

Effect of Ammonium Sulfate Application Levels on the Growth and Yield of IR-28 Rice

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ABSTRACT--- *The study was conducted in JICA Tsukuba experimental rice field RE-2 from April 2018 to September 2018, to determine the effect of different levels of nitrogen fertilizer (Ammonium sulfate) application, on the growth and the yield of IR-28 rice. Four levels of nitrogen fertilizer were applied; 0kg.ha⁻¹ (N0), 40kg.ha⁻¹(N40), 60kg.ha⁻¹(N60) and 80kg.ha⁻¹(N80). For each of the four levels, part of the fertilizer was applied as basal dressing prior to transplanting, and the rest was applied as top-dressing at the panicle initiation stage. The experimental design was a Randomized Complete Block Design (RCBD) with four treatments and three replications. The plant length, the tiller number and the leaf colour were measured for growth data. The number of panicles per m², the number of spikelets per panicle, the spikelet fertility rate, the 1000 grains weight, and the calculated yield were determined for yield components assessment. The plant length and the tiller number were significantly higher in N80 and N60 compared to N40 and N0, and N80 showed the highest values. There was no significant difference among the four nitrogen levels in terms of the number of panicles per m² and the number of spikelets per panicle. The spikelet fertility rate and the 1000 grains weight were significantly higher in N80, N60 and N40 compared to N0, and no significant difference was observed among the three. Calculated yield values were higher in N80 (5.74 tons.ha⁻¹) and N60 (5.38 tons.ha⁻¹) compared to N40(4.88 tons.ha⁻¹) and N0 (4.36 tons.ha⁻¹), but there were no significant differences among the four treatments (5% Level of HSD). These results suggest that a high yield of rice can be achieved through the application of high amounts of nitrogen fertilizers. N60 nitrogen level can be recommended for optimum yield of IR-28. Although N80 showed higher yield and yield components, N60 is the best and the most economical nitrogen level required for optimum yield of IR-28.*

Keywords--- Oryza sativa L, Agronomy, Physiology, Nitrogen amount

1. INTRODUCTION

Rice (*Oryza sativa* L.) for many decades was the staple food only for the people in Asia. Nowadays, rice has become the main staple food that provides food calories to people all over the world. The consumption of rice has exponentially increased in the world over the past twenty years. Currently, it is reported that more than half of the world population eats rice as a staple food (Muthayya et al.,2014). The world's population is estimated to reach 9 billion people by 2050. Considering the major role rice is now playing in people's nutrition across the world, rice production must increase by at least 50% to be able to feed the world population (FAO, 2008). There are two main ways through which rice production can be increased in the world; expansion of rice cultivation lands and increase in the yield of rice per unit area (Tang et al., 2017). The best way rice production can be increased in a sustainable manner nowadays is by increasing the yield of rice per unit area. An increase in rice yield of at least 1% is needed every year to meet the increasing global demand for rice (Normile, 2008).

Numerous yield improvement practices have been developed in the past decades. Some of these practices are the use of high yielding varieties, irrigation, planting density, seedling age, and the use of fertilizers. The use of fertilizers is the most widely adopted practice, and the most efficient also. Nitrogen fertilizers are the main fertilizers used in rice cultivation. In Asia, the number one producer of rice in the world, reports have shown that the high yield levels of rice grains achieved currently is because of excessive use of nitrogen (N) fertilizers (Ladha & Reddy, 2003; Samonte et al., 2006). In China,

the use of high amounts of nitrogen fertilizers is reported to have become a common practice for agricultural production (Gong et al, 2011; Ju et al., 2015; Tian et al., 2016). Nitrogen fertilizers are widely used because, of all the nutrient elements that are required for plant growth and development, nitrogen is the most important and often the main limiting factor for adequate growth and development of plants (Kant, 2018). Nitrogen (N) has a determining role in crop production because and is a key component in the biomass accumulation process (Salvagiotti & Miralles, 2008). Nitrogen constitutes a building block for plant protoplasm and acts as a catalyst for other nutrients. Nitrogen is also reported to be the most active factor that determines soil fertility (Agreen, 1985; Cassman et al.,2002). Studies have reported that rice yield is significantly increased when nitrogen fertilizers are applied during its cultivation (Cassman et al.,2002; Guo *et al.*, 2010). Nitrogen is a key element in the rice grain filling process that determines grains yield and quality (Gregersen et al.,2008). The amount of nitrogen fertilizer to be applied during rice cultivation however remains ambiguous. Some studies have reported that the use of high amounts of nitrogen (N) fertilizer application is a conventional method used to increase rice yield (Zhang et al.,2015; Zheng et al., 2016). Other studies reported that high amounts of nitrogen fertilizer application could cause the plant to lodge and decrease the rice yield in a very significant way. Also, it is reported that excessive nitrogen fertilizer application during rice cultivation significantly reduces rice quality (Zhu et al., 2017; Carley et al.,2009). Furthermore, applying high amounts of nitrogen fertilizer in an unchecked manner is detrimental to the rice plant and to the environment (Guo et al., 2010; La Croix et al., 2005; Liu et al., 2013; Tan et al., 2011).

In addition, although Nitrogen is important for rice plant, if it is applied in high amounts, it may modify the soil productivity by altering microbial communities (Xun et al., 2016). Also, a significant positive correlation has been reported to exist between nitrogen leaching and the rate of nitrogen applied (Li et al., 2007). Some studies have further reported that increasing the amount of nitrogen fertilizer application, does not increase the grain yield (Huang et al., 2007; Yan et al., 2009). Nitrogen Use Efficiency (NUE) is low in rice plant as the plant uses 20-30% of the applied nitrogen fertilizer (Peng et al.,2006). Thus, applying nitrogen fertilizer at the appropriate amounts could improve rice NUE and yield. Previous studies have revealed that morphological mechanisms of rice yield increase in response to optimized nitrogen (N) management (Zhou et al., 2017). NUE depends largely in the nitrogen management practices especially the amount of nitrogen fertilizer applied (Ju et al., 2009; Yan et al.,2009; Peng et al.,2006). It was reported that, to enhance NUE in rice, N fertilizer application should be reduced during the mid-tillering stage and increased at the time of panicle initiation stage (Zhong et al., 2007; Yan et al.,2009; Zhang et al. 2013). This experiment was conducted to determine the effect of different amounts of Nitrogen fertilizer applications on the growth and yield of IR-28 (Indica variety).

2. MATERIALS AND METHODS

2.1. Study site

The research was conducted at the rice experimental field RE-2 of Japan International Cooperation Agency Tsukuba in Japan. The City of Tsukuba also known as “Tsukuba Science City” is located at 36° 12’ N Latitude, 140 ° 0559 longitude and at 33 m altitude. The work was conducted from April 2018 to October 2018.

2.2. Nursery Preparation and Management

An Indica type rice (*Oryza sativa L.*) genotype IR-28 was used for this experiment. Rice seed was selected using a salt solution of 1.13 specific gravity determined with a hydrometer. Selected seeds were disinfected by soaking them into a mixture of Benlate- T (Thiram-benomyl mixt) fungicide at 0.5% of 100grams with 20 litres of water for 24 hours. About 600 grams of treated seed were then soaked into a running water machine at 17 °C for six to seven days for pre-germination. Pre-germinated seeds were then sowed into nursery boxes of 60cm x 30cm dimensions containing soil, and at the rate of 50 g of pre-germinated seed per box. After the seeds were sowed, the nursery boxes were placed in a greenhouse to raise rice seedlings. The nursery was monitored at least twice a day to control water supply, diseases, insects, and to measure and record the temperature inside the greenhouse.

2.3. Land Preparation

The experimental field RE-2 of 422 m² (28.7 m x 14.7 m) dimension was ploughed using a tractor. A power tiller machine was used for a second tilling to loosen the soil before flooding the field. Samples of soil were collected for chemical analysis JICA Tsukuba Soil laboratory. Soil samples were analyzed, and total carbon content, total nitrogen content, available phosphorous, exchangeable potassium and pH were determined (table1). After flooding the field, plot layout and fencing were done, and basal fertilization was applied. After basal fertilization, puddling was conducted in each of the spit plot using a small puddling machine.

Table 1. Chemical composition of the soil of the experimental field (RE-2).

Total C (g.kg ⁻¹)	Total N (g.kg ⁻¹)	Available P (Bray2) (mg.kg ⁻¹)	Exchangeable K cmol (+).kg ⁻¹	pH (H ₂ O)
14.1	1.2	83.2	0.335	6.2

2.4. Transplanting

Seedlings at the age of 30 days were manually transplanted. Transplanting was done in straight lines at a density of 30cm x 15cm; 30 cm between the lines and 15 cm inside the lines. The plant density was 22.22 hills per m². Before transplanting, seedlings were treated with a systemic fungicide to prevent and combat fungal diseases that may arise during cultivation. Transplanted seedlings had a plant age in leave number of 4.3, and a plant length of 17.4cm.

2.5. Fertilization

Basal fertilization was done prior to puddling by application of granular fertilizers containing Nitrogen, Phosphorus and Potassium, on all the plots. Phosphoric acid, chemically known as Phosphorus Pentoxide (P₂O₅) was applied at the rate of 100 Kg ha⁻¹, to supply Phosphorus to the soil. Potassium Oxide (K₂O) also known as Potash, was applied at the basal at the rate of 100 Kg ha⁻¹ at the basal. Phosphoric acid and Potassium Oxide were applied only at the basal and the amounts were the same for all the plots. Four levels of nitrogen fertilizer (Ammonium Sulfate) amount was applied during this experiment; N0, N40, N60, and N80. Ammonium Sulfate fertilization was done in two applications and at two different stages. The first application was done at basal prior to puddling, and the second application was done as top dressing at the panicle initiation stage (Table 2).

Experimental design

A Randomized Complete Block Design (RCBD) with six treatments and three replications were used for this experiment. The experimental field was divided into 18 spit plots, each of which had a dimension of 4 m x 3.42 m.

Table 2. Nitrogen fertilizer applications levels

Label	Nitrogen Amount (kg ha ⁻¹)		Total (kg ha ⁻¹)
	Basal (prior to puddling)	Top dressing (PI)	
N0	0	0	0
N40	20	20	40
N60	25	35	60
N80	35	45	80

2.6. Data Collection

Growth data

The first data were collected seven days after transplanting and continued weekly until harvest. Growth data were collected from 6 hills per plot. Three growth parameters were determined during this experiment. Plant length was measured weekly using a meter ruler. The length of the plant was measured from the base of the stem (hill) to the tip of the tallest leaf. The number of tillers per hill was counted and recorded manually once a week. The colour of the leaves was determined using a Leaf Color Chart (LCC), an instrument that is commonly used in the determination of rice leaves greenness, and which indicates the nitrogen content in the leaves.

Determination of yield components

The number of panicles per 1m² was determined by counting the total number of panicles and the number of sterile panicles of 10 hills at three different survey points in the sample field and then deducting the number of sterile panicles from the total number of panicles. The value obtained after the deduction represented the number of fertile panicles. To determine the number of spikelets per panicle, we started by counting the number of spikelets of a total of 10 hills collected from three separate survey points. Three hills were collected from the first point, four hills from the second point, and three hills from the third point. At each survey point, the number of spikelets of the highest panicle and second-lowest panicle of the hills were counted and the average number of spikelets per panicle was calculated. The number of spikelets per 1m² was calculated by multiplying the number of spikelets per panicle by the number of fertile panicles per 1m². The determination

of the filled grain percentage or ripening ratio was obtained from the ratio of the number of filled grains to the total number of spikelets. To separate filled grains from unfilled grains, water of 1.0 specific gravity was used. 1000-grains weight was obtained and adjusted to 14% moisture content. Data on the culm length, panicle length, and internode length were later collected for other yield-related analyses.

Data Analysis

Microsoft Excel 2013 for Windows was used for data processing and statistical analyses were done using analysis of variance (ANOVA). Differences among means were separated by Fisher's least significant difference (LSD) test and Tukey's HSD Least Significance Difference test at the 0.05 probability level ($p < 0.05$). Microsoft Excel 2013 for windows was used to produce tables and graphs, and all error bars indicated standard errors of means.

3. RESULTS

3.1. Growth parameters

3.1.1. Plant length

The data of the plant length of IR-28 rice in response to four nitrogen fertilizer (Ammonium Sulfate) application amounts are shown in Table 3. N60 and N80 significantly increased the plant length compared to the N0 and N40. Although plant length in N80 appeared to be slightly higher compared to N60, there was no significant difference between the two treatments. The lowest values of plant length were observed in the control treatment N0 (Figure 1).

3.1.2. Tiller number

The data of the number of tillers per hill of IR-28 rice in response to four nitrogen fertilizer application amounts is shown in Table 4. From the time of transplanting till the maximum tillering stage, the tiller number was significantly increased by N80 and N60 compared to other treatments (Figure 2). The lowest number of tillers per hill were observed in the control treatment N0 (Table 4).

3.1.3. Leaf color

The data presented in Table 5 represent the changes in leaf color of rice plant in response to four nitrogen fertilizer application amounts. The leaf color was significantly increased by N40, N60 and N80 compared to the control N0. However, a decrease in leaf color was observed in all the treatments, from the beginning of active tillering until the time of top-dressing application (Figure 3).

3.2. Yield components

The yield components of IR-28 rice in response to four amount of nitrogen fertilizer applications are shown in Table 5. Although the highest number of panicles per m^2 was observed in N80, there was no significant difference among the four treatments at a 5% level of significant difference. The number of spikelets per panicle showed an increasing trend within the treatments as the nitrogen application was increased, but there was no significant difference between the four treatments. N80 and N60 showed higher values of calculated yield values (Table 5), but there was no significant difference between the four treatments at a 5% level of significant difference. The spikelet fertility rate (Figure 4) and 1000 grains weight (Figure 5) were significantly increased in N80, N60 and N40 compared to N0.

Table 3: Plant height (cm) of IR-28 rice as influenced by different levels of Nitrogen (N) fertilizer application.

Plant Length	17DAT		24DAT		31DAT		38DA T		46DA T		52DA T		59DA T		66DA T		73DA T		79DA T		87DA T	
	N0	30.4	b	32.3	c	34.8	b	37.1	c	40.9	b	45.4	b	50.2	b	55.7	b	65.2	c	73.6	c	87.0
N40	33.7	ab	35.5	b	38.7	a	41.3	b	45.4	ab	49.1	ab	53.3	ab	63.2	a	71.4	b	87.9	b	95.3	a
N60	34.7	a	36.6	b	39.5	a	42.9	ab	48.1	a	51.8	a	56.2	a	66.6	a	76.8	a	93.4	ab	98.4	a
N80	34.5	a	38.0	a	40.7	a	44.5	a	49.7	a	52.9	a	58.5	a	67.7	a	76.8	a	94.4	a	99.5	a

In a column means with the same letter are not significantly difference at 5 % level by HSD

Table 4: Number of Tillers of IR-28 rice as influenced by different levels of Nitrogen (N) fertilizer application.

Tiller No,	17DAT	24DAT	31DAT	38DAT	46DAT	52DAT	59DAT	66DAT	73DAT	79DAT	87DAT
N0	5 b	8 c	8 c	8 c	8 c	9 c	9 b	9 b	10 b	10 b	10 b
N40	9 a	13 b	14 b	14 b	13 b	13 b	12 a	11 ab	11 ab	11 b	10 b
N60	10 a	14 ab	15 ab	16 ab	15 ab	14 ab	14 a	11 a	12 a	11 ab	11 ab
N80	11 a	17 a	18 a	18 a	17 a	17 a	15 a	12 a	13 a	13 a	12 a

In a column means with the same letter are not significantly difference at 5% level by HSD

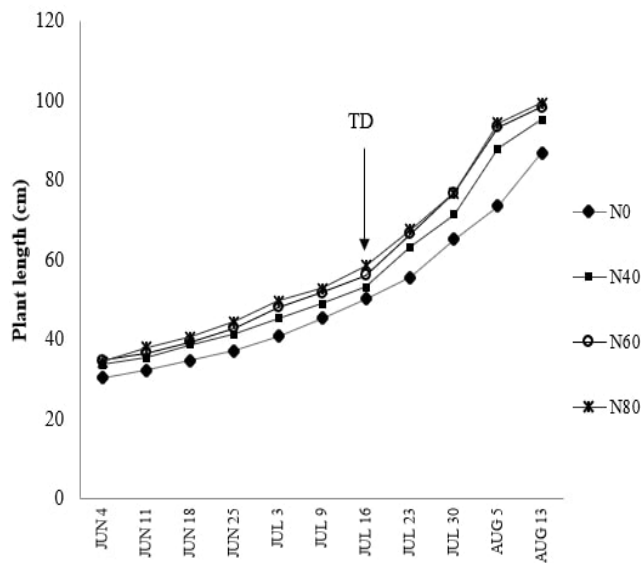


Figure 1. Plant length of rice as influenced by ammonium sulfate fertilizer applications levels. Data presented are means of three replicates. N0 = No application of $(\text{NH}_4)_2\text{SO}_4$, N40 = 40 $\text{kg}\cdot\text{ha}^{-1}$ of $(\text{NH}_4)_2\text{SO}_4$, N60 = 60 $\text{kg}\cdot\text{ha}^{-1}$ $(\text{NH}_4)_2\text{SO}_4$, N80 = 80 $\text{kg}\cdot\text{ha}^{-1}$ $(\text{NH}_4)_2\text{SO}_4$

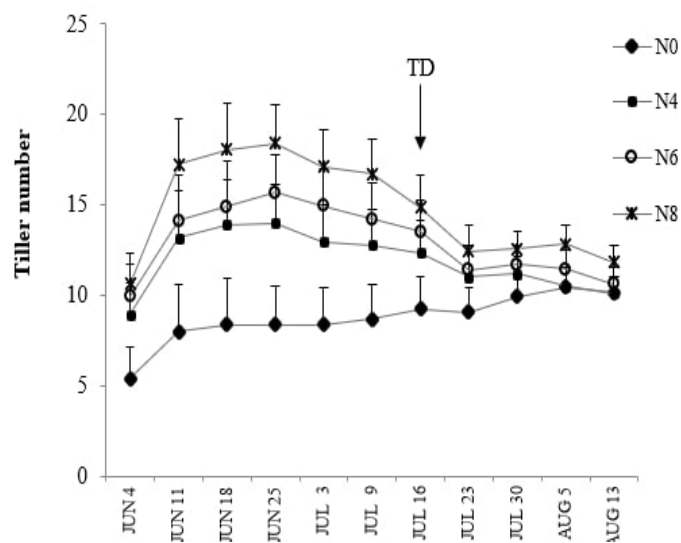


Figure 2. Tiller number of rice as influenced by ammonium sulfate fertilizer applications levels.

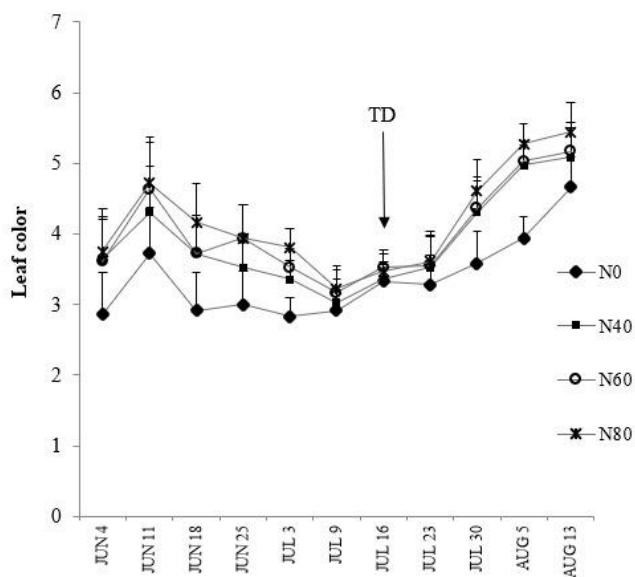


Figure 3. Leaf color of rice as influenced by ammonium sulfate fertilizer applications levels.

Table 5. Yield and Yield Components of IR-28 rice as affected by different Nitrogen fertilizer levels.

	No. of Panicles /m ²	(%)	No. of Spikelet/ panicle	(%)	Spikelet fertility. (%)	1000 grain weight (g) at 14% Moist	(%)	Calculated Yield (ton/ha)	(%)
N0	222.22	(100) a	92.61	(100) a	84.55 (100) b	25.13b	(100) b	4.36	(100) a
N40	229.62	(103) a	89.10	(96) a	91.16 (108) a	26.13a	(104) ab	4.88	(112) a
N60	266.67	(120) a	85.11	(92) a	90.99 (108) a	26.08a	(104) ab	5.38	(123) a
N80	266.67	(120) a	85.66	(92) a	92.10 (109) a	27.15a	(108) a	5.74	(131) a

Values in the table are means of three replicates. Within a column, means with the same letter do not differ significantly at 0.05 probability level.

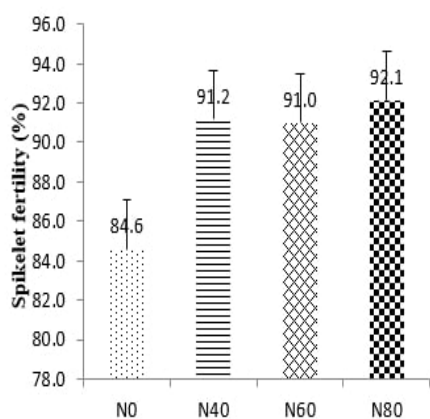


Figure 4. Spikelet fertility as affected by Nitrogen fertilizer levels.

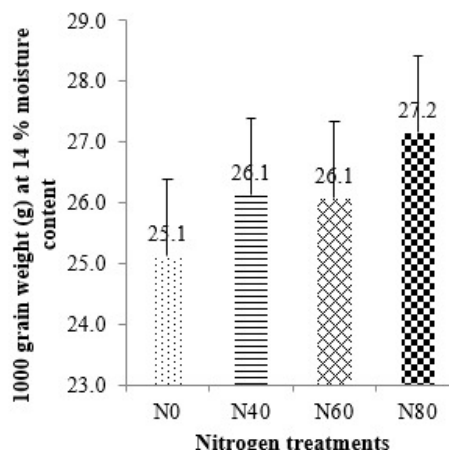


Figure 5. 1000 grain weight of rice at 14 % moisture content as affected by Nitrogen fertilizer levels.

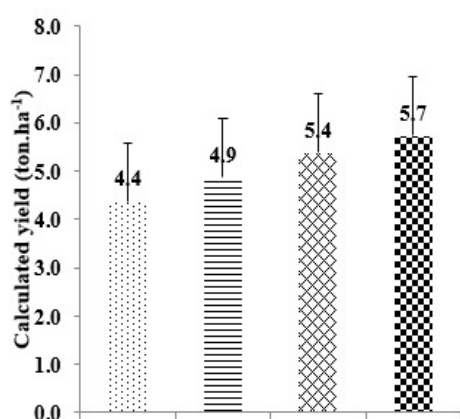


Figure 6. Calculated yield of rice as affected by Nitrogen fertilizer levels.

4. DISCUSSION

4.1. Growth parameters

4.1.1. Plant length

We found that high amounts of Ammonium sulfate fertilizer applications during rice cultivation significantly increase the yield plant length of IR-28 rice. N60 and N80 significantly increased the plant length of rice compared to N0 and N40 (Figure 1). These results were consistent with the findings of Li et al., 2013, that concluded that increased amounts of nitrogen input significantly increased the plant length and basal internode length of rice. The increase in plant length that results from the application of a high amount of nitrogen fertilizer could be explained by the fact that nitrogen is the most important component that supports plant growth. It was previously reported that nitrogen plays a key role in plant development due to the role it plays in the production of chlorophyll, which is crucial for photosynthesis (Munoz-Huerta et al., 2013). Nitrogen has also been reported to be the primary building block for plant protoplasm, which enhances speedy shoot growth.

Some previous studies have reported that nitrogen promotes plant growth and increases the number of internodes and the length of internodes, thus an increase in nitrogen fertilizer application increases the plant length (Gasim, 2001). However, although a high amount of nitrogen fertilizer increased the plant length, it may also cause some unwanted effects on the plant. Previous studies have reported that there is a significant correlation between lodging and plant length (UNAN et al., 2013). Some studies concluded that taller plants may have higher yields, but they may also have a high risk of lodging (Xue et al., 2019). Other studies concluded that a taller rice plant is more susceptible to lodging and to have a poor response to nitrogen (Yoshida, 1978).

4.1.2. Tiller number

We found that the highest number of tillers per hill were obtained from high amounts of Ammonium sulfate fertilizer application; N60 and N80 (Figure 2). This result was consistent with numerous previous studies that came to similar conclusions. Cassman et al., 1998, reported that the high yield of rice that Asian farmers are experiencing nowadays is a result of high amounts of nitrogen fertilizer applications. Studies conducted by Yosef in 2012, concluded that high amounts of nitrogen applications increased the tiller number of rice plants. Thieu et al., 2014, also reported that by application of high amounts of nitrogen fertilizer significantly increased the tiller number. The increase in the number of tillers resulting from the application of high nitrogen fertilizer amounts can be explained by the fact that nitrogen (N) is needed to produce chlorophyll which is crucial for photosynthesis (Munoz-Huerta et al., 2013).

However, although high amounts of nitrogen fertilizer increased tiller number, it is worth noting that, higher quantities of nitrogen fertilizer may enhance the production of non-productive tillers. Previous studies have reported that, increased amounts of nitrogen promoted tiller heterogeneity in rice (Wang et al., 2016). Moreover, the number of productive tillers per plant is one of the most important agronomic traits associated with the grain yields of rice crops (Deng et al., 2020).

4.1.3. Leaf color

N40, N60 and N80 significantly increased the leaf colour of the plants compared to the control N0. The likely reason for this observation could be that nitrogen fertilizer applications increased the amount of available nitrogen in the soil which plants absorbed immediately (Figure 3). The leaf colour of the plants helps the farmers know the nitrogen content in the leaves and understand when to apply fertilizer (Tao et al., 2020). Studies have concluded that the level of leaf nitrogen is affected by the rate of nitrogen application (Taylaran et al., 2011). However, for the nitrogen applied to be reflected in the leaf colour, a good root system of the plant is required. The root is the main organ responsible for nitrogen uptake, and root morphology and functioning determine a plant's ability to uptake nitrogen (Jiang et al., 2017; Hu et al., 2018).

4.2. Yield components

We found that there were no significant differences among the four treatments in terms of the number of panicles per m², the number of spikelets per panicles and the calculated yield of IR-28 even though N80 N60 have higher yield values (Figure 6). These results were completely unexpected because several previous studies have reported different findings. Studies concluded that, there was a significant increase of rice yield after applications of high nitrogen amounts (Cassman et al., 2002; Guo et al., 2010). Some other studies concluded that, the high levels of rice yield that is being achieved by Asian farmers nowadays depend on large amounts of nitrogen fertilizers applications (Cassman et al., 2002).

Other previous studies concluded that applying nitrogen topdressing at the panicle initiation stage, as we did in our experiment, significantly increased the number of spikelets per panicles (Ding & Maruyama, 2004; Kamiji et al., 2011). Nevertheless, these results could be explained by the fact that top dressing was done at panicle initiation stage and fertilizer was not applied at tillering stage. Numerous previous studies have shown that applying nitrogen at the tillering stage of rice increases the number of tillers per hill and consequently the number of panicles per m², which is the most important component for determining rice yield. Liang et al., 2014, reported that, the more a rice plant could produce tillers, the

higher its panicle number would be. Similarly, Liao et al., 2019 reported that the tiller number per plant determines the panicle number (Liao et al., 2019).

Also, the number of panicles per m² was previously reported to be the most important components of rice yield and 87% of variations in rice yield are caused by the number of panicles (Fageria & Baligar, 1999). Thus, the non-application of nitrogen fertilizer at tillering stage affected the tillering ability of the plants and subsequently affected the number of panicles per m², which affected the yield. However, our findings were consistent with some previous studies that concluded that rice grain yield does not increase proportionately with increasing amounts of nitrogen fertilizer (Huang et al., 2007; Huang et al., 2008; Yan et al., 2009).

N40, N60 and N80 significantly increased the spikelet fertility rate (Figure 4) and the 1000 grains weight (Figure 5) of IR 28 rice compared to N0. These results were consistent with numerous previous studies that concluded that, nitrogen is a major determinant in the seed filling process, and its application at panicle initiation stage increases the number of filled spikelets per panicles (Gregersen et al., 2008). Furthermore, studies have reported that, applying nitrogen fertilizer at panicle initiation stage increases the fertility rate of the spikelets, because nitrogen improves photosynthetic capacity and promotes carbohydrates accumulation (Mae, 1997).

5. CONCLUSION

The level of nitrogen (N) fertilizer applied during rice cultivation affects the growth and the yield of rice. The plant height and the number of tillers were increased by high levels of nitrogen fertilizers applications. Also, the number of panicles per m² and the number of spikelets per panicle increased with high amounts of nitrogen fertilizers. By increasing the number of panicles per m² and the number of spikelets per panicles, high levels of nitrogen fertilizer application subsequently increased the yield of IR-28 rice. Although N80 showed higher calculated yield and yield components than N60, we conclude that N60 is the best nitrogen level to apply for optimum yield of IR-28 because it gives a good yield, and it is more economical than N80.

6. CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

7. ACKNOWLEDGEMENT

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