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Physiological and Yield Responses of Five Rice Varieties to Nitrogen Fertilizer Under Farmer's Field in IADA Ketara, Terengganu, Malaysia

(Tindak Balas Fisiologi dan Hasil Lima Varieti Beras terhadap Baja Nitrogen di Ladang Peladang, IADA Ketara, Terengganu, Malaysia)

Muhamad Faiz Che Hashim, Asniyani Nur Haidar, Khairudin Nurulhuda*, Farrah Melissa Muharam, Zulkarami Berahim, Zed Zulkafli, Siti Najja Mohd Zad & Mohd Razi Ismail

ABSTRACT

Studies have suggested that the need for site-specific fertilizer application to reduce waste of resources as soil nutrients varies across the Malaysian rice fields. The present study was aiming to determine the physiological and yield responses of five rice varieties (MR269, MR297, MR220CL2, MR219, and UPUTRA) treated with three nitrogen (N) rates under farmer's field condition. The experiment design was a split-plot randomized complete block design, in which N rates, i.e. low (76 kg N ha^{-1}), farmers' practice (109 kg N ha^{-1}) and high (142 kg N ha^{-1}), were the main plot while rice varieties were the sub-plot. In general, harvestable panicle yield including the grain (at 14% moisture content) of all varieties was between 5.4 and 7.4 t ha^{-1} under all N treatments. Among all varieties, MR220CL2 recorded significantly higher yield, irrespective of N treatments. The physiological responses of rice varieties to N treatments, however, were mostly non-significant except for panicle biomass. Specifically, each variety recorded different biomass partitioning percentages for different organs at different growth phases. In the case of N treatments, there was no significant difference in the yield between 30% low and 30% high N rates as compared to farmer's practice at harvest. Hence, it could be suggested that farmers may reduce N application of about 30% without significant reduction in harvestable panicle yield for this specific plot. However, the potential N uptake might also be affected by unaccounted factors such as the availability of micronutrients and planting density.

Keywords:MR269; MR297; MR220CL2; MR219; nitrogen management; rice yield

ABSTRAK

Kajian telah mencadangkan tentang keperluan aplikasi baja khusus untuk lokasi berbeza bagi mengurangkan pembaziran sumber kerana kandungan nutrien tanah sawah di seluruh Malaysia adalah berbeza. Kajian ini bertujuan untuk menentukan tindak balas fisiologi dan hasil untuk lima varieti padi (MR269, MR297, MR220CL2, MR219 dan UPUTRA) yang dirawat dengan tiga kadar nitrogen (N) dalam keadaan ladang petani. Reka bentuk uji kaji adalah reka bentuk blok lengkap rawak beserta plot berpecah dengan kadar N, iaitu rendah (76 kg N ha^{-1}), amalan petani (109 kg N ha^{-1}) dan tinggi (142 kg N ha^{-1}), adalah plot utama sementara varieti padi adalah faktor subplot. Secara keseluruhan, kesemua varieti tersebut menghasilkan antara 5.4 hingga 7.4 t ha^{-1} hasil panikel termasuk bijirin (pada kadar kelembapan 14%) untuk ketiga-tiga rawatan N. Hasil panikel MR220CL2 adalah berbeza dengan ketara dan tertinggi berbanding dengan varieti lain tanpa mengira rawatan N. Kecuali untuk biojisim panikel, kajian mendapati tiada tindak balas N pada kebanyakan sifat fisiologi tumbuhan. Secara khusus, setiap varieti mempunyai peratusan pembahagian biojisim yang berbeza untuk organ dan fasa pertumbuhan yang berbeza. Untuk rawatan N, tidak ada perbezaan yang signifikan antara kadar N rendah 30% dan tinggi 30% berbanding dengan kadar N amalan petani pada waktu menuai. Maka, dapatlah disarankan supaya petani mengurangkan penerapan N sekitar 30% tanpa penurunan yang signifikan dalam hasil panikel yang dapat dituai untuk plot khusus ini. Walau bagaimanapun, potensi pengambilan N mungkin juga dibatasi oleh faktor yang tidak diambil kira dalam kajian seperti ketersediaan mikronutrien dan kepadatan tumbuhan.

Kata kunci: MR269; MR297; MR220CL2; MR219; pengurusan nitrogen; hasil padi

Introduction

Rice is a staple food for more than half of the world's population. In 2012, rice was cultivated on about 156 million ha worldwide (FAO 2013). In Malaysia, around 680,000 ha is used for paddy cultivation, which takes place over two seasons (DOA 2015). The self-sufficiency level for rice in Malaysia was 71% in the year 2015, and the Malaysian government aims to increase the self-sufficiency level to 80% by the year 2020 (Ismail 2017; Omar et al. 2019).

The frequently used rice varieties in the year 2014 were MR220CL2, where this variety was cultivated at 50% of the overall granary area in Malaysia, followed by MR219 at 24%, MR263 at 13%, MR220 at 5%, MR269 at 3% and other varieties at 5% (DOA 2015). All these varieties are categorized as indica rice (Alhasnawi et al. 2017; Atabaki et al. 2018; Yao et al. 2018; Zuraida et al. 2011). In the year 2015, a new variety currently called UPUTRA was developed by Miah et al. (2015) and Tanweer et al. (2015).

The grain yield of high yielding varieties such as MR219, MR220, and MR269 can potentially reach up to 10 t ha^{-1} (Ismail 2005, 2001; Yusob et al. 2014). In the year 2015, the rice yield (at 14% moisture content) in the main season was the highest at IADA Barat Laut Selangor with an average value of 6.1 t ha^{-1}, and the lowest at IADA Seberang Perak with an average value of 3.9 t ha^{-1}, while the average value of all major granary areas was 4 t ha^{-1} (DOA 2016).

Due to the importance of nitrogen (N) management, studies were conducted to understand the response of rice varieties to N treatments. Field experiments were conducted to evaluate the N surplus for three conditions, i.e. the farmers' practices, optimized N management and optimized N and water management in Guangdong Province in South China (Liang et al. 2016). The study found that the yearly N surplus in the farmers' practices was 31% higher than the two optimized conditions. The grain yield, recovery efficiency, partial factor productivity and agronomic efficiency of applied N in the two optimized conditions were substantially higher than farmers' practices. The effects of four N rates and planting density on the crop growth and development (aboveground biomass, harvest index, leaf photosynthetic features, grain yield, and yield components) of three japonica rice varieties over two years were investigated (Zhou et al. 2019). The harvested grain yield had a significantly positive correlation with the stomatal conductance, net photosynthesis rate, transpiration rate, SPAD value, leaf area index, panicles per unit area and spikelets per panicle (Zhou et al. 2019).

Recent studies were directed to improve growth and yield attributes of specific Malaysian rice varieties using various approaches. For instance, Dorairaj et al. (2017) demonstrated silicone added in fertilizer improved lodging resistance and yield attributes of MR219. Masni and Wasli (2019) analysed rice variety MRM16 under different fertilizer rates in a pot-based study. Kareem et al. (2014) studied the effect of osmotic and hormonal priming on rice grain yield of MR219 and saw that that priming of all forms improved the early growth performance of MR219.

However, few published reports on comparison of N responses of Malaysian rice varieties to N treatment under field conditions exist. The few studies include the work by Saidon et al. (2014) who reported potential grain yield and the yield attributes for eight rice varieties under recommended fertilizers and farm management. The study showed that varieties MR272, M278, MR283, and MR284 had higher yield potential than MR211 MR219, MR253, and MR263. Ismail et al. (2014) observed the effects of different combinations of N and K rates on rice grain yields of MR269 and MR284 for two seasons and found that the yield was affected by N, but not by K. They further observed that the amount of N needed to produce the highest yield differed between the two seasons. In addition, multi-location verification trials of Malaysian rice varieties typically considered no more than three varieties at a time; the performance of a new variety was compared to another two best performing varieties at a given time. For instance, MR219 was compared to MR84 (Ismail 2001) while MR220 was compared to MR84 and MR219 (Ismail 2005). Furthermore, all these studies only observed the final grain yield and the corresponding yield attributes, but not the development of crop physiological traits of rice crop throughout the growth stages.

Subsidy fertilizer was introduced in Peninsular Malaysia in 1979 (MARDI 2008), and rice farmers in Malaysia continue to be heavily subsidised. In 2016, approximately USD112 million was spent by the Malaysian Government for rice fertilizer subsidy (Bernama 2016). Sustainable rice cultivation technology including fertilization and irrigation recommendations have been provided by MARDI (2008). In practice, some farmers adjust the timing and amount of fertilizers based on their experiences. However, it has been shown that soil nutrients vary across the rice fields in IADA granary (Aishah et al. 2010), and this suggests the need for implementation of site-specific fertilizer application to reduce waste of resources.

In light of the limited understanding of responses of Malaysian rice varieties to N treatments under field conditions, the objective of this study was to investigate the physiological and yield responses across five varieties treated with three N rates including the farmer's practice rate under field conditions. Five popular varieties were selected for this study, namely MR269, MR297, MR220CL2, MR219, and UPUTRA.

MATERIALS AND METHODS

STUDY SITE DESCRIPTION

A field experiment was carried out from February 2018 to June 2018 at a 0.4 ha lot in IADA KETARA rice granary, Besut, Terengganu, Malaysia (5.717497N, 102.492691E). The study area is an active cultivation area, and it has been operated by a local farmer since the year 1999. In IADA KETARA, rice is typically cultivated from August to February of the following year (main season) and from March to July (off-season). The term off-season refers to a season when there is lack of precipitation in the area. However, in IADA KETARA, enough water supply can be ensured during the off-seasons through proper irrigation resulting in comparable rice grain yields between main and off seasons (DOA 2015). The average yield produced from IADA KETARA granary area is 5.5 t ha^{-1} (DOA 2016; Ismail 2005).

A weather station was installed at the experimental lot and micrometeorological data were recorded from early January 2018 to late August 2018. The average sunshine hours were 12 h day^{-1}. A cumulative rainfall of 747 mm was recorded from January 2018 to August 2018. The ambient temperature ranged between 24 and 33 °C. The average solar radiation was 10471 W m^{-2} day^{-1}. The average wind speed was 0.25 m s^{-2}.

Prior to the experiment, 54 soil-core samples were collected at 18 random locations, and three depths (0 to 20 cm, 20 to 40 cm and 40 to 60 cm from the ground surface) at each location. Site inspection showed that the roots did not exceed 60 cm from the ground surface due to presence of a hardpan. The 54 soil-core samples were pulverized to pass a sieve with 2 mm opening. Next, the 54 samples were analysed for contents of total nitrogen (TN) and total carbon (TC) using the TruMac equipment. Then, the 54 samples were subsampled and composited into 18 samples, and subsequently analysed for organic matter (OM) using the loss on ignition method and soil texture using the pipette method. Table 1 shows the soil physical properties at the study area, and Table 2 shows the soil requirements for paddy cultivation in Malaysia.

TABLE 1. Soil physical properties at the study area

Soil property (%)	Depth from the ground surface (cm)	Average value	Minimum value	Maximum value
TC	0-20	1.89	1.38	2.47
	20-40	1.14	0.48	2.01
	40-60	0.62	0.18	2.10
TN	0-20	0.20	0.14	0.26
	20-40	0.16	0.05	0.23
	40-60	0.18	0.02	0.13
OM	0-20	5.53	5.05	5.80
	20-40	4.15	3.84	5.16
	40-60	3.80	2.93	4.68
Clay	0-20	57.96	55	61
	20-40	58.78	57	61
	40-60	57.59	56	61
Sand	0-20	12.26	11	14
	20-40	13.44	11	20
	40-60	14.71	9	24
Silt	0-20	32.84	30	36
	20-40	30.52	26	37
	40-60	31.18	28	35

TC is total carbon, TN is total nitrogen and OM is organic matter

TABLE 2. Soil requirements for paddy cultivation in Malaysia

Soil property	Value	References
pH	5.5 to 6.5	Aishah et al. (2010); DOA (2008)
Organic carbon (%)	2 to 3	Aishah et al. (2010)
Total nitrogen (%)	0.2 to 0.3	Aishah et al. (2010)
Available phosphorus (mg kg^{-1})	> 40	Aishah et al. (2010); DOA (2008)

EXPERIMENTAL DESIGN

The experiment design was a split-plot randomized complete block design. The main plot was N rate arranged in four blocks while the sub-plots were rice varieties. There were three N treatments: 1) low (76 kg N ha^{-1}), 2) farmers' practice (109 kg N ha^{-1}) and 3) high (142 kg N ha^{-1}). The low N treatment was 30% less, while the high N treatment was 30% more than the farmers' practice. Five rice varieties selected were MR269, MR297, UPUTRA, MR219, and MR220CL2. In overall, there were a total of 60 subplots, whereby each subplot had an area of 11 × 5 m.

Preparation of the land was initiated in early January 2018. The experimental area was tilled twice using a tractor. This was followed by creating bunds to separate the subplots. In order to reduce risk of N seepage through the bunds, the bunds were covered with polyethylene plastic. The plastic was extended below the bund to 20 cm below the ground surface. The third tillage was conducted manually using a scrapper. The experimental subplots were then continuously flooded between 5 and 10 cm depth throughout the experimental duration, except for the last 15 days prior to harvesting whereby the ponded water was drained.

Rice seeds were manually broadcasted into the subplots under flooded conditions at a rate of 2 kg per subplot. Manual broadcast of seeds is the current practice by the majority of farmers in Peninsular Malaysia. The total N fertilizer was split into three applications: 39% was applied at 18 days after sowing (DAS), 42% at 39 DAS and 19% at 55 DAS. The first application was in the form of compound fertiliser N:P:K at 12:20:10, and the second was in the form of urea and the third application was compound fertiliser N:P:K:MgO at 17:3:25:2.

PLANT PHYSIOLOGICAL SAMPLING

Samplings were conducted based on the rice plant growth stages mainly vegetative, reproductive, and ripening (DOA 2008). For a variety with a maturation period of 95 to 100 DAS, the vegetative phase is from 1 to 60 DAS, the reproductive phase from 60 to 80 DAS and the ripening phase from 81 to 110 DAS (DOA 2008). For a variety with a maturation period of 120 DAS, the vegetative phase is from 1 to 55 DAS, the reproductive phase is from 56 to 90 DAS and the ripening phase is from 91 to 120 DAS (DOA 2008).

Plant physiological properties measured included number of plants (NOP), average height, average chlorophyll content of the first fully expanded leaves, green leaf biomass (GL), dead leaf biomass (DL), stem biomass (St), panicle (includes grain) biomass (SO) and leaf area index (LAI). These measurements were made at three different growth phases of paddy, specifically at 34 to 37 DAS (mid vegetative), 62 to 65 DAS (early reproductive) and 83 to 86 DAS (early ripening) (DOA 2008).

Two quadrants, each with a size 0.5 × 0.5 m, were randomly distributed in each subplot. The number of plants (hills) in each quadrant was counted. Then, the plants above ground were sampled from each quadrant. The stems of plants were cut just above the ground surface, with roots excluded. Then, the average plant height was measured for each quadrant using a standard meter stick. The relative chlorophyll content of the first fully expanded leaves was measured using the Minolta SPAD 502 meter (Minolta Corp., Osaka, Japan). The plants were then carefully packed and stored in a container filled with ice and subsequently transported to the lab.

Upon arrival at the lab, the organs of the plants were separated. The leaf area of the fresh green leaves was measured using several benchtop leaf area meters (Li-Cor 3100C, Li-cor Inc., Lincoln, NE, USA). The separated crop organs were sun-dried in a greenhouse until a constant weight was reached. Subsequently, the dry weight of green leaves, dead leaves, stems and panicles (grains) were weighed. A maximum of 30 quadrants were analysed each day using the benchtop leaf area meters, and all 120 samples were completed within four days.

The dried green leaves were pulverized using Retsch Cross Beater Mill SK 100 grinder until the ground leaves can pass through a sieve with an opening of 1 mm. Then, 0.25 g leaf samples were wet digested with a mixture of sulphuric acid and hydrogen peroxide at 285 °C (Miller & Miller 1948). The digested solution was then analyzed for the total N content (%) using the LACHAT quikChem FIA+ 8000 series autoanalyzer (Danaher Corp., Loveland, CO, USA).

Values of LAI and biomass of separated organs were found to be affected by the number of plants (NOP) per quadrant. Preliminary analysis showed that the NOP, LAI, and biomass between the quadrants within the same subplot highly vary due to uneven distribution of seeds during seed broadcast. Seedlings may be transplanted to avoid bias in the plant physiological data due to variation in the number of plants per square area, but in this study, the broadcast technique was applied to emulate the actual practice by majority farmers in Peninsular Malaysia. Interactions between plants in a seedling transplanting scheme versus a seed broadcast scheme may differ due to competition for available nutrients, water and space between plants.

Due to broadcasting of seeds, the uneven distribution of seeds indicates that care must be taken to ensure representative sampling. A representative sampling may be achieved by increasing the number of quadrants per subplot, but this approach increases labour and financial cost.

Instead, the leaf area index (LAI), green leaf (GL) biomass, dead leaf (DL) biomass, stem (St) biomass and panicle (SO) biomass from the first, second, and third samplings were normalized by the NOP per quadrant to achieve a representative sampling as follows (1).

$$Normalized\ variable = \frac{Value\ of\ crop\ variable\ in\ a\ quadrant}{NOP\ per\ quadrant} \times Average\ NOP\ per\ quadrant \quad (1)$$

where NOP is the number of plants (hills). The average NOP per quadrant was 37, which was calculated from a total of 360 quadrants obtained from the three samplings.

For the fourth sampling, the normalization of final panicle biomass was modified as follows because the NOP for each quadrant was not measured (2).

$$Normalized\ final\ panicle = \frac{Value\ of\ final\ panicle\ in\ a\ quadrant}{Average\ NOP\ per\ subplot} \times Average\ NOP\ per\ quadrant \quad (2)$$

The average NOP per subplot was calculated by summation of NOP for the respective subplot throughout the three samplings and then divided by a total of six quadrants.

YIELD SAMPLING

The fourth sampling was conducted at 104 to 105 DAS (ripening stage) to determine the biomass of the panicle (grain) yield just before the field was harvested. This time, four randomly placed quadrants were used to sample the panicle from each subplot. The harvested yield at 14% moisture content was calculated from the dried harvested yield using the dry basis moisture content formula.

DATA ANALYSIS

The two-way Analysis of Variance (ANOVA) was conducted on the rice plant physiological properties followed by mean separation analysis using the Least Square Mean. The statistical analyses were performed using the PROC GLIMMIX procedure in SAS version 9.4.

RESULTS AND DISCUSSION

PHYSIOLOGICAL AND YIELD RESPONSES OF RICE VARIETIES TO N TREATMENTS

Table 3 shows the results of rice plant physiological traits for three N treatments and five rice varieties. In overall, the effects of N and varieties on the plant traits were inconsistent throughout the growth phases. The effect of N on %N in green leaf, relative chlorophyll content (SPAD-N) and LAI were non-significant throughout the growth phases. Interaction between N treatments and varieties was observed only for the panicles at the early reproductive phase.

Table 4 shows the mean separation for rice plant physiological traits in response to N treatments. The N treatments were significant for biomass of dead leaf and stem at mid vegetative phase, biomass of panicle at early reproductive phase, and plant height and biomass of green leaf and panicle at early ripening phase. At mid vegetative phase, the high N treatment (142 kg N ha^{-1}) produced the highest dead leaf biomass and is significantly different to the low N treatment (76 kg N ha^{-1}). The high N treatment also produced the highest stem biomass and was significantly different to the low and farmer's N (109 kg N ha^{-1}) treatments. In contrast, at early reproductive phase, the low N treatment (76 kg N ha^{-1}) resulted in the highest panicle biomass and was significantly different to the high and farmer's N treatments. At the early ripening phase, the plant height was tallest for the high N treatment and was significantly different to the other two treatments. Meanwhile, the green leaf and panicle biomass were the highest for the low N treatment and was significantly different to the farmer's N treatment.

Table 5 shows the mean separation for significant rice plant physiological traits for variety effects. At mid-vegetative phase, green leaf %N content of MR220CL2 was the highest and significantly different among all varieties. At early reproductive phase, plant height, relative chlorophyll content and biomass of panicle were the highest for MR220CL2. Further inspection showed that the relative chlorophyll content changed positively with the panicle biomass at the reproductive phase; relative chlorophyll content and panicle biomass of MR220CL2 were the highest of all varieties, while those of MR269, UPUTRA, and MR219 were the lowest. At the early ripening phase, plant height, LAI and green leaf biomass were the lowest for MR220CL2. Nevertheless, the panicle biomass of MR220CL2 remained the highest and significantly different among all five varieties at this phase. Except for the previously mentioned trends, there was no defined trend between most of the significant physiological properties and the varieties. At harvesting, the panicle biomass of MR220CL2 remained the highest, followed by the MR269 and MR297, the UPUTRA, and the MR219, respectively.

The average harvested panicle (grain) yield at 14% moisture content in decreasing order were 7.4, 6.1, 6.1, 5.7 and 5.4 t ha^{-1} for MR220CL2, MR269, MR297, UPUTRA, and MR219, respectively. These values were higher than the average grain yield at 14% moisture content observed at IADA KETARA granary area, which is about 5 t ha^{-1} (DOA 2015).

All varieties selected for this study are high yielding. However, the two-way ANOVA showed that the panicle biomass of MR220CL2 was significantly the highest compared to the other varieties, irrespective of N treatments at all growth phases and harvesting. The next highest panicle biomass at harvest were MR269, MR297, UPUTRA and finally MR219.

Maturity periods of MR219, MR269 and MR297 are comparable to each other, namely between 105 and 111 DAS, 104 and 109 DAS and 105 and 110 DAS, respectively (DOA 2008). The minimum maturity period reported for MR219, MR269, and MR297 were met at harvesting. Meanwhile, UPUTRA has a maturity period of 115 to 120 days. The decision to harvest UPUTRA earlier than the maturity period had to be made to avoid invasion of rats that would consume the grains since other neighbouring plots had been harvested.

The average harvested yield results may be partly explained by the breeding history of the different rice varieties. MR219 is a cross between MR151 and MR137 (Ismail 2001), while UPUTRA is produced by transferring two blast resistance genes from Pongsu Seribu into MR219 (Tanweer et al. 2015). Therefore, it is expected that MR219 and UPUTRA would share many similar characteristics, which may explain similar yield between MR219 and UPUTRA at harvesting. Meanwhile, MR269 and MR297 are produced from different parents than those of UPUTRA and MR219. The yield of MR269 and M297 was significantly different than those of MR219 at harvesting. The harvested yield of MR220CL2 was higher than that of MR219. MR220 is a sister line to MR219 and similarly is a cross between MR151 and MR137, hence sharing most of the characteristics of MR219 including the maturity period (Ismail 2001). MR220CL2 is a cross between MR220 and an American rice variety. The resultant variety has two strikingly different characteristics than those of MR219 and MR220: short plant maturity, which is between 95 and 105 days, and herbicide resistant compared to other Malaysian rice varieties (Azmi et al. 2012).

Except for MR220CL2, overall, there was no defined relationship between most of the significant crop physiological properties and the varieties as described in the results. Based on the results, two inferences are deduced. First, results of this study suggest that each rice variety has different biomass partitioning percentage for different plant organs at different growth phases. Second, the total aboveground biomass and separate organ biomass alone may not be used to predict the harvested yield; higher crop organ biomass may not result in higher harvested yield, i.e. in the case of MR220CL2 where lower crop organ biomass resulted in high yield. Similarly, Zhou et al. (2019) reported lack of correlation between harvested yield with the total aboveground biomass of three japonica rice varieties in field studies conducted in Liaoning Province, China.

Farmers in the east of Peninsular Malaysia who are dependent only on the subsidised fertilisers would apply around 109 kg N ha^{-1} per season. However, results from our study showed that increasing the total amount of N to 142 kg N ha^{-1} did not increase the harvested yield for the specific study area. Either rate is lower than the farmer's fertiliser practices in China, where the fertilizer applications range from 180 to 350 kg N ha^{-1} as reported in Guo et al. (2019) and Liang et al. (2019). Liang et al. (2019) observed the average harvested yield for the optimized N (166 and 134 kg N ha^{-1} in early and late seasons, respectively) was 7.2 t ha^{-1}, which was 9.5% higher than that of farmer's fertiliser practices (199 and 203 kg N ha^{-1} in early and late seasons, respectively). Similarly, Guo et al. (2019) observed the average harvested yield for japonica varieties for optimized N (240 kg N ha^{-1}) was higher than that of the farmer's fertiliser practice (350 kg N ha^{-1}).

The two-way ANOVA showed that the effects of N on rice plant physiological properties other than the panicle were little throughout the growth phases. Significant effect of N was observed on the panicle biomass at early reproductive and early ripening phases, while not observed at harvesting. The average initial soil N at the study site prior to the experiment was 2%, which is just about the reported soil N required for paddy cultivation. Based on these observations, it appears that the plant N requirement may have been met towards the harvesting phase even with the low N treatment.

Except at the mid-vegetative phase, the two-way ANOVA also showed that there was no significant effect of either the N treatments or the varieties on the green leaf %N content at all growth phases. A closer inspection of the data showed that average green leaf %N content for all N treatments and varieties at mid-vegetative, early reproductive, and early ripening phases were 2.29, 2.03, and 1.86%, respectively. The green leaf N content did not exceed 3% at all growth phases. Overall, it may be that the critical %N content in the leaves, which is 2% as reported in Singh et al. (1995), was met at both reproductive and early ripening phases. However, the green leaf %N content at mid-vegetative phase for the high N treatment was about two times lower than the reported critical %N content, i.e. 4.4% in Singh et al. (1995). Due to this and the lack of difference in the green leaf %N content between the low and high N treatments at all growth phases, it may be inferred that the N uptake was limited by factors unaccounted in this study, in particular the availability of micronutrients and effects of planting density. Micronutrients such as boron could be a limiting factor (Atique-ur-Rehman et al. 2018). Meanwhile, Zhou et al. (2019) found significant interactions between varieties, plant density and optimum N rate. Tian et al. (2017) suggest that higher rice planting densities resulted in less N inputs, while more N was needed to improve single plant actual tiller ability under low density to offset the reduced planting density. It is also plausible that the applied N is lost to the environment via ammonia volatilization at early growth phase (Lin et al. 2012).

TABLE 3. Two-way ANOVA of rice plant physiological traits for two independent variables: nitrogen treatments (N) and varieties (V)

DAS	Treatment effect	Physiological properties									
		Df	Height	%N	SPAD-N	LAI	GL	DL	St	SO	Total ABG
34 to 37 Mid vegetative	Effect of N	2	0.1145	0.1976	0.3016	0.3166	0.0687	0.0266	0.0112	N/A	0.0099
	Effect of V	4	0.1155	**0.0100**	0.0683	0.3968	0.6760	0.8350	0.5481	N/A	0.5818
	Effect of NxV	8	0.6681	0.4999	0.8282	0.5815	0.7943	0.6880	0.4187	N/A	0.6270
62 to 65 Early reproductive	Effect of N	2	0.2909	0.8498	0.2312	0.4590	0.3745	0.0516	0.1287	**0.0097**	0.0701
	Effect of V	4	**0.0091**	0.2128	**0.0004**	0.5998	0.4968	**0.0362**	0.2042	**<0.0001**	0.2494
	Effect of NxV	8	0.3510	0.3147	0.1454	0.9475	0.9293	0.1563	0.5523	**0.0040**	0.6921
83 to 86 Early ripening	Effect of N	2	**0.0051**	0.7792	0.3821	0.2919	**0.0363**	0.9166	0.0618	**0.0227**	**0.0204**
	Effect of V	4	**0.0063**	0.3760	0.5239	**0.0017**	**0.0030**	0.6638	0.3251	**<0.0001**	**0.0021**
	Effect of NxV	8	0.6187	0.4146	0.4681	0.6491	0.5001	0.8674	0.0843	0.8373	0.5157
104 to 105 Harvesting	Effect of N	2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.9023	N/A
	Effect of V	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**<.0001**	N/A
	Effect of NxV	8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.8737	N/A

Note that statistical results were based on the normalized values of LAI, GL, DL, St, SO and Total ABG ((1) and (2)). DAS is day after sowing, N is nitrogen, V is variety, %N is percent N in green leaf, SPAD-N is relative chlorophyll content in leaf, LAI is leaf area index, GL is biomass of green leaf (g $quadrant^{-1}$), DL is biomass of dead leaf (g $quadrant^{-1}$), St is biomass of stem (g $quadrant^{-1}$), SO is biomass of panicle (g $quadrant^{-1}$), Total ABG is total aboveground biomass (g $quadrant^{-1}$), N/S is not significant and N/A is not available. Values of p less than 0.05 are significant. The df denotes degree of freedom

TABLE 4. Mean separation for significant rice plant physiological traits for N effects

DAS/ Phase	Physiological properties												
	Height		%N green leaf	SPAD-N	LAI	GL		DL		St		SO	
34 to 37 Mid vegetative phase	N/S		N/S	N/S	N/S	N/S		142 kg N ha^{-1}	2.96^{a}	142 kg N ha^{-1}	41.12^{a}	N/A	
								109 kg N ha^{-1}	2.69^{ab}	109 kg N ha^{-1}	35.07^{b}		
								76 kg N ha^{-1}	2.04^{b}	76 kg N ha^{-1}	34.17^{b}		
62 to 65 Early re-productive phase	N/S		N/S	N/S	N/S	N/S		N/S		N/S		76 kg N ha^{-1}	8.36^{a}
												142 kg N ha^{-1}	5.59^{b}
												109 kg N ha^{-1}	3.91^{b}
83 to 86 Early ripening phase	142 kg N ha^{-1}	0.92^{a}	N/S	N/S	N/S	76 kg N ha^{-1}	66.4^{a}	N/S		N/S		76 kg N ha^{-1}	148.68^{a}
	109 kg N ha^{-1}	0.89^{b}				142 kg N ha^{-1}	62.96^{ab}					142 kg N ha^{-1}	141.14^{ab}
	76 kg N ha^{-1}	0.87^{b}				109 kg N ha^{-1}	57.14^{b}					109 kg N ha^{-1}	124.79^{b}
104 to 105 Harvesting	N/A											N/S	

Note that statistical results were based on the normalized values of LAI, GL, DL, St and SO ((1) and (2)). DAS is day after sowing, N is nitrogen, V is variety, %N is percentage of N in leaf, SPAD-N is relative chlorophyll content in leaf, LAI is leaf area index, GL is biomass of green leaf (g quadrant^{-1}), DL is biomass of dead leaf (g quadrant^{-1}), St is biomass of stem (g quadrant^{-1}), SO is biomass of panicle (g quadrant^{-1}), Total ABG is total aboveground biomass (g quadrant^{-1}), N/A is not available and N/S is not significant

TABLE 5. Mean separation for significant rice plant physiological traits for variety effects

DAS/ Phase	Physiological properties														
	Height		%N green leaf		SPAD-N		LAI		GL		DL		St	SO	
34 to 37 Mid vegetative phase	N/S		MR220CL2	2.52^{a}	N/S		N/S		N/S		N/S		N/S	N/A	
			UPUTRA	2.34^{b}											
			MR297	2.31^{b}											
			MR219	2.30^{b}											
			MR269	2.20^{b}											
62 to 65 Early reproductive phase	MR220CL2	0.79^{a}	N/S		MR220CL2	37.00^{a}	N/S		N/S		MR220CL2	14.77^{a}	N/S	MR220CL2	19.48^{a}
	MR269	0.76^{ab}			MR297	36.64^{ab}					UPUTRA	14.39^{a}		MR297	5.85^{b}
	MR219	0.74^{b}			MR269	35.47^{bc}					MR269	13.63^{a}		UPUTRA	1.88^{c}
	MR297	0.73^{b}			UPUTRA	34.86^{c}					MR219	12.51^{ab}		MR219	1.52^{c}
	UPUTRA	0.71^{b}			MR219	34.41^{c}					MR297	10.51^{b}		MR269	1.04^{c}
83 to 86 Early ripening phase	MR269	0.93^{a}	N/S		N/S		UPUTRA	7.40^{a}	UPUTRA	70.82^{a}	N/S		N/S	MR220CL2	198.94^{a}
	MR219	0.91^{ab}					MR269	6.69^{a}	MR269	65.78^{ab}				MR297	149.53^{b}
	UPUTRA	0.89^{bc}					MR219	6.38^{a}	MR297	61.79^{ab}				UPUTRA	127.52^{c}
	MR297	0.88^{bc}					MR297	6.29^{a}	MR219	61.33^{b}				MR269	111.55^{cd}
	MR220CL2	0.86^{c}					MR220CL2	4.83^{b}	MR220CL2	51.20^{c}				MR219	103.49^{d}
104 to 105 Harvesting	N/A													MR220CL2	160.14^{a}
														MR269	130.94^{b}
														MR297	130.93^{b}
														UPUTRA	121.80^{bc}
														MR219	116.16^{c}

Note that statistical results were based on the normalized values of LAI, GL, DL, St and SO ((1) and (2)). DAS is day after sowing, N is nitrogen, V is variety, %N is percentage of N in leaf, SPAD-N is relative chlorophyll content in leaf, LAI is leaf area index, GL is biomass of green leaf (g quadrant^{-1}), DL is biomass of dead leaf (g quadrant^{-1}), St is biomass of stem (g quadrant^{-1}), SO is biomass of panicle (g quadrant^{-1}), Total ABG is total aboveground biomass (g quadrant^{-1}), N/A is not available and N/S is not significant

Conclusion

This study investigates the physiological and yield responses across five high-yielding Malaysian rice varieties treated with three nitrogen rates including the farmer's practice rate under field conditions. The physiological traits included the plant height, %N in green leaf, SPAD-N value, LAI, biomass of panicle (grain), green and dead leaf, and stem. The average harvested panicle (grain) yield at 14% moisture content in decreasing order were 7.4, 6.1, 6.1, 5.7, and 5.4 t ha^{-1} for MR220CL2, MR269, MR297, UPUTRA, and MR219, respectively. Among all the varieties, MR220CL2 recorded significantly higher panicle yield, irrespective of N treatments. The physiological responses of rice varieties to N treatments, however, were mostly non-significant except for panicle biomass. In the case of N treatments, there was no significant difference in the yield between 30% low and 30% high N rates as compared to farmer's practice at harvest. This finding signifies that a periodic sampling of soil nutrient contents may result in substantial reduction in fertilizer subsidy, in addition to environmental benefits. Each variety recorded different biomass partitioning percentages for different organs at different growth phases.

Based on the %N content in green leaves, we hypothesise that the N uptake may be limited by unaccounted factors like availability of micronutrients and planting density. Therefore, assessment of these factors' interaction with N may be crucial in attempting to increase the rice yield. This study further shows a lack of N response on rice plant physiology across multiple varieties. Currently, there is a lack of reference on comparison of N responses of Malaysian rice varieties. Thus, findings in this study may serve as an impetus for continued field experiments across multiple seasons and locations in Malaysia.

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Muhamad Faiz Che Hashim & Khairudin Nurulhuda*
Department of Biological and Agricultural Engineering
Faculty of Engineering
Universiti Putra Malaysia
43400 Serdang, Selangor Darul Ehsan
Malaysia

Asniyani Nur Haidar & Farrah Melissa Muharam
Department of Agriculture Technology
Faculty of Agriculture
Universiti Putra Malaysia
43400 Serdang, Selangor Darul Ehsan
Malaysia

Khairudin Nurulhuda*
Smart Farming Technology Research Centre
Universiti Putra Malaysia
43400 Serdang, Selangor Darul Ehsan
Malaysia

Zulkarami Berahim & Mohd Razi Ismail
Institute of Tropical Agriculture and Food Security
Universiti Putra Malaysia
43400 Serdang, Selangor Darul Ehsan
Malaysia

Siti Najja Mohd Zad & Zed Zulkafli
Department of Civil Engineering
Faculty of Engineering
Universiti Putra Malaysia
43400 Serdang, Selangor Darul Ehsan
Malaysia

*Corresponding author; email: k_nurulhuda@upm.edu.my

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