

*Original Research Article*

**Effect of Nitrogen Management at the Reproductive Phase in Transplanted Rice**

**Running title: Nitrogen Management at the Reproductive Phase of Rice**

**ABSTRACT**

Top dress of nitrogen (N) at reproductive stage is harmful or useful for rice cultivation.

**Comment [mN1]:** the formulation of the problem has not been described at the beginning of the abstract

Considering the above facts, two field experiments were designed at Bangladesh Rice Research Institute (BRRI) farm, Gazipur, Bangladesh during the transplanting Aman season (July to November), 2018-19 and the Boro season (December to May), 2019-20 to evaluate the effect of four different nitrogen management on growth, yield attributes, yield and nitrogen uptake by rice variety BRRI dhan75 and BRRI dhan89. The experiment was laid out in a randomized complete block design involving four different N management at different stages (Active tillering, Panicle initiation, Flowering and Heading) replicated three times. Results revealed that 69 kg N ha<sup>-1</sup> (29.5 kg as basal + 29.5 kg at 15 DAT + 10 kg ha<sup>-1</sup> at heading) would be a better option for higher yield in T. Aman rice While 120 kg N ha<sup>-1</sup> (23 kg as basal + 40 kg at 20 DAT + 40 kg at 40 DAT + 17 kg ha<sup>-1</sup> at heading) significantly improved growth, yield attributes and grain yield as well as nitrogen uptake by grain and straw. From the results, it can be said that application of N @10 kg ha<sup>-1</sup> for T. Aman rice and N @17 kg ha<sup>-1</sup> only at the heading stage would reduce sterility and give a higher yield than BRRI recommended management. Hence, the study suggests that nitrogen management at the reproductive phase gives better performance to the transplanted aman and boro rice crops.

**Keywords:** Aman rice; Boro rice; nitrogen management; reproductive phase; sterility.

## 1. INTRODUCTION

Rice is one of the most important cereal crops in the world and contributes to food security in several developing countries including Bangladesh. With increase in population, the demand for rice is increasing over the years. Nutrient management is a prime strategy to achieve the demand for food and sustainable production for the rapidly increasing population in the world and to improve food and nutritional quality [1, 2]. Among the plant nutrients, nitrogen (N) is the most essential element in determining the yield potentiality of intensified agricultural systems [3]. In order to exploit the full yield potential of modern rice cultivars, N fertilizer application is necessary in most rice soils. Nitrogenous fertilizer has an immense effect on rice yield throughout a positive influence on the production of effective tillers [4]. But the efficiency of added N fertilizer in rice depends on N sources, rate of N as well as management practices as evidenced by the  $^{15}\text{N}$  tracer studies [5, 6]. Nitrogen not only enhances the yield of rice but also reduces spikelet sterility. Nitrogen is required in an adequate amount in the early, mid tillering and panicle initiation stages for better grain development [7]. According to ICAR, there are two stages of rice crop growth when N is essential; early vegetative-promotes tillering leading to higher yield and panicle initiation stage- which helps to produce more spikelets and heavier grains per panicle. Sometimes in Bangladesh, farmers become unable to apply urea (3<sup>rd</sup> top dress) due to a lack of irrigation water or do not follow the prescribed fertilizer schedule due to early recession of floodwater in intensive boro cultivation area (haor area) and inundation due to heavy rainfall or severe flood in T. Aman season. Therefore, the farmers in these areas achieve lower yields. To escape flash flood, farmers have to go for early crop establishment allowing it prone to sterility problem. Grain yield reduction in rice is often associated with spikelet sterility, which in turn, usually reflects the effects of adverse growing conditions on reproductive

development [8]. The application of urea at the reproductive stage of rice crops, increased sterility and reduced grain yield [9]. So, it needs to investigate whether the top dress of urea at reproductive stage is harmful or useful for rice cultivation. This study was undertaken to evaluate the response of different modern varieties with the application of different nitrogen management for obtaining optimum yield by reducing spikelet sterility of T. Aman and Boro rice.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Period

The experiment was conducted at BRRRI farm, Gazipur, Bangladesh during the T. Aman season (July to November), 2018-19 and the Boro season (December to May), 2019-20.

### 2.2 Treatments and Design

The study evaluates the effects of four different nitrogen management in the form of urea. In T. Aman season, fertilizer rate was (N:P:K:S @ 69:10:41:16 kg ha<sup>-1</sup> and N was splitted as, T<sub>0</sub> = No fertilizer, T<sub>1</sub> = 23 kg as basal + 23 kg at 15 DAT + 23 kg at before panicle initiation (BPI) (BRRRI recommended practice), T<sub>2</sub> = 29.5 kg as basal + 29.5 kg at 15 DAT + 10 kg ha<sup>-1</sup> at 10 days after PI (DAPI), T<sub>3</sub> = 29.5 kg as basal + 29.5 kg at 15 DAT + 10 kg ha<sup>-1</sup> at 20 days after PI (DAPI)/Booting and T<sub>4</sub> = 29.5 kg as basal + 29.5 kg at 15 DAT + 10 kg ha<sup>-1</sup> at heading stage. In Boro season, Fertilizer rate was (N:P:K:S:Zn @ 120:18:75:40:4 kg ha<sup>-1</sup> and N was splitted as, T<sub>0</sub> = No fertilizer, T<sub>1</sub> = 40 kg at 15 DAT + 40 kg at 30 DAT + 40 kg at BPI (BRRRI recommended), T<sub>2</sub> = 23 kg as basal + 40 kg at 20 DAT + 40 kg at 40 DAT + 17 kg at 10 days after PI (DAPI), T<sub>3</sub> = 23 kg as basal + 40 kg at 20 DAT + 40 kg at 40 DAT + 17 kg at 20 days after PI (DAPI)/Booting, T<sub>4</sub> = 23 kg as basal + 40 kg at 20 DAT + 40 kg at 40 DAT + 17 kg at heading stage. The experiment was conducted in RCB design with three replications.

### 2.3 Planting Material

**Comment [mN2]:** add the characteristics of the soil at the experimental site

Short duration variety BRR1 dhan75 for T. Aman season and long duration variety BRR1 dhan89 for Boro season were used as test crops.

#### **2.4 Collection and Preparation of Initial Soil Sample**

The initial soil samples were collected before land preparation from a 0-15 cm soil depth by means of an auger from different locations covering the whole experimental plot and mixed thoroughly to make a composite sample. After the collection of soil samples, the plant debris was picked up and removed. Then the sample was air-dried and sieved through a sieve and stored in a clean plastic container for chemical analysis.

#### **2.5 Fertilization**

The full doses of PKSZ were applied as basal doses during the final land preparation of individual plots. The BRR1 recommended dose of urea in inbred Boro varieties 120 kg ha<sup>-1</sup> and short duration T. Aman varieties 69 kg ha<sup>-1</sup> respectively. Urea was applied to the T<sub>1</sub> treatment plot in three equal splits on 15, 30 and 55 DAT for BRR1 dhan89 and in case of BRR1 dhan75, the splits were 0, 15 and 45 DAT, respectively.

#### **2.6 Uprooting of Seedlings and Transplanting**

Twenty-five days old seedlings of BRR1 dhan75 and forty-day-old seedlings of BRR1 dhan89 respectively were uprooted from the nursery beds carefully. Seedlings were transplanted in the well-puddled experimental plots. Spacing was given 20 cm × 20 cm for BRR1 dhan75 and BRR1 dhan89. Two seedlings for BRR1 dhan75 and BRR1 dhan89 were transplanted hill<sup>-1</sup>. Seedlings of some hills died off and these were replaced by gap filling after one week of transplanting with seedlings from the same source. Manual weeding was done for three times at 15 DAT, 30 DAT and 50 DAT followed by first, second and third top dressing of urea. Irrigation was done by alternate wetting and drying from transplanting to the maximum tillering stage. From panicle

initiation (PI) to the hard dough stage, a thin layer of water (2-3 cm) was kept on the plots. Water was removed from the plots during the ripening stage.

### **2.7 Harvesting and processing**

The crop of each plot was harvested separately at full maturity when 80% of the grains become golden yellow in color. At maturity, plants of 5 m<sup>2</sup> area were harvested for the determination of yield and yield components. The grain yield was adjusted at a 14% moisture level. The vegetative plant parts were oven-dried at 72 °C to constant weight and then weighed to calculate the stem dry weight of the respective stage.

**Comment [mN3]:** Add the irrigation and pest control techniques used during the experiment

### **2.8 Data Collection**

Data were collected on the following parameters - plant height, leaf area, number of tillers, total dry matter, number of filled grains, spikelet sterility, spikelet sterility at the top, the middle and bottom portion of panicle, 1000-grains weight, and grain yield.

The percentage of sterility was calculated by the following formula;

$$\text{Sterility (\%)} = (\text{Number of sterile spikelets per panicle} \div \text{number of total spikelets per panicle}) \times 100$$

From the sample hills m<sup>-2</sup>, each panicle was divided into three equal parts by eye estimation. The apical, middle and lower parts were termed the top, middle, and bottom portions of the panicle, respectively. The sterility pattern for each portion was calculated.

### **2.9 Determination of Nitrogen**

Straw and grain N concentration was measured at maturity. After dry weight measurement, straw and grain were ground using a mixer mill homogenizer. Approximately 0.5 g sample was used to measure N concentration using an Auto nitrogen analyzer. Nitrogen uptake at maturity was calculated according to Bremner and Mulvaney [10].

## 2.10 Statistical Analysis

The data were analyzed statistically. Analysis of variance were performed using cropstat7.2 software. The mean differences among treatments were compared by multiple comparison tests using the least significant test at the 0.05 probability label [11].

## 3. RESULTS AND DISCUSSION

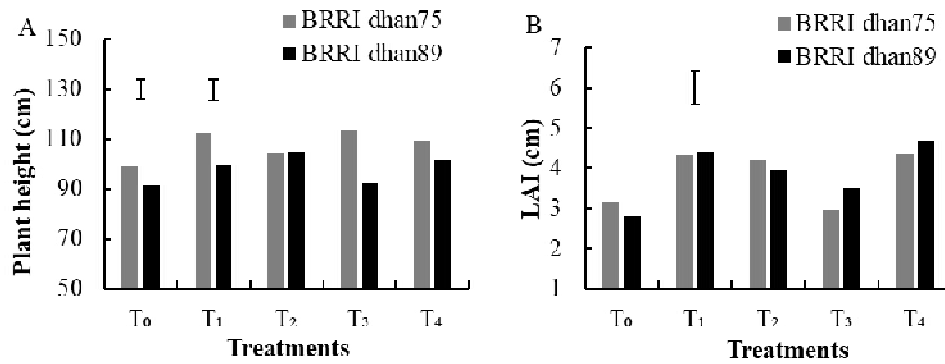
### 3.1 Initial Soil Status

Initial soil status of the experimental field was pH = 6.5, Total N = 0.13%, P = 40.1  $\mu\text{g/g}$ , K = 0.146 me/100 g, S = 14.06  $\mu\text{g/g}$  and Zn = 0.81  $\mu\text{g/g}$ .

### 3.2 Growth Characters

#### 3.2.1 Plant Height and Leaf Area Index

Plant height differed significantly among cultivars and nitrogen managements. Plant height of BRR1 dhn75 ranged from 98.8 to 113.6 cm and BRR1 dhan89 from 91.07 to 104.6 cm among the treatments. The LAI was significantly affected by N application at the heading stage in both varieties. The highest LAI was observed in the T<sub>4</sub> treatment (4.37 and 4.39) followed by the T<sub>1</sub> treatment (4.35 and 4.70) in BRR1 dhan75 and BRR1 dhan89. The lowest LAI was observed in T<sub>3</sub> treatment (2.96 and 3.39) followed by T<sub>0</sub> treatment (3.15 and 2.82), respectively (Fig. 1).

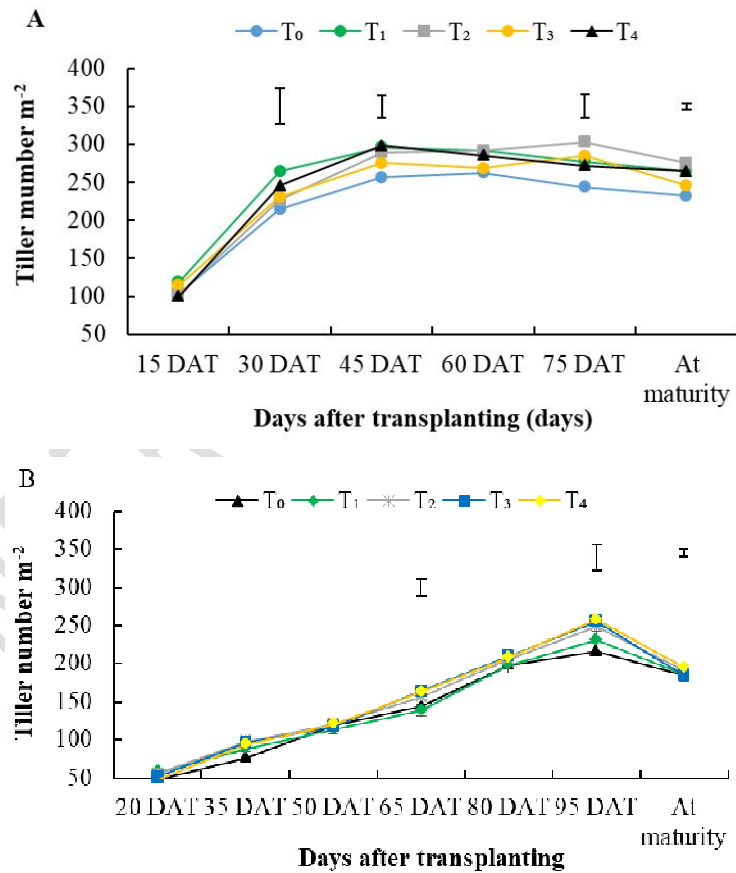


**Fig.1: Plant height (A) and Leaf area index (B) of BRR1 dhan75 (T. Aman) and BRR1 dhan89 (Boro) at heading stage influenced by different N management (Vertical bars represent the LSD at 5% level of significance)**

### 3.2.2 Tillering Pattern

In T. Aman season, tiller number was recorded from 15 DAT and continued up to 90 DAT. It was significantly varied with N management techniques at 45 DAT and at 90 DAT. The maximum number of tillers (297 per m<sup>2</sup>) was observed at 45 DAT for all N management techniques except T<sub>0</sub> treatment (256 per m<sup>2</sup>). T<sub>1</sub>, T<sub>2</sub>, and T<sub>4</sub> produced a higher number of tillers up to 60 DAT and then declined slightly for all N management (Fig. 3A). In Boro season, tiller number was recorded from 20 DAT and continued up to maturity. It was significantly varied

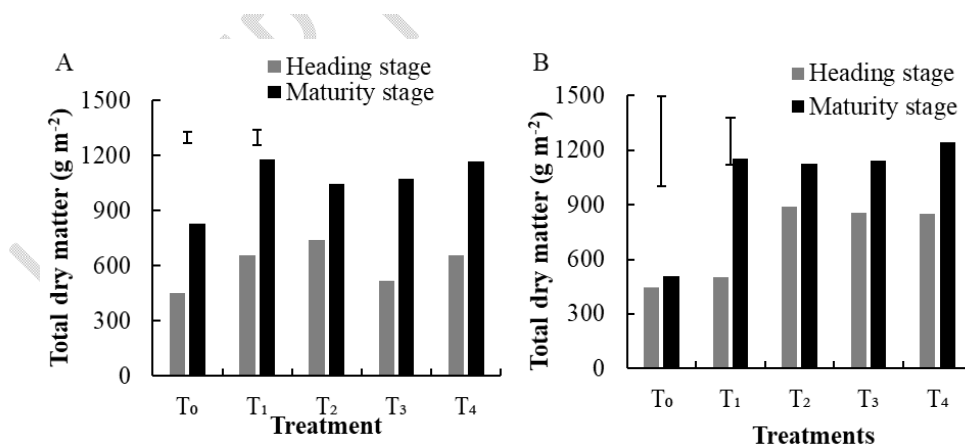
with N management techniques at 80-95 DAT. The maximum number of the tiller (258 m<sup>-2</sup>) was observed at 95 DAT for all N management techniques except T<sub>0</sub> and T<sub>1</sub>. The lowest number of tiller (202-213 m<sup>-2</sup>) was observed in T<sub>0</sub> and T<sub>1</sub> at 65-95 DAT, it was due to a lack of nitrogen fertilization. At maturity tiller number declined slightly for all N management (Fig. 3B). The results are in conformity work of Zhang et al. [12] who found that integrative crop management with judicious use of the N fertilizer not only increased grain yieldbut also enhanced agronomic performance with an improved tillering ability.



**Fig.3. Tillering pattern of BRRI dhan75 (a) and BRRI dhan89 (b) affected by different N management (Vertical bars represent the LSD at 5% level of significance).**

### 3.2.3 Total Dry Matter

Accumulation of dry matter is essential for crop yield formation, and dry weight is an extensively used parameter for assessing the growth conditions of plants. As shown in figure 2 A, B, the total dry matter gradually increased until the maturity (MA) stage in both varieties, which was also significantly affected by nitrogen fertilizer. The treatment T<sub>1</sub> produced the highest dry matter (1176.7 g m<sup>-2</sup>) in comparison to other treatments of N management in BRRI dha75. The rapid increase of dry matter was observed at the heading stage. During maturity, the highest dry matter (1241.1 g m<sup>-2</sup>) was found from T<sub>4</sub> treatment in BRRI dhan89 (Fig. 2 A & B). Lower dry matter yield is associated with a higher temperature at the heading stage in the Boro season than in the T. Aman season [13]. The higher dry matter with proper nitrogen management was due to an increased amount of photosynthate accumulation which was provided by more availability of photosynthetically active radiant.



**Fig. 2: Dry matter production ( $\text{g m}^{-2}$ ) of BRR1 dhan75 (a) and BRR1 dhan89 (b) affected by different N management (Vertical bars represent the LSD at 5% level of significance)**

### 3.3 Yield and Yield components

Among N management treatments, BRR1 recommended management ( $T_1$ ) and  $29.5 \text{ kg}$  as basal +  $29.5 \text{ kg}$  at 15 DAT +  $10 \text{ kg ha}^{-1}$  at the heading stage ( $T_4$ ) gave the significantly the highest grain yield ( $5.5$  and  $5.2 \text{ t ha}^{-1}$ ). More grain and biomass yield might be explained by the higher capability of the rice cultivar to utilize more nitrogen through a better growth pattern and more dry matter. It is confirmed that an increase in aboveground-biomass production through the nitrogen application during the reproductive stage is the primary factor in increasing grain number in rice [14]. In T. Aman season, the lowest grain yield was observed in  $T_0$ ,  $T_2$  and  $T_3$  treatment ( $4.2$ ,  $4.62$  and  $4.95 \text{ t ha}^{-1}$ ), respectively. No significant difference was observed in grains panicle<sup>-1</sup>, thousand grain weight (g), straw yield and harvest index. Yoshida [9] reported that grain weight is a fairly stable yield component and trait of the cultivar. The result agreed with the findings of Fageria et al. [15] who have also found that N rates had no effect on grain weight of rice genotypes. There was significant difference among different N management techniques in panicle  $\text{m}^{-2}$  and sterility (%) (Table 1a). Highest sterility (%) was found in  $T_0$  ( $42.2\%$ ) and lowest sterility% was found in  $T_1$  ( $26.3\%$ ),  $T_4$  ( $32.8\%$ ),  $T_3$  ( $36.7\%$ ) and  $T_2$  ( $40.3\%$ ), respectively. In Boroseason, among N management treatments  $T_5$  produced the highest grain yield ( $7.64 \text{ t ha}^{-1}$ ) followed by  $T_3$  ( $7.35 \text{ t ha}^{-1}$ ). The lowest grain yield was observed from  $T_1$  and  $T_0$  treatment ( $6.17$  and  $4.66 \text{ t ha}^{-1}$ ) respectively. There was significant difference among different N management techniques in panicle<sup>-2</sup> and sterility (%) (Table 1b). Fageria et al. [15] reported that

spikelet sterility in irrigated rice is a genotypic trait and can be reduced with proper management of N which is consistent with our results.

**Table 1 a. Yield and yield components affected by different N management in BRRI**

**dhan75**

| Treatments  | Panicle<br>m <sup>-2</sup> | Grains<br>panicle <sup>-1</sup> | 1000<br>grain wt.<br>(g) | Grain<br>yield<br>(t ha <sup>-1</sup> ) | Sterility<br>(%) |
|---|----------------------------|---------------------------------|--------------------------|---|------------------|
| T <sub>0</sub> = No fertilizer  | 223                        | 86                              | 22.6                     | 4.20                                    | 42.2             |
| T <sub>1</sub> = 23 kg as basal + 23 kg at 15 DAT + 23 kg at BPI (BRRI recom. practice)                   | 267                        | 82                              | 22.1                     | 5.50                                    | 26.3             |
| T <sub>2</sub> = 29.5 kg as basal + 29.5 kg at 15 DAT + 10 kg ha <sup>-1</sup> at 10 days after PI (DAPI) | 295                        | 88                              | 22.4                     | 4.62                                    | 40.3             |
| T <sub>3</sub> = 29.5 kg as basal + 29.5 kg at 15 DAT + 10 kg ha <sup>-1</sup> at 20 days after PI (DAPI) | 279                        | 90                              | 23.0                     | 4.95                                    | 36.7             |
| T <sub>4</sub> = 29.5 kg as basal + 29.5 kg at 15 DAT + 10 kg ha <sup>-1</sup> at heading                 | 264                        | 82                              | 23.4                     | 5.22                                    | 32.8             |
| LSD <sub>(0.05)</sub>   | 7.40                       | NS                              | NS                       | 0.30                                    | 4.58             |
| CV (%)  | 5.5                        | 20.5                            | 5.7                      | 3.2                                     | 6.9              |

**Table 1 b. Yield and yield components affected by different N management in BRRI**

**dhan89**

| Treatment  | Panicle<br>m <sup>-2</sup> | Grains<br>panicle <sup>-1</sup> | 1000<br>grain wt.<br>(g) | Grain<br>yield<br>(t ha <sup>-1</sup> ) | Sterility<br>(%) |
|--|----------------------------|---------------------------------|--------------------------|---|------------------|
| T <sub>0</sub> = No fertilizer   | 175                        | 125                             | 24.0                     | 4.66                                    | 11.2             |
| T <sub>1</sub> = 40 kg at 15 DAT + 40 kg at 30 DAT + 40 kg at BPI (BRRI recom.)                        | 189                        | 138                             | 24.3                     | 7.35                                    | 17.1             |
| T <sub>2</sub> = 23 kg as basal + 40 kg at 20 DAT + 40 kg at 40 DAT + 17 kg at 10 days after PI (DAPI) | 180                        | 156                             | 23.4                     | 6.67                                    | 15.4             |
| T <sub>3</sub> = 23 kg as basal + 40 kg at 20 DAT + 40 kg at 40 DAT + 17 kg at 20 days after PI        | 177                        | 113                             | 23.0                     | 6.71                                    | 34.0             |

(DAPI)/Booting

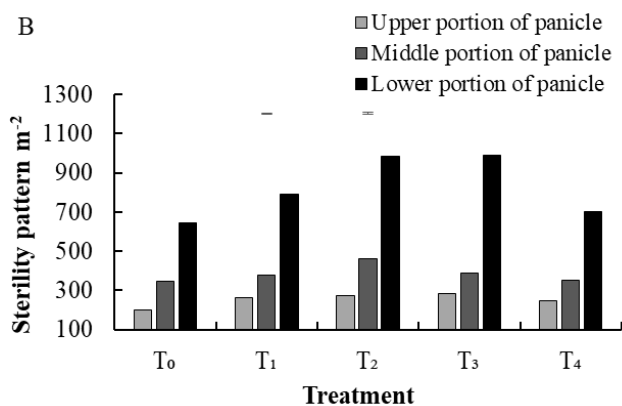
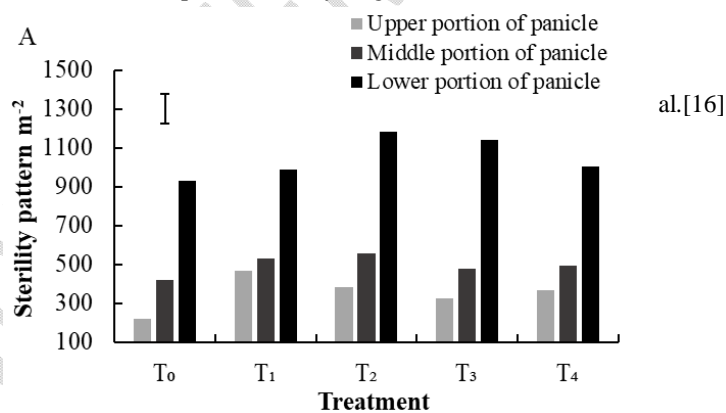
|  |       |      |      |      |       |
|--|-------|------|------|------|-------|
| T <sub>4</sub> = 23 kg as basal + 40 kg at 20 DAT + 40 kg at 40 DAT + 17 kg at heading stage | 191   | 157  | 24.0 | 7.64 | 14.6  |
| LSD <sub>(0.05)</sub>  | 10.01 | NS   | NS   | 1.23 | 14.30 |
| CV (%)   | 3.2   | 17.4 | 8.0  | 10.4 | 44.8  |

### 3.3.1 Sterility pattern at the top, middle and bottom portion of panicle

Nitrogen management showed significant variation for producing the spikelet sterility pattern at top, middle and bottom portion of panicle in both varieties. In most of the cases, the highest sterility was found at bottom portion and lowest at top portion of panicle whereas middle portion of panicle showed intermediate level of sterility. In both varieties, highest spikelet sterility was found for T<sub>0</sub> for bottom and middle portions (except top portion) followed by T<sub>3</sub>, T<sub>2</sub>, T<sub>4</sub>, and T<sub>1</sub>.

In top portion T<sub>4</sub> showed the lowest spikelet sterility (Fig. 4 A, B). The results are in conformity with

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**Fig. 4: Sterility pattern (m<sup>-2</sup>) of BRRRI dhan75 (A) and BRRRI dhan89 (B) affected by different N management (Vertical bars represent the LSD at 5% level of significance).**

### 3.3.2 Nitrogen uptake

In T. Aman season, treatment T<sub>1</sub> [69 Kg Nha<sup>-1</sup>: 23 kg as basal + 23 kg at 15 DAT + 23 kg at BPI (BRRRI recommended practice)] showed significantly the highest nitrogen uptake compared to other treatments (Table 2 A). In Boro season, T<sub>1</sub> [120 kg/ha: 40 kg at 15 DAT + 40 kg at 30 DAT + 40 kg at BPI (BRRRI recommended)] and T<sub>3</sub> [120 kg/ha: 23 kg as basal + 40 kg at 20 DAT + 40 kg at 40 DAT + 17 kg at 20 days after PI (DAPI)/Booting] showed the significantly highest nitrogen uptake compared to other treatments (Table 2 B). The grain N uptake, straw N uptake increased during the Boro season, while an opposite trend was seen during the T. Aman season. The results are obtained in line with Deng et al. [17].

**Table 2 a. Effect of different N management on nitrogen uptake (kg ha<sup>-1</sup>) of BRRRI dhan75**

| Treatments | Nitrogen uptake (kg ha <sup>-1</sup> ) |       |
|------------|--|-------|
|            | Straw                                  | Grain |

|   |       |       |
|---|-------|-------|
| T <sub>0</sub> = No fertilizer  | 17.81 | 23.42 |
| T <sub>1</sub> = 23 kg as basal + 23 kg at 15 DAT + 23 kg at BPI (BRRRI recom. practice)                  | 25.62 | 78.13 |
| T <sub>2</sub> = 29.5 kg as basal + 29.5 kg at 15 DAT + 10 kg ha <sup>-1</sup> at 10 days after PI (DAPI) | 15.13 | 48.51 |
| T <sub>3</sub> = 29.5 kg as basal + 29.5 kg at 15 DAT + 10 kg ha <sup>-1</sup> at 20 days after PI (DAPI) | 18.1  | 57.2  |
| T <sub>4</sub> =29.5 kg as basal+29.5 kg at 15 DAT+10 kg ha <sup>-1</sup> at heading                      | 24.5  | 77.10 |
| LSD <sub>(0.05)</sub>   | 7.04  | 8.94  |
| CV (%)  | 18.5  | 8.3   |

**Table 2 b. Effect of different N management on nitrogen uptake (kg ha<sup>-1</sup>) of BRRRI dhan89**

| Treatments   | Nitrogen uptake (kg ha <sup>-1</sup> ) |       |
|--|--|-------|
|  | Straw                                  | Grain |
| T <sub>0</sub> = No fertilizer   | 7.68                                   | 33.83 |
| T <sub>1</sub> = 40 kg at 15 DAT + 40 kg at 30 DAT + 40 kg at BPI (BRRRI recommended)                          | 19.40                                  | 85.33 |
| T <sub>2</sub> = 23 kg as basal + 40 kg at 20 DAT + 40 kg at 40 DAT + 17 kg at 10 days after PI (DAPI)         | 22.6                                   | 71.80 |
| T <sub>3</sub> = 23 kg as basal + 40 kg at 20 DAT + 40 kg at 40 DAT + 17 kg at 20 days after PI (DAPI)/Booting | 19.5                                   | 85.2  |
| T <sub>4</sub> = 23 kg as basal + 40 kg at 20 DAT + 40 kg at 40 DAT + 17 kg at heading stage                   | 18.4                                   | 82.40 |
| LSD <sub>(0.05)</sub>  | NS                                     | 14.10 |
| CV (%)   | 37.0                                   | 10.9  |

#### 4. CONCLUSIONS

This study revealed the N management; application of 69 kg N ha<sup>-1</sup> ( $\frac{1}{3}$  as basal +  $\frac{1}{3}$  at 15 DAT +  $\frac{1}{3}$  at BPI) (T<sub>1</sub>) followed by 69 kg N ha<sup>-1</sup> (29.5 kg as basal + 29.5 kg at 15 DAT + 10 kg ha<sup>-1</sup> at heading) (T<sub>4</sub>) would be a better option for higher yield in T. Aman rice. While 120 kg N ha<sup>-1</sup> ( $\frac{1}{3}$  at 15 DAT +  $\frac{1}{3}$  at 30 DAT +  $\frac{1}{3}$  at BPI) (T<sub>2</sub>) and 120 kg N ha<sup>-1</sup> (23 kg as basal + 40 kg at 20 DAT + 40 kg at 40 DAT + 17 kg ha<sup>-1</sup> at heading) (T<sub>5</sub>) would be a better option for higher yield by reducing sterility% in Boro rice. From the results, it can be said that application of N @10 kg ha<sup>-1</sup> only at heading stage would be reduce sterility and gave higher yield than BRRRI recommended management.

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**Comment [mN4]:** references are still very minimal and have not used the Medley or Zetero applications

**Comment [mN5]:** very old reference, look for the latest, at least the last 10 years

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**Comment [mN6]:** very old reference, look for the latest, at least the last 10 years