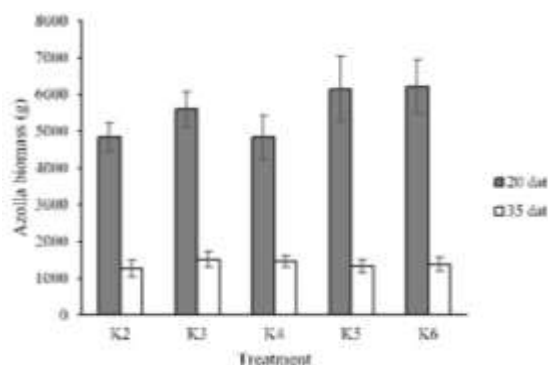


Fig. III. Azolla biomass at 20 and 35 DAT; bars show means \pm standard deviation, n=4.

treatment, number of effective tillers was less than total tiller per hill. The rice treated with K3 resulted in both the highest total tiller and effective tiller. Crops treated without N fertilizer + Azolla 100 kg ha⁻¹ were unable to produce high number of total and effective tillers (Fig. IV).

Rice yield was strongly affected by the number of fertile and sterile spikelet. High number of fertile spikelet resulted in higher yield. In this study, different N application rates and Azolla cultivation significantly affected the percentage of fertile spikelet. K3 treatment had the highest number of fertile spikelet and the lowest number of sterile spikelet. However, this was not significantly different compared to K2, K4, and K5 (Fig. V. A). Grain weight hill⁻¹ ha showed similar pattern with percentage of fertile spikelet. The highest grain weight was found in K3 treatment (60.63 g) and the lowest in K6 (45 g) (Fig. V. B).

N fertilizer application and Azolla cultivation did not significantly affect N content in leaves at vegetative stage (Fig. VI. A), leaf senescence (Fig. VI. B), grain (Fig. VI. C), and straw (Fig. VI. D). However, the rice planted in K2 treatment showed the highest N content in straws, as well as in leaves at vegetative stage and

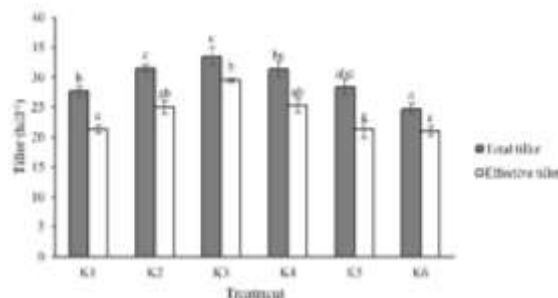


Fig. IV. Number of total and effective tillers; bars (means \pm standard deviation, n=4) with different letters are significantly different based on hsd 0.05.

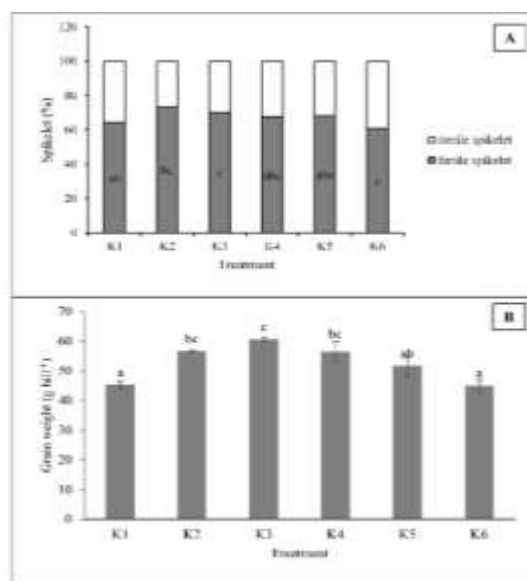


Fig. V. Percentage of fertile and sterile spikelet (A) and grain weight (B); bars (means \pm standard deviation, n=4) with different letters are significantly different based on hsd 0.05.

leaf senescence. In average, the highest grain N content was found in K4 where plants did not receive N fertilizer. This indicates that N application did not affect N uptake by the plants.

Nitrogen use efficiency

The N application rate strongly correlated with agronomic efficiency (Fig. VII. A), physiological efficiency (Fig. VII. B), agro-physiological efficiency (Fig. VII. C), utilization efficiency (Fig. VII. D), nutrient efficiency ratio (Fig. VII. E), and apparent recovery efficiency (Fig. VII. F).

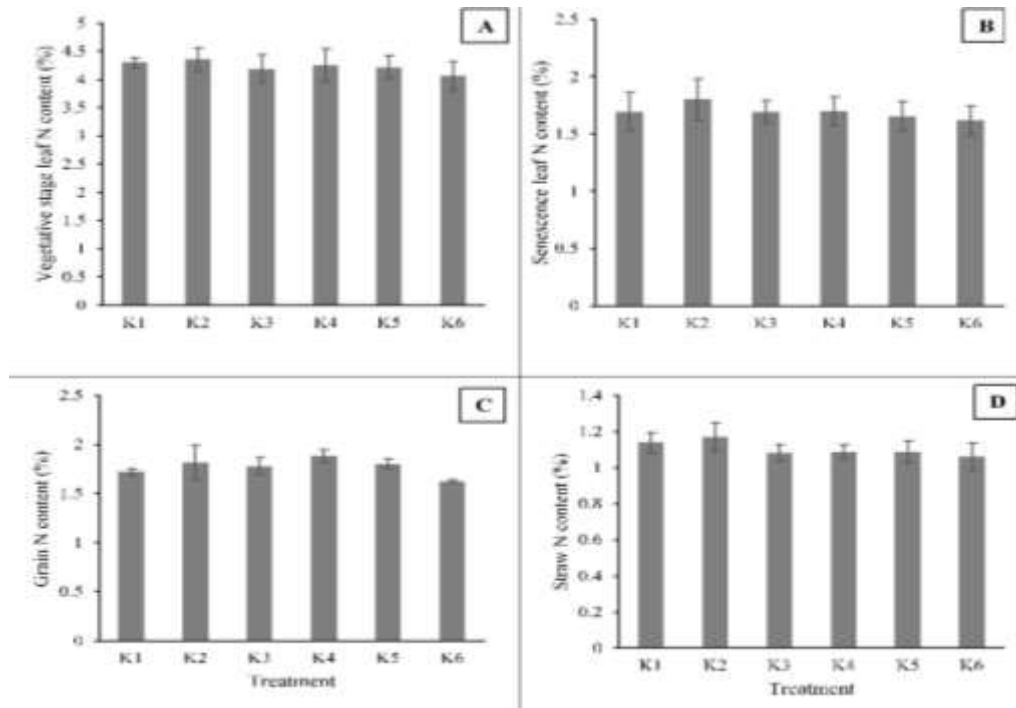


Fig. VI. Crop N content; vegetative stage leaf (A), senescence leaf (B), grain (C), stem (D); bars are means ± standard deviation, n =4.

Table 2
Correlation of growth and yield components with nitrogen efficiency

The variables observe	Efficiency nitrogen (r ²)					
	AE	PE	APE	UE	NER	ARE
1.Plant height	0.443	0.702	0.632	0.776	0.823	0.443
2.Number of tillers hill ⁻¹	0.752	0.981	0.965	0.995	0.531	0.624
3.Dry weight of straw hill ⁻¹	0.086	0.211	0.176	0.345	0.959	0.427
4.Number of panicles hill ⁻¹	0.582	0.738	0.678	0.826	0.845	0.532
5.Number of grains hill ⁻¹	0.446	0.614	0.555	0.730	0.929	0.535
6.Weight of grains hill ⁻¹	0.377	0.677	0.651	0.821	0.903	0.773
7.1000 grains weight	0.544	0.273	0.195	0.247	0.327	0.003

AE=agronomic efficiency, PE= physiological efficiency, APE= agrophysiological efficiency, UE= utilization efficiency, NER= nutrient efficiency ratio, ARE=apparent recovery efficiency

Application of N fertilizer resulted in high N physiological efficiency, agro-physiological efficiency, utilization efficiency, and nutrient efficiency ratio (98.7%, 99.9%, 90.0% and 90.0%, respectively). Similar to the other observed variables, treatment K3 had higher N efficiency.

Growth and yield components of rice correlated with nitrogen efficiency (Table 2). The average growth and yield components showed the strong correlation with nutrient efficiency ratio. Plant height, plant dry weight, number of panicle, number of grain, and grain weight variables

significantly and positively correlated with nutrient efficiency ratio (82.3%, 84.5%, 92.9%, and 90.35, respectively). On the other hand, the number of tiller significantly correlated with physiological efficiency, agro-physiological efficiency, and utilization efficiency (98.1%, 96.5%, and 99.5% respectively). In contrast, weight of 1000 grains had a very small correlation with nitrogen efficiency.

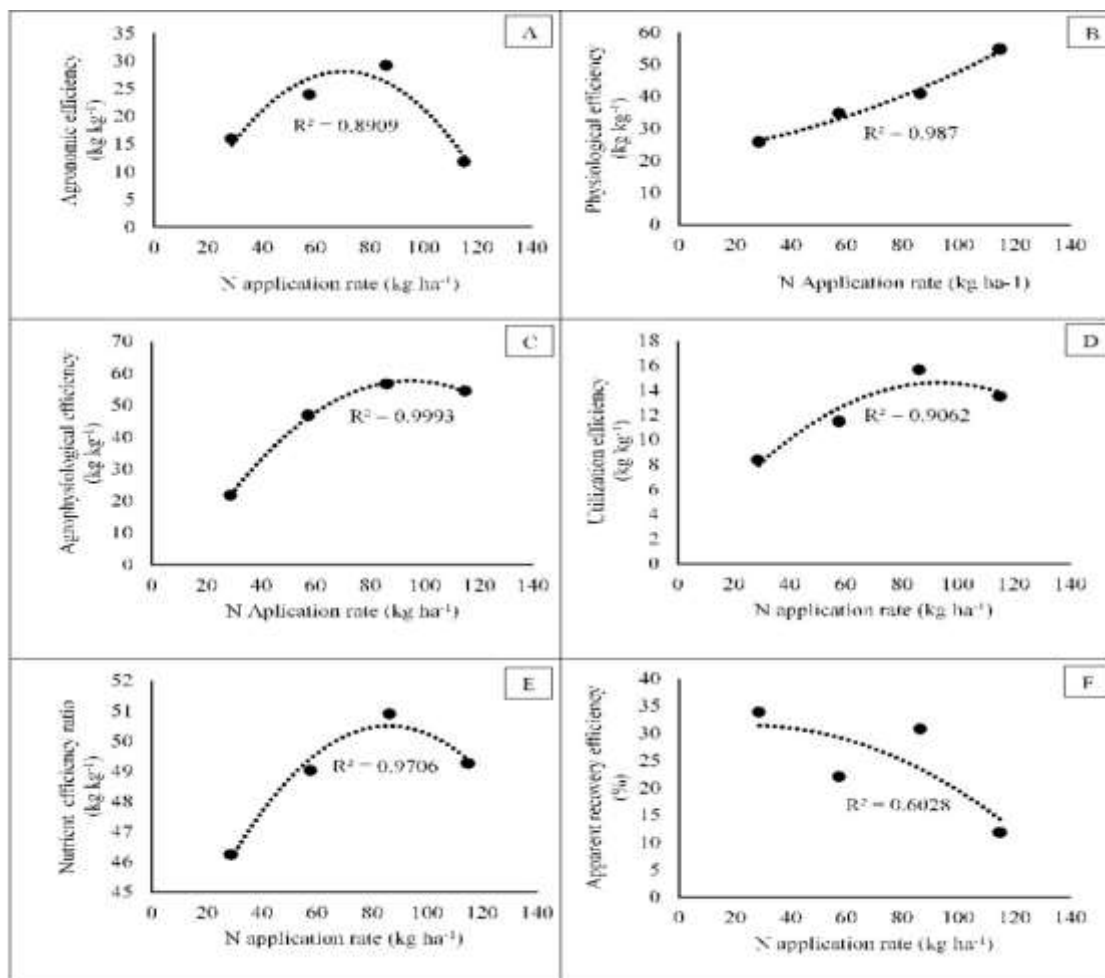


Fig. VII. Agronomic efficiency (A), physiological efficiency (B), agro-physiological efficiency (C), utilization efficiency (D), nutrient efficiency ratio (E), apparent recovery efficiency (F) of N on different application rates

Discussion

Nitrogen application and Azolla cultivation in paddy field resulted in different nitrogen availability for rice growth. Azolla contains 3.04% nitrogen so that the treatment supplied nitrogen for K2, K3, K4, K5, and K6 as 93.60, 109.61, 96.69, 114.91, and 116.56 kg ha⁻¹ respectively. Azolla also contributed to supplying P-total, K-total, and C-organic contents as 41.54, 119.074, and 696.454 kg ha⁻¹, respectively.

In our study, application of N 86 kg ha⁻¹ + Azolla 1000 kg ha⁻¹ could increase rice growth and yield due to sufficient nitrogen availability at both vegetative and generative stages. Sufficient nutrient availability during rice vegetative stage improves its ability to produce assimilates for generative stage which is essential for producing the grains. However, study area was characterized

with low nitrogen availability and require extra supply for supporting rice growth.

This study confirmed that the application of N 86 kg ha⁻¹ + Azolla 1000 kg ha⁻¹ (K3) led to the highest plant height, shoot dry biomass, and number of total tillers during rice vegetative stage. This finding is similar to previous studies that Azolla cultivation in paddy field increased plant height, number of effective tillers, leaf area, and dry biomass accumulation (Bhuvaneshwari dan Singh, 2015). It indicates that applied N fertilizer with the addition of N fixation by Azolla sufficiently provided for rice vegetative growth. Kollah et al (2015) reported that symbiosis between Azolla and cyanobacteria supplied 30-60 kg ha⁻¹ nitrogen fixation. Furthermore, Azolla would be easily decomposed in paddy field and supply more nitrogen for rice growth (Raja, et al., 2012). On the other hand, rice plants grown under

K1 and K6 treatments (both without nitrogen application) showed the lowest vegetative and generative growth as compared to those with nitrogen fertilizer addition. This study revealed that in order to optimize rice growth, Azolla cultivation should be combined with 50% of nitrogen recommended application rate (Baigi et al. 2013).

Reduction of N application for 25% (K3) increased agronomic efficiency, physiological efficiency, agro-physiological efficiency, utilization efficiency, nutrient efficiency ratio, and apparent recovery efficiency. Higher nitrogen application tends to decrease agronomic efficiency, physiological efficiency, agro-physiological efficiency, utilization efficiency, nutrient efficiency ratio, and apparent recovery efficiency. It is assumed that rice could not absorb all nutrient applied and increase the possibility of nutrient loss. Fageria et al. (2011) and Fageria (2014) reported 50-70 nitrogen loss through leaching, run off, and denitrification. Furthermore, Eagle et al (2001) reported that N physiological efficiency decreased as N fertilizer application increased. N requirement and N uptake are dependent on plant growth stage and excessive N supply would disturb rice growth and ecosystem stability, increasing production cost (Zheng et al. 2007). Plant height, dry biomass, number of panicles, and number and weight of grains highly correlated with nutrient efficiency ratio. Kollah et al. (2016) reported that Azolla is a source of biological nitrogen. Rice utilized N optimally during the growing season and improved its growth and grain quantity and quality (Sainju, 2013; Fageria, 2014).

Number of tillers strongly correlates with physiological efficiency, agro-physiological efficiency, and utilization efficiency. Physiological efficiency indicates biological yield; agro-physiological efficiency indicates grain yield; and apparent recovery efficiency indicates absorbed nutrient. This finding showed that number of tillers was affected by the amount of N uptake by plant. The high number of tillers will produce higher biological components and yield. This is similar to the findings of Fageria (2014) who reported that number of tillers positively correlated with grain weight.

Different nitrogen application rates did not affect the growth of Azolla. The highest Azolla

biomass was found in K6 with no N application. Sabetraftar et al. (2015) reported that *Azolla microphylla* is a photoautotrophic aquatic organism which is strongly influenced by phosphorus availability (in the form of phosphate). Phosphorus is a major limiting nutrient for rapid growth of Azolla. Phosphorus supply enhance sporulation and sufficient phosphorus increases the ability of Azolla to grow without nitrogen supply. In this study, Azolla growth decreased at 35 DAT due to light competition with rice. From 28 DAT, rice shoot covered approximately about 65% of the ground and inhibited azolla growth. Pabby et al. (2003) demonstrated that growth rate and nitrogen fixation activity of Azolla was affected by light intensity.

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