

Physiological and Yield Performance of Commercial Rice Varieties under Cyclic Water Stress in Malaysia

(Fisiologi dan Prestasi Hasil Varieti Padi Komersial di bawah Tekanan Air Kitaran di Malaysia)

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ABSTRACT

The production of drought-tolerant rice varieties in Malaysia and the information regarding the response of local varieties to water stress are still lacking. Therefore, this experiment was conducted to determine the growth, physiological performance, molecular response, and yield of ten available rice varieties, namely MR 219, MR 220-CL2, MR 297, MRQ 76, Vietnam Hybrid, UKM RC2, UKM RC8, Putra 1, MR 303, and MR 307, under ten days of cyclic water stress. The experiment was arranged in a Randomized Complete Block Design (RCBD) with three replications. Plant height, tiller number, photosynthesis rate, stomatal conductance, transpiration rate, chlorophyll content, biomass partitioning, genotyping of SSR markers, days of harvest, and yield component were measured. Results showed that water limitations reduced tiller number per hill, while plant height, leaf dry matter, and panicle length were enhanced. It was found that MR 297 had the shortest plant height, while MR 220-CL2 had a short maturity period, a shorter panicle length, and an enhanced filled grain percentage. Putra 1 and UKM RC8 showed a higher photosynthesis rate, stomatal conductance, and transpiration rate under water limitation at 99 days after sowing (DAS). Under well-watered conditions, the total grain weight per pot of Putra 1 and MR 307 was enhanced compared to MR 219. Meanwhile, under water limitation, the total grain weight per pot of UKM RC2, MR 220-CL2, MR 307, MR 297, and Vietnam Hybrid was comparable to MR 219 and slightly enhanced in UKM RC8, Putra 1, and MR 303. Among the tested varieties, MR 220-CL2 can be selected based on early maturity criteria for the potential development of drought-tolerant varieties.

Keywords: Drought; *Oryza sativa*; photosynthesis rate; stomatal conductance; yield component

ABSTRAK

Pengeluaran varieti padi tahan kemarau di Malaysia dan maklumat mengenai tindak balas varieti tempatan terhadap tekanan air masih kurang. Justeru, penyelidikan ini dijalankan untuk menentukan pertumbuhan, prestasi fisiologi, tindak balas molekul dan hasil sepuluh varieti padi yang ada, iaitu MR 219, MR 220-CL2, MR 297, MRQ 76, Vietnam Hybrid, UKM RC2, UKM RC8, Putra 1, MR 303 dan MR 307 di bawah kitaran sepuluh hari tekanan air. Uji kaji telah disusun dalam Reka Bentuk Blok Lengkap Rawak (RCBD) dengan tiga ulangan. Ketinggian tanaman, kadar fotosintesis, kekonduksian stomata, kadar transpirasi, kandungan klorofil, pembahagian biojisim, genotip penanda SSR, hari penuaian dan komponen hasil diukur. Keputusan menunjukkan bahawa air yang terhad mengurangkan bilangan anak setiap rumpun, manakala ketinggian tanaman, berat kering daun dan panjang tangkai bertambah baik. Didapati bahawa MR 297 mempunyai ketinggian terendah manakala MR 220-CL2 mempunyai tempoh matang yang pendek, panjang tangkai yang lebih pendek dan peratusan biji berisi yang dipertingkatkan. Putra 1 dan UKM RC8 menunjukkan kadar fotosintesis, kekonduksian stomata dan kadar transpirasi adalah lebih tinggi di bawah tekanan air pada 99 hari selepas menyemai (DAS). Di bawah keadaan pengairan yang baik, jumlah berat bijirin setiap pot di

dalam Putra 1 dan MR 307 telah dipertingkatkan berbanding MR 219. Sementara itu, di bawah tekanan air, jumlah berat bijirin setiap pot UKM RC2, MR 220-CL2, MR 307, MR 297 dan Vietnam Hibrid adalah setanding dengan MR 219 dan dipertingkatkan sedikit dalam UKM RC8, Putra 1 dan MR 303. Antara varieti yang diuji, MR 220-CL2 boleh dipilih berdasarkan kriteria kematangan awal untuk potensi pembangunan varieti tahan kemarau.

Kata kunci: Kadar fotosintesis; kekonduksian stomata; kemarau; komponen hasil; *Oryza sativa*

INTRODUCTION

Rice, a major food crop produced in Malaysia, was cultivated on 165.2 million hectares (ha) worldwide. Approximately 87% and 27% of this harvested area were in Asia and Southeast Asia (SEA), respectively (FAOSTAT 2021). The average production of rice in Malaysia is about 4.4 tons/ha, contributing to 63% of the self-sufficiency level (SSL) (Department of Agriculture 2021). The Malaysian government, through the National Agrofood Policy under the 12th Malaysian Plan, aims to increase the SSL of rice to 80% by the year 2030 (Ministry of Agriculture and Food Industries 2021). The impact of climate change caused high uncertainty in the seasonal rainfall pattern throughout the country, where minimal rainfall occurred during the dry season, contributing to extreme drought and increasing temperatures that threatened the potential rice production (Tan et al. 2021; Zulkafli et al. 2021). A study conducted by Vaghefi et al. (2016) using the DSSAT crop simulation model showed that rice yield is expected to reduce by 12% in the main season and 31.3% in the off-season until 2030 due to temperature increases and changes in rainfall patterns.

Over the past two decades, several high-yielding rice varieties have been officially released in Malaysia, such as MR 219 (2002), MR 220 (2003), MR 220-CL2 (2010), MR 269 (2012), MARDI Sempadan 303 or MR 303 (2018), MARDI Sebernas 307 or MR 307 (2018), specialty rice MRQ 76 (2012), and disease-resistant varieties known as MARDI SIRAJ 297 or MR 297 (2016) (Sunian et al. 2022). Another two varieties, UKM RC2 and UKM RC8, were officially released in 2019 for being more durable, resistant to diseases, and submergence-tolerant (Asmuni, Ismail & Abd Aziz 2019). Following that, Putra 1, a new high-yielding and blast-resistant variety developed since 2015 by Miah et al. (2015) and Tanweer et al. (2015), was officially released in 2021. Among these varieties, MR 297, MR 220-CL2, and MR 219 are Malaysia's top three popular planted paddy varieties (MAFI 2019). However, these newly developed varieties' tolerance to drought is limited.

Drought resistance traits in rice were categorized into four categories, which are drought escape, drought avoidance, drought tolerance, and drought recovery (Susanto et al. 2019). Drought escape mechanisms can

be achieved through the involvement of rapid plant development by early flowering and a shorter vegetative stage to allow the completion of the full life cycle prior to a coming drought (Shavrukov et al. 2017). Meanwhile, drought avoidance can be carried out by cultivars that are able to decrease water loss, for instance, by leaf rolling and decreased stomatal conductance. This study aimed to characterize these newly commercial rice varieties on growth, physiological, molecular, and yield performance under limited water conditions. The performance of these varieties was compared to MR 219, a well-established and accepted variety by 85% of farmers in Malaysia in 2010, which has been used for over 20 seasons but has been identified as sensitive to drought stress (Kamarudin et al. 2020; Sunian et al. 2022). MR 219 may be high-yielding but is also susceptible to environmental changes and requires ample water with an average of 1,240 mm per season or 86.4 m³ ha⁻¹ day⁻¹ (8.64 mm day⁻¹) (MARDI 2002; Talei et al. 2013). Understanding the morphological and physiological changes of these varieties in response to limited water is vital for developing drought-tolerant varieties and maximizing rice productivity.

MATERIALS AND METHODS

PLANT MATERIAL, SOIL PROPERTIES AND PLANT ESTABLISHMENT

The seeds were collected from Haji Mad Nor Sdn. Bhd., Sekinchan, Selangor, Malaysia, DOA, and the National Rice Genebank located at MARDI Seberang Perai, Pulau Pinang. Three seeds of each variety were grown in a pot (35 cm width × 35 cm diameter × 38 cm height) containing approximately 17 kg of soil under the glasshouse. The soil type is a clay loam texture (29% sand, 21% silt, and 50% clay) with a pH of 6.1, 1.9% organic carbon, and contained 0.81% total N, 24 mg kg⁻¹ available P, and 15 mg kg⁻¹ available K from the east coast granary area in Peninsular Malaysia. The NPK fertilizer was applied at the rates of 1.5 g pot⁻¹ (140 kg ha⁻¹), 0.7 g pot⁻¹ (100 kg ha⁻¹), and 0.7 g pot⁻¹ (100 kg ha⁻¹) at 15, 50, and 70 DAS. Urea was top-dressed at a rate of 0.4 g pot⁻¹ (80 kg ha⁻¹) at 35 DAS, and standard rice growing practices were followed according to MADA (2015).

TREATMENTS AND EXPERIMENTAL DESIGN

The experiment was conducted as a factorial experiment design comprised of two water levels (well-watered and ten days of cyclic water stress or water limited) and ten rice varieties (MR 219, MR 220-CL2, MR 297, MRQ 76, Vietnam Hybrid, UKM RC2, UKM RC8, Putra 1, MR 303, and MR 307). MR 303, MR 307, UKM RC2, UKM RC8, and Putra 1 are among the newly released varieties from 2018 and 2021, while others, such as MR 219, MR 220-CL2, and MR 297, were commonly used by farmers in Malaysia. Well-watered treatment was maintained at 5 cm by irrigation throughout the rice cultivation periods. The cyclic water-stress treatment, or water limitation, commenced on the 30th DAS, where re-watering was made after ten days of withholding water (Berahim et al. 2019). The experiment was arranged in a Randomized Complete Block Design (RCBD) with three replications.

PLANT HEIGHT, TILLER NUMBER, CHLOROPHYLL CONTENT AND BIOMASS PARTITIONING

The measurement of plant height was done from the plant's base to the leaf tip, as described by Zain et al. (2014). The number of tillers per hill was calculated by a fully expanded tiller at 99 DAS. The chlorophyll content of the leaves was taken by a Minolta SPAD 502 Plus Chlorophyll Meter (Delta T, UK). Three plants were collected on harvesting day from each treatment. The plant sample was divided into leaves, culms, and grains and oven dried at 65 °C for three consecutive days before being weighted using a digital balance (QC35EDE-S Sartorius, Germany).

PHOTOSYNTHESIS, STOMATAL CONDUCTANCE AND TRANSPIRATION RATE

The measurement was performed on fully expanded young leaves on three plants for each treatment between 09:00 and 11:00 am at 99 DAS using an infrared gas analyzer Li-6400XT portable photosynthesis system (IRGA, Licor Inc., Lincoln, NE, USA). The CO₂ reference rate and photosynthetic photon flux density (PPFD) were set at 400 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and 1,000 $\text{mmol m}^{-2}\text{s}^{-1}$, respectively.

GENOTYPING OF SSR MARKERS

Seeds of ten different rice varieties were sterilized in 50% sodium hypochlorite (NaOCl) for an hour, then rinsed thoroughly and soaked in distilled water for 24 h. The seeds were then sown directly into peatmoss in planting trays at 25-30 °C. After 14 days, leaf tissue was harvested from each variety and stored in a 5 mL polypropylene conical tube at -80 °C.

Total genomic DNA was extracted from leaf tissues using the method described by Doyle (1991) with minor modifications: Approximately 250 mg of leaf tissue was ground to a very fine powder using a mortar and pestle as well as liquid nitrogen. The sample was then transferred into a 2 mL tube containing 1 mL of CTAB extraction buffer (CTAB (2% w/v), sodium chloride (1.4 M), EDTA (20 mM, pH 8.0), and Tris-HCL (100 mM, pH 8.0)). Five μL of 2-mercaptoethanol (0.5%) was added with 1 μL of RNase A (20 mg/mL) into the 2 mL tube and mixed by inversion. The mixture was then placed in a 65 °C water bath for 30 min. After cooling to room temperature, 1 mL of chloroform/isoamyl alcohol (24:1) was added. The samples were then centrifuged for 10 min at 10,000 rpm. The supernatant was transferred into a new tube containing 800 μL of chloroform/isoamyl alcohol (24:1) and centrifuged for 7 min at 10,000 rpm. The supernatant was transferred into a new tube containing 2/3 of the supernatant volume of ice-cold isopropanol. The DNA was left to precipitate for 30 min at -20 °C. The isopropanol was then discarded, and the pellet was washed twice with 70% ethanol. The pellet was allowed to dry for at least 30 min to let the ethanol evaporate. Finally, the DNA pellet was resuspended in 100 μL of nuclease-free water. The DNA yield and purity were assessed using a nanodrop spectrophotometer. Gel electrophoresis was used for further analysis using 1% gel stained with GelRed and run at 80V for 90 min in 1X TAE buffer. The gels were imaged and documented using the Gel Doc XR+ gel documentation system.

SSR markers associated with drought tolerance traits in rice were used to screen the ten local rice varieties. The SSR markers used were as follows: 1) marker RM260 (Bashier et al. 2018), 2) marker RM201 (Aboulila et al. 2015), 3) marker RM302 (Salunkhe et al. 2011), 4) marker RM212 (Salunkhe et al. 2011), 5) marker RM28048 (Susilowati et al. 2017), 6) marker RM511 (Shamsudin et al. 2016), 7) marker RM520 (Mehmood et al. 2021), 8) marker RM6703 (Barik et al. 2020), 9) marker RM170 (Barik et al. 2020) and 10) marker RM517 (Afiukwa et al. 2016).

A polymerase chain reaction (PCR) was performed using PCR Red Taq Mix in a 50 μL reaction mixture composed of 2X Taq master mix 25 μL , F/R primer (10 μM) 2 μL , <500 ng genomic DNA, and PCR grade water. The thermocycler was set under the following conditions: initial denaturation at 95 °C for 3 min, denaturation at 95 °C for 15 s, and extension at 72 °C for 30 s for 30 cycles. The PCR product was evaluated using gel electrophoresis on a 2% gel stained with GelRed and run at 90 volts for 60 min. The gels were imaged and documented using the Gel Doc XR+ gel documentation system.

The molecular weight of each band was determined by comparing their migration distance with a 100-base-pair DNA ladder. The band profiles for each SSR primer were scored for distinct and reproducible bands as present (1) or absent (0). The band size was measured using GelAnalyzer 19.1 (www.gelanalyzer.com) by Istvan Lazar Jr., PhD, and Istvan Lazar Sr., PhD, CSc. Each gel measurement used the 100-kb ladder inserted as a reference measurement.

YIELD COMPONENTS

The weight of grain and the thousand grain weight were calculated after drying (72 h at 65 °C) using a digital balance. For each treatment, three uniform panicles were selected from each replication. Panicle length, the number of spikelets per panicle, and the filled grain proportion per panicle were manually counted according to Yoshida (1981).

$$\text{Filled grain percentage} = \frac{\text{Number of filled grain}}{(\text{Filled} + \text{Unfilled grains})} \times 100\%$$

STATISTICAL ANALYSIS

The analysis of the data was performed using the Statistical Analysis System (SAS 9.2), and significant differences among the treatments were compared using the Least Significant Different (LSD) at $P \leq 0.05$.

RESULTS

Under water limitation at 99 DAS, plant height, leaf dry matter, and panicle length were greater compared to well-watered; however, tiller numbers were reduced, with no significant effect on the relative chlorophyll content, culm dry matter, days of panicle initiation, or days of harvest (Table 1). The rice varieties had a significant effect on the plant height. MR 297 was the shortest (72 cm), followed by MR 220-CL2, UKM RC2, UKM RC8, and Putra 1, which had comparable plant heights (85-91 cm), while MR 303, MR 307, and MRQ 76 had taller plant heights (96-101 cm) compared to MR 219 (84 cm) (Table 1).

As shown in Table 1, tiller number per hill was significantly reduced in Vietnam Hybrid, MR 303, and MR 307 (6-8 tillers), while MR 220 CL-2, UKM RC2, UKM RC8, MR 297, MRQ 76, and Putra 1 had comparable tiller numbers per hill (9-11 tillers) compared to MR 219 (11 tillers). Besides that, the rice varieties also significantly affected the panicle length. MRQ 76, Vietnam Hybrid, and MR 220-CL2 showed the shortest panicle length (23-24 cm), followed by MR 297, Putra 1, UKM RC2, UKM RC8, and MR 307 (25-28 cm), while the longest panicle length belonged to MR 303 (29 cm) compared to MR 219 (27 cm) (Table 1).

Rice varieties also exerted a significant effect on the relative chlorophyll content at 99 DAS (Table 1). MRQ 76 significantly had the lowest relative chlorophyll content compared to MR 219 and could be harvested earlier with Vietnam Hybrid at 101-106 DAS. Following that, MR 220-CL2, which had the highest leaf dry matter production compared to MR 219, can be harvested about one week prior to MR 219 and the other varieties.

PHOTOSYNTHESIS RATE, STOMATAL CONDUCTANCE AND TRANSPIRATION RATE

At 99 DAS, under well-watered conditions, MRQ 76 and UKM RC2 showed a greater photosynthesis rate than MR 219. Following that, MR 297, Putra 1, MR 220-CL2, MR 303, UKM RC8, and MR 307 were the lowest in Vietnam Hybrid compared to MR 219. Meanwhile, under water-limited conditions, Putra 1 and UKM RC8 showed a greater photosynthesis rate than MR 219, followed by MR 297, UKM RC2, MR 220-CL2, MR 303, MRQ 76, and MR 307, and the lowest were found in Vietnam Hybrid (Figure 1(a)).

Under well-watered conditions, MR 220-CL2 had greater stomatal conductance than MR 219, followed by UKM RC2, MR 297, Putra 1, MR 303, UKM RC8, MR 307, MRQ 76, and the lowest in Vietnam Hybrid. Interestingly, under water-limited conditions, Putra 1 showed greater stomatal conductance than MR 219, followed by MR 297, MR 220-CL2, UKM RC8, UKM RC2, MR 303, MR 307, Vietnam Hybrid, and the lowest in MRQ 76 (Figure 1(b)).

Under well-watered conditions, the transpiration rates of MR 220-CL2, MR 303, Putra 1, UKM RC2, and MR 297 were comparable to MR 219, followed by UKM RC8, MR 307, MRQ 76, and the lowest in Vietnam Hybrid. In addition, under water limitations, Putra 1 had an improved transpiration rate compared to MR 219. The transpiration rate of MR 219 was comparable with MR 220-CL2 and MR 297 and was reduced in MR 303, UKM RC8, UKM RC2, MR 307, Vietnam Hybrid, and the lowest in MRQ 76 compared to MR 219 (Figure 1(c)).

SSR ANALYSIS

Based on the gel images, SSR markers RM201, RM302, RM212, RM28048, RM511, RM520, RM6703, and RM170 were present in all rice genotypes (Figure 2; Tables 2 & 3). SSR markers RM260 and RM517 showed a discernible band for all genotypes except in Vietnam Hybrid and MR 307, respectively (Figure 2; Tables 2 & 3). RM260, RM212, and RM28048 showed heterozygous alleles in MR 219, MR 303, and MR 220-CL2, respectively, while RM302 showed heterozygous alleles in two genotypes, MR 219 and MR 303 (Figure 2 and Tables 2 & 3).

TABLE 1. Agronomic and physiological traits of *Oryza sativa* L. under cyclic water stress from different varieties

Treatments	Plant height (cm)	Tiller number per hill	Panicle length (cm)	Relative chlorophyll content (SPAD unit)	Leaves dry matter (g)	Culm dry matter (g)	Total shoot dry matter (g)	Days of panicle initiation	Days of harvest
<u>Water Level</u>									
Well-watered	83.13±2.1 ^b	10±0.4 ^a	25.42±0.4 ^b	44.62±0.6 ^a	19.65±1.6 ^b	37.93±1.1 ^a	57.58±2.1 ^a	80±0.9 ^a	116±1.2 ^a
Water limited	92.90±2.4 ^a	9±0.4 ^a	27.68±0.4 ^a	45.82±0.6 ^a	23.19±1.1 ^a	36.83±1.5 ^a	60.02±2.3 ^a	81±1.0 ^a	117±1.3 ^a
<u>Rice Varieties</u>									
MR 219	84.83±3.4 ^{cd}	11±1.9 ^a	27.18±1.3 ^{bed}	44.73±1.3 ^{ab}	18.68±3.7 ^{bc}	41.73±3.7 ^a	60.41±5.2 ^a	84±1.6 ^{ab}	122±0 ^a
MR 220-CL2	85.25±2.6 ^{cd}	9±0.9 ^{abc}	24.17±0.2 ^{cd}	44.30±1.0 ^{abc}	29.41±3.0 ^a	38.84±2.9 ^a	68.25±5.9 ^a	79±0 ^c	114±0 ^d
MR 297	72.58±2.7 ^e	10±0.5 ^a	25.87±1.1 ^{de}	47.13±0.6 ^a	14.01±1.8 ^c	35.17±2.7 ^a	49.18±1.1 ^a	82±0.6 ^{bc}	120±0.4 ^b
MRQ 76	101.42±6.6 ^a	11±0.7 ^a	23.37±0.3 ^f	40.58±1.2 ^c	24.41±6.0 ^{ab}	33.57±3.3 ^a	57.97±7.7 ^a	79±2.4 ^c	106±1.0 ^c
Vietnam Hybrid	76.42±3.1 ^{de}	6±0.5 ^d	24.13±0.7 ^{ef}	42.88±2.2 ^{bc}	20.37±2.0 ^{bc}	35.68±1.5 ^a	56.05±3.4 ^a	68±0 ^d	101±0 ^f
UKM RC2	85.17±2.4 ^{cd}	9±0.9 ^{abc}	27.98±0.7 ^{abc}	45.98±0.7 ^{ab}	19.88±2.4 ^{bc}	31.51±2.5 ^a	51.39±4.0 ^a	82±1.2 ^{bc}	120±0.4 ^b
UKM RC8	85.58±6.7 ^{cd}	9±0.8 ^{abc}	28.55±0.2 ^{ab}	45.92±0.7 ^{ab}	20.76±2.4 ^{bc}	39.97±2.2 ^a	60.72±3.2 ^a	83±0.1 ^{ab}	119±0.6 ^c
Putra 1	91.17±3.5 ^{bc}	11±0.4 ^a	26.67±0.5 ^{cd}	45.60±1.5 ^{ab}	20.32±1.7 ^{bc}	39.62±2.5 ^a	59.94±3.1 ^a	86±0.7 ^a	122±0 ^a
MR 303	96.33±4.0 ^{ab}	7±1.0 ^{cd}	29.18±0.8 ^a	47.07±0.4 ^a	23.12±2.9 ^{ab}	38.93±4.9 ^a	62.05±7.0 ^a	83±1.0 ^{ab}	120±0.4 ^b
MR 307	101.42±5.8 ^a	8±0.8 ^{bed}	28.45±1.1 ^{abc}	48.0±1.7 ^a	23.25±2.4 ^{ab}	38.77±1.96 ^a	62.02±3.9 ^a	84±1.2 ^{ab}	120±0.4 ^b
<u>F-test</u>									
Water level	0.0001	0.1953	≤0.0001	0.1528	0.0388	0.5697	0.4183	0.3832	0.2615
Varieties	≤0.0001	0.0126	≤0.0001	0.0112	0.0298	0.3583	0.2375	≤0.0001	≤0.0001
Water level x varieties	0.2240	0.9968	0.0856	0.8841	0.0579	0.5675	0.6553	0.8308	0.2695
CV	9.89	24.26	5.80	7.08	29.86	19.79	19.65	3.78	0.78

*Mean values followed by similar letters within a column were not significantly different at $P \leq 0.05$ by the LSD test. CV: Coefficient of variation

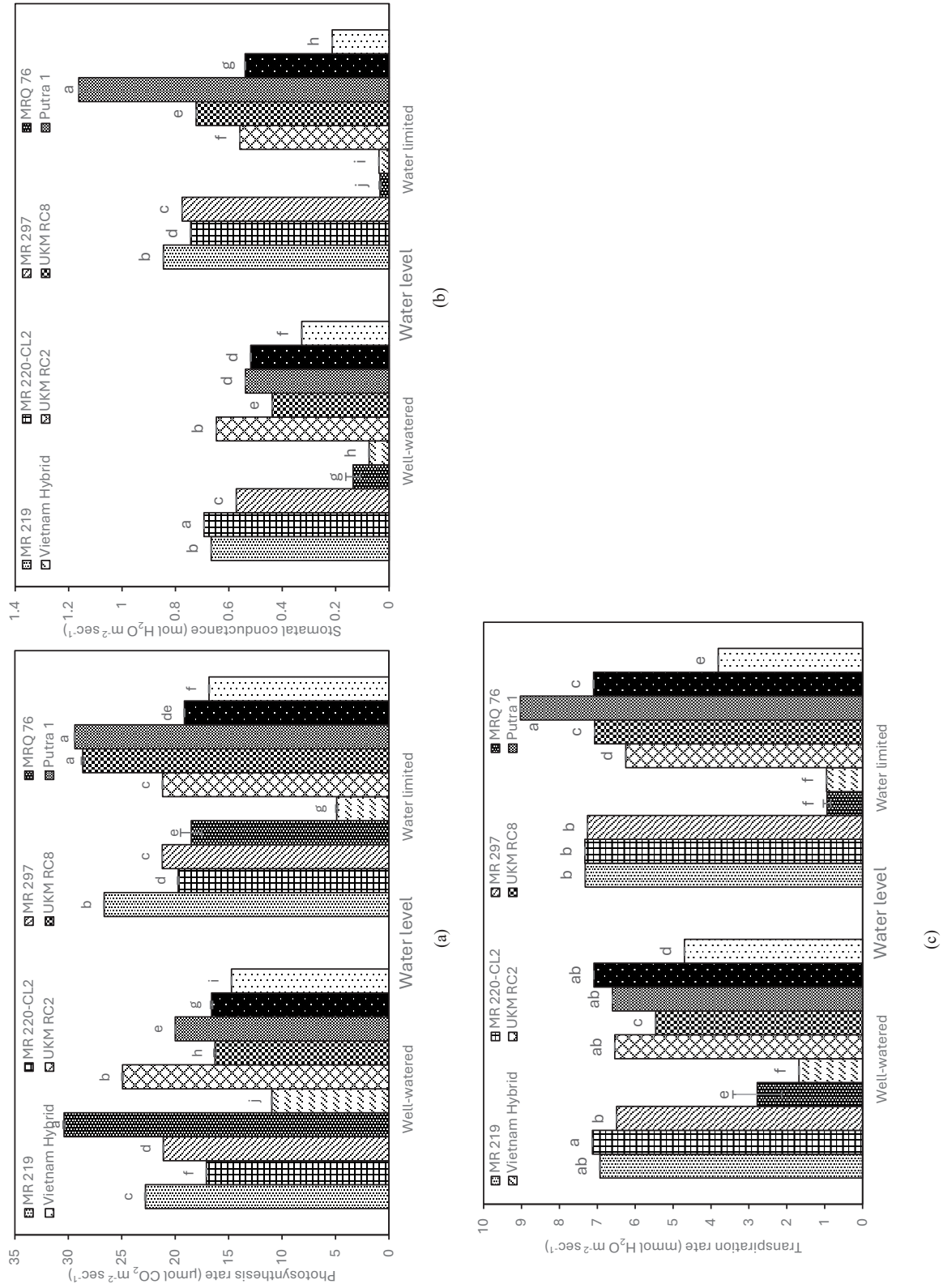


FIGURE 1. Photosynthesis rate (a), stomatal conductance (b) and transpiration rate (c) on different water levels from different rice varieties at 99 DAS

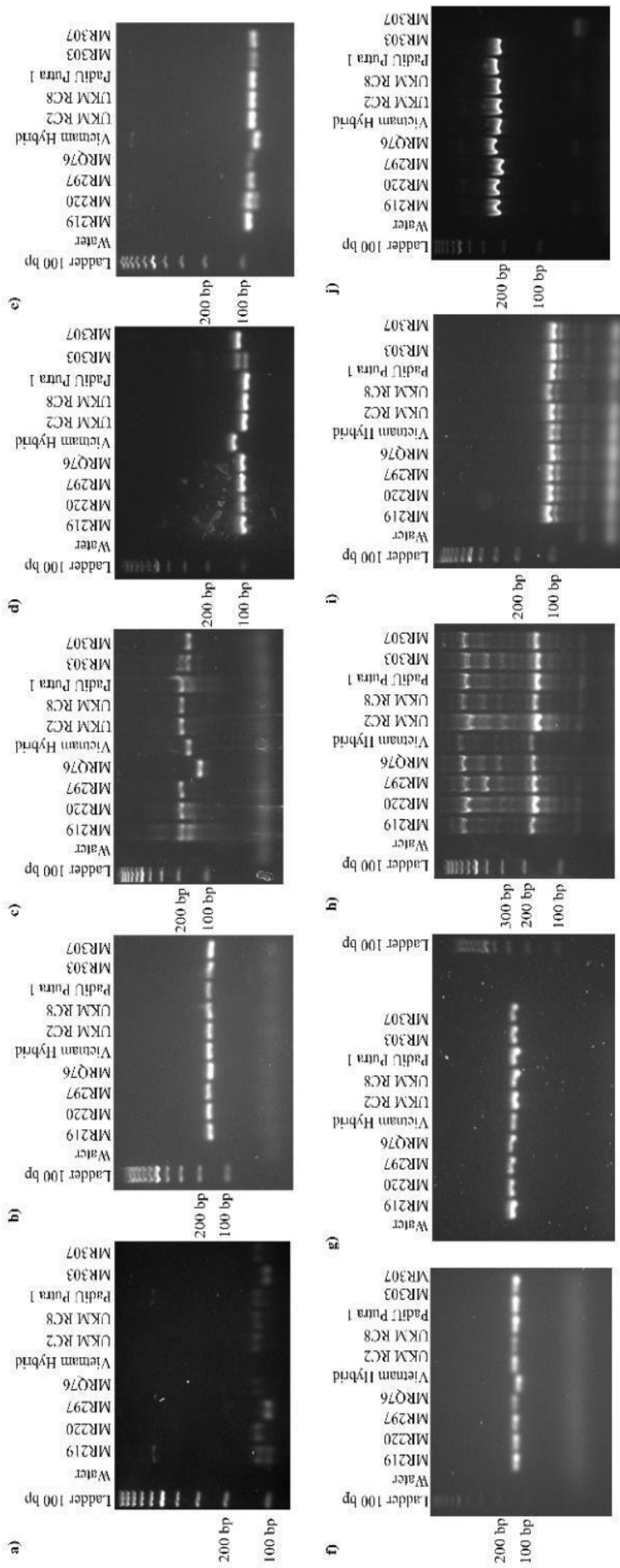


FIGURE 2. DNA profile of SSR markers associated with drought tolerance traits for 10 rice varieties (MR 219, MR 220-CL2, MR 297, MRQ 76, Vietnam Hybrid, UKM RC2, UKM RC8, Putra 1, MR 303, MR 307) in 2% gel stained with GelRed. a) marker RM260 associated with deep root dry weight and grain yield per plot b) marker RM201 associated with increased root length and drought tolerance c) marker RM302 associated with leaf rolling and leaf drying d) marker RM212 associated with leaf rolling and leaf drying e) marker RM28048 associated with panicle per plant, flag leaf length, panicle length, and percent seed set f) marker RM511 associated with increased harvest index, biomass yield, plant height, and number of days to flowering g) marker RM520 associated with leaf dry weight, stem dry weight, total shoot dry weight, and leaf drying score h) marker RM6703 associated with effects on days to 50% flowering in stress, chlorophyll A and relative chlorophyll content i) marker RM170 associated with grain yield per plots, number of productive tillers, panicle length, drought score, spikelet fertility j) marker RM517 associated with days to 50% flowering

TABLE 2. The presence of SSR marker visible by band

Rice varieties	RM260	RM201	RM302	RM212	RM28048	RM511	RM520	RM6703	RM170	RM517
MR 219	1	1	1	1	1	1	1	1	1	1
MR 220-CL2	1	1	1	1	1	1	1	1	1	1
MR 297	1	1	1	1	1	1	1	1	1	1
MRQ 76	1	1	1	1	1	1	1	1	1	1
Vietnam Hybrid	0	1	1	1	1	1	1	1	1	1
UKM RC2	1	1	1	1	1	1	1	1	1	1
UKM RC8	1	1	1	1	1	1	1	1	1	1
Putra 1	1	1	1	1	1	1	1	1	1	1
MR 303	1	1	1	1	1	1	1	1	1	1
MR 307	1	1	1	1	1	1	1	1	1	0

* 1 indicates presence, 0 indicates absence

TABLE 3. Size of band (bp) for each SSR marker with comparison to a 100 bp DNA ladder

Rice varieties	RM260	RM201	RM302	RM212	RM28048	RM511	RM520	RM6703	RM170	RM517
MR 219	127/115	156	173/152	114	111	134	290	175	122	214
MR 220-CL2	127	156	173	114	111/104	134	290	167	118	214
MR 297	115	156	173	114	108	134	290	167	118	197
MRQ 76	127	156	128	114	108	134	290	167	118	229
Vietnam Hybrid	0	156	152	129	104	127	250	175	118	197
UKM RC2	127	156	173	114	108	134	290	167	118	197
UKM RC8	127	156	173	114	108	134	290	167	122	197
Putra 1	127	156	173	114	108	134	290	167	118	229
MR 303	115	156	173/152	126/114	108	134	290	167	118	197
MR 307	127	156	152	126	108	134	290	167	118	0

YIELD COMPONENT OF RICE VARIETIES UNDER WELL-WATERED AND WATER-LIMITED CONDITIONS

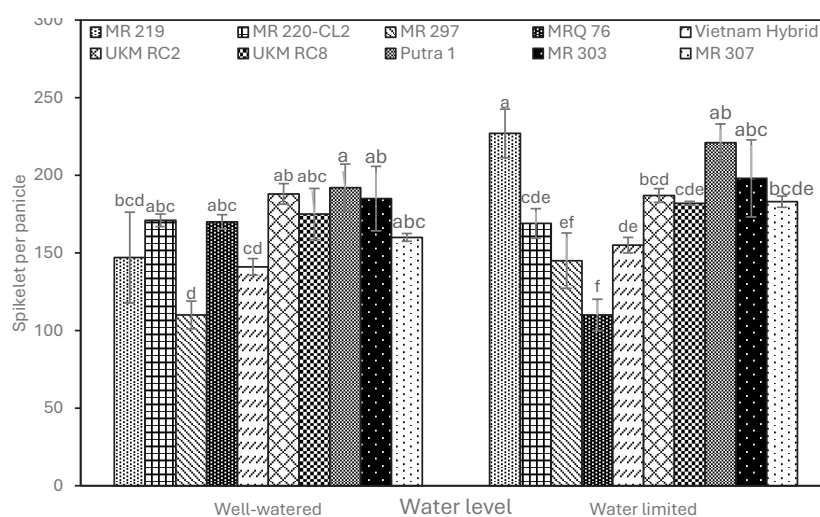
Rice varieties had a significant effect on the yield component traits at different water levels. Under well-watered conditions, Putra 1 showed the greatest spikelets per panicle compared to MR 219 (Figure 3(a)). Meanwhile, under water limitation, spikelet per panicle was comparable between Putra 1 and MR 303, with MR 219, and was significantly reduced in UKM RC2, MR 307, UKM RC8, MR 220-CL2, Vietnam Hybrid, MR 297, and the lowest in MRQ 76 compared to MR 219 (Figure 3(a)).

Interestingly, under well-watered conditions, Vietnam Hybrid and MR 220-CL2 (83-84%) had the highest filled grain percentage as compared to MR 219 (67%). Similarly, under water limitations, Vietnam Hybrid and MR 220-CL2 (88-89%) had the highest filled grain percentage, followed by MR 303, UKM RC8, MR

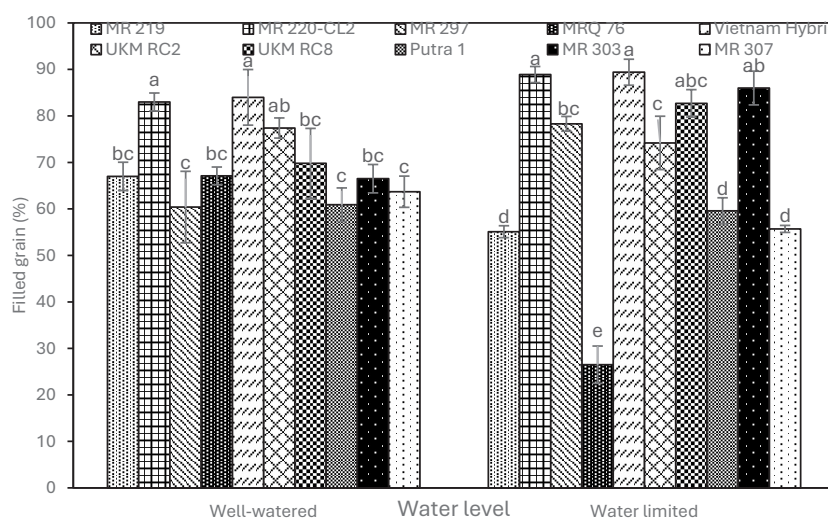
297, and UKM RC2 (74-86%), as compared to MR 219 (55%). MRQ 76 had the lowest percentage of filled grain (26.5%) under water limitations (Figure 3(b)).

Under well-watered and water-limited conditions, the result showed MR 307 had the greatest thousand grain weight (25 g) compared to MR 219 (21 g). MRQ 76 had the lowest (13 g) thousand grain weight under water limitations among the varieties (Figure 3(c)).

As can be seen, rice varieties significantly affected the total grain weight per pot under different water levels (Figure 3(d)). Under well-watered conditions, Putra 1 and MR 307 showed the highest (72-75 g) total grain weight per pot compared to MR 219. The other seven varieties, which were UKM RC2, UKM RC8, MRQ 76, MR 303, MR 220-CL2, Vietnam Hybrid, and MR 297, had comparable (41-66 g) total grain weight per pot with MR 219 (53 g). Meanwhile, under water limitations, it can be observed



(a)



(b)

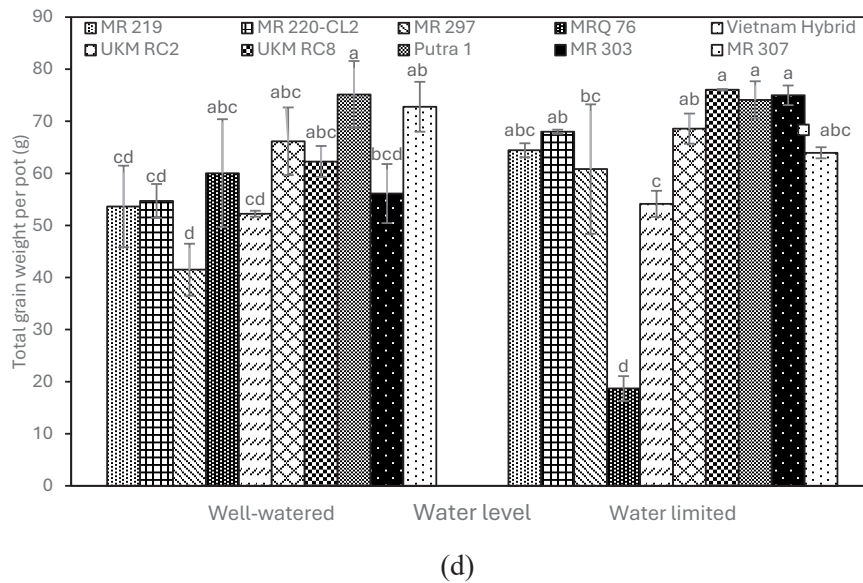
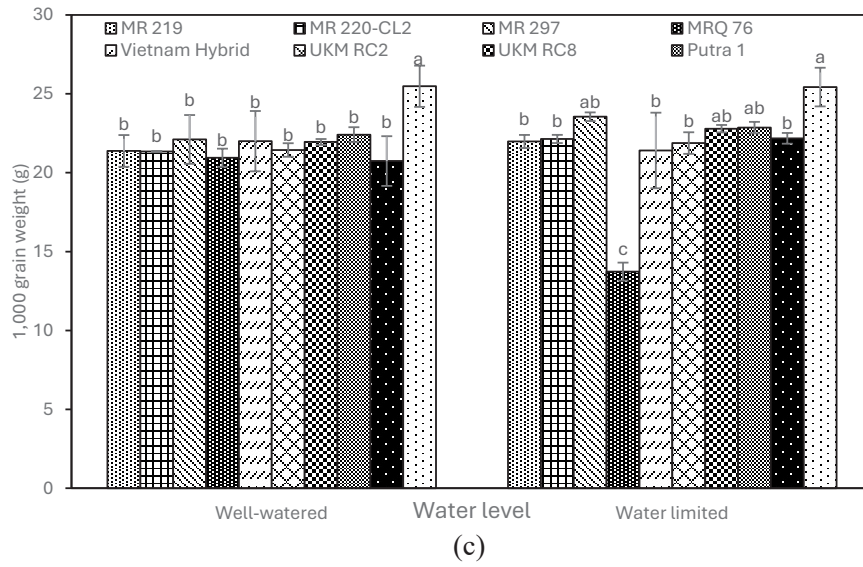


FIGURE 3. Spikelets per panicle, filled grain percentage, thousand grain weight and total grain weight per pot under well-watered and water limited conditions from different rice varieties

that the total grain weight per pot showed a significant difference among rice varieties. The total grain weight per pot of eight varieties, which were UKM RC8, MR 303, Putra 1, UKM RC2, MR 220-CL2, MR 307, MR 297, and Vietnam Hybrid, was comparable (54-76 g) with MR 219 (64 g) and the lowest in MRQ 76 (18 g).

RELATIONSHIP BETWEEN AGRONOMIC, PHYSIOLOGICAL AND YIELD TRAITS

In this study, plant height was positively correlated with culm dry matter, total shoot dry matter, panicle length, and spikelet per panicle (Table 4). Varieties

with longer panicles also have greater plant height. The result indicated that days of harvest for rice correlated well with chlorophyll content, photosynthesis, stomatal conductance, and transpiration rate, implying that senescence occurred in the early maturity variety. Besides that, days of harvest had a positive correlation with panicle length, spikelet per panicle, thousand grain weight, and total grain weight per pot. The results in Table 4 showed that there was a positive and highly significant correlation between total grain weight per pot with panicle length, and spikelet per panicle. In general, high dry matter production in the leaves would be displayed in greater total grain weight per pot as well.

TABLE 4. Pearson's correlation coefficients matrix among agronomic, physiological and yield traits

Var	PH	TN	PHO	STM	TR	RCC	LDM	SDM	TDM	DPI	DOH	PL	SPP	FGP	TGW	TPW
PH	1.0															
TN	-0.04	1.0														
PHO	0.24	0.40**	1.0													
STM	-0.08	0.23	0.59***	1.0												
TR	-0.07	0.19	0.54***	0.94***	1.0											
RCC	0.17	-0.03	0.01	0.29*	0.34**	1.0										
LDM	0.32	0.07	0.08	-0.04	-0.05	-0.11	1.0									
SDM	0.10*	0.17	-0.04	0.12	0.13	0.22	0.24	1.0								
TDM	0.28*	0.15	0.03	0.04	0.04	0.07	0.80***	0.77***	1.0							
DPI	0.23	0.33*	0.54***	0.54***	0.61***	0.45**	-0.02	0.26*	0.15	1.0						
DOH	0.04	0.23	0.48***	0.70***	0.81***	0.46**	-0.09	0.16	0.04	0.81***	1.0					
PL	0.34**	-0.01	0.14	0.29*	0.36**	0.42**	0.08	0.12	0.12	0.39**	0.56***	1.0				
SPP	0.31*	0.15	0.33*	0.44**	0.44**	0.14	0.28*	0.15	0.27*	0.29*	0.38**	0.64***	1.0			
FGP	-0.29*	-0.27*	-0.21	0.13	0.17	-0.005	0.20	-0.09	0.07	-0.42**	-0.13	0.05	0.12	1.0		
TGW	-0.04	0.01	-0.001	0.25*	0.31*	0.36**	0.08	0.06	0.09	0.10	0.38**	0.43**	0.35**	0.4**	1.0	
TPW	0.14	0.08	0.24	0.41**	0.46**	0.21	0.38**	0.06	0.29*	0.23	0.47**	0.51***	0.66***	0.37**	0.6***	1.0

*Var: Variable, PH: Plant height, TN: Tiller number, PHO: Photosynthesis rate, STM: Stomatal conductance, TR: Transpiration rate, RCC: Relative chlorophyll content, LDM: Leaf dry matter, SDM: Culm dry matter, TDM: Total shoot dry matter, DPI: Days of panicle initiation, DOH: Days of harvest, PL: Panicle length, SPP: Spikelet per panicle, FGP: Filled grain percentage, TGW: Thousand grain weight, TPW: Total grain weight per pot, *P<0.05; **P<0.01; ***P<0.001

DISCUSSION

In the present study, under water-limited treatment, plant height and panicle length showed improvement, probably due to the high accumulation of sugars, starch, and redistributed resources that promoted growth recovery and adaptation to water stress (Dien, Mochizuki & Yamakawa 2019). Similarly, under drought stress, Darmadi et al. (2021) found a 5.6% increment in plant height during the vegetative stage and a 0.4% increment during the reproductive stage of rice varieties. Plant height was determined by both genetic and environmental factors that affect lodging, biomass, and yield (Wu et al. 2022). In this study, different varieties were used to determine the plant height, which ranged from 72-101 cm. Varieties with plant heights below 110 cm were classified as semi-dwarf varieties (Elixon et al. 2017). MR 297 was found to be the shortest variety (72 cm) in this study. Shorter plant height is one of the preferable criteria for selection of the variety in rice cultivation to prevent lodging (Dorairaj & Govender 2023).

In this study, MR 220-CL2 is one of the varieties that was harvested earlier on 114 DAS than MR 219. Hashim et al. (2022) also found that MR 220-CL2 has a short plant maturity that can be harvested earlier, between 95 and 105 days. Besides that, Vietnam Hybrid and MRQ 76 varieties were also harvested earlier, at 101-106 DAS, than MR 219. Early maturity variety is an important trait for drought escape mechanisms and a suitable choice when drought is predictable (Pantuwan et al. 2002). In drought-prone areas, short-duration varieties were commonly used, in which some varieties reached maturity after 80 days (Khush 1997). Such varieties usually escape terminal drought, but they are not necessarily drought-resistant (Farooq et al. 2009). Besides that, Vietnam Hybrid and MRQ 76 had lower chlorophyll content due to less accumulation of chlorophyll at maturity (Yilmaz & Gokmen 2016). When reaching the maturity period of 101-106 DAS, both Vietnam Hybrid and MRQ 76 induced a low sink demand in the leaves, reduced stomatal conductance, and a reduced transpiration rate. Pan et al. (2017) showed that low sink demand decreased leaf photosynthesis due to sink feedback regulation.

In this study, Putra 1 and UKM RC8 had a greater photosynthesis rate, stomatal conductance, and transpiration under water stress. In response to water deficit stress, ion- and water-transport systems across membranes function to control turgor pressure changes in guard cells and stimulate stomatal closure (Osakabe et al. 2014). Genotypes with taller heights had greater overall plant size, intercepted more light, increased

transpiration, and consequently reduced plant water status (Kamoshita et al. 2004). In a water-stress plant, a higher transpiration rate implies the maintenance of leaf moisture and leaf area for the continuity of photosynthetic activity and the production of assimilate, which is significantly connected with high carbohydrate production (Cabuslay, Ito & Alejar 2002). Zhao et al. (2020) also obtained a higher photosynthesis rate for winter wheat under mild water stress.

In this study, we examined a combination of several SSR markers. SSR markers RM 201, 302, 212, 28048, 511, 520, 6703, and 170 were present in all rice genotypes. This indicates the markers are widespread and likely not directly linked to the specific drought tolerance trait in our experimental setting. The lack of variation in the SSR marker was due to the similar sets of germplasm used in the experiment (Freeg et al. 2016). On the other hand, the RM 260 marker was absent in the Vietnam Hybrid genotype, while the marker RM 517 was absent in the MR 307 genotype. These observations suggest potential variations or genetic differences in these genotypes. The presence of heterozygous alleles for specific markers could also potentially contribute to the variation in the expression of the associated drought-tolerant trait. These heterozygous allele patterns should indicate either a seed mixture or true heterozygosity remains in these cultivars (Xu, Beachell & McCouch 2004). The presence and absence of SSR markers, along with the provided traits, provide valuable insight into the genetic architecture underlying the desired drought tolerance trait in rice. The selection of markers used within this study is also subjected to numerous traits that serve as potential markers for marker-assisted selection breeding. Information on the genetic relatedness of germplasm could be used to select parental genotypes to broaden genetic material and improve rice varieties (Pathaichindachote et al. 2019).

Under water stress conditions, the early maturity varieties in this study, which were MRQ 76, Vietnam Hybrid, and MR 220-CL2, had a reduction in panicle length and spikelet per panicle, while total grain weight per pot was only enhanced in MR 220-CL2. The enhanced yield production in MR 220-CL2 may be related to the highest leaf dry matter production, as also shown by the positive correlation between the total grain weight per pot and the leaf dry matter. Therefore, the yield production of varieties in this study was closely related to dry matter accumulation. Cheng et al. (2022) stated that high sink capacity is the internal driving force of high rice grain yield and can improve the photosynthetic capacity of

rice at the grain filling stage. MR 220-CL2 also had an enhanced filled grain percentage, showing an efficient process of assimilate partitioning into the grain.

Under well-watered conditions, the total grain weight per pot of Putra 1 and MR 307 was enhanced compared with MR 219. Another variety, UKM RC2, UKM RC8, MRQ 76, MR 303, MR 220-CL2, Vietnam Hybrid, and MR 297, were comparable with MR 219. The enhanced grain weight per pot of MR 307 was contributed partly by longer panicle length in this variety, while Putra 1 had the highest number of tillers and an enhanced photosynthesis rate. In this study, there was a positive correlation between the total grain weight per pot and the days of harvest. Therefore, the days of harvest are a critical factor that will affect yield production, especially for the late-mature variety. In this study, Putra 1 had a longer duration to harvest (122 days), produced more tillers per hill, and improved grain weight per pot. A longer period of maturity resulted in more days available for canopy photosynthesis, contributing to higher biomass production and thus higher crop yield (Yang et al. 2019).

CONCLUSIONS

The outcomes of the current study have shown that, among all the varieties, MR 297 had the shortest plant height. The local Malaysian variety, MR 220-CL2, had a short maturity period, which can be harvested one week prior to MR 219, a shorter panicle length, and an enhanced filled grain percentage, as well as a comparable grain weight per pot with MR 219. The newly released varieties tested in this study, which are Putra 1 and MR 307, had an enhanced total grain weight per pot under well-watered conditions. There was slightly enhanced total grain weight per pot of UKM RC8, Putra 1, and MR 303, while total grain weight per pot of UKM RC2, MR 307, MR 297, and Vietnam Hybrid was comparable with MR 219 under water-limited conditions. Based on this finding, shorter plants, short maturation, and high yielding traits can be used as suitable criteria for drought stress varietal development without hampering the yield to avoid a dry period.

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