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Drought Tolerance of Five Selected Rice (*Oryza sativa* L.) Genotypes based on Morphological Physio-biochemical and Phenotypic Polymorphism

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ABSTRACT

Droughts are a significant cause of reduced rice yields in South Asia, particularly in rainfed areas, where they affect over 23 million hectares of cultivated land. The prediction is that 15 million hectares of flooded-irrigated rice crops in Asia will become drought-prone by the end of 2025. This present study used a Malaysian commercial established variety, the MR219, which possesses high yield potential but is drought sensitive, as a recurrent parent and was screened with four selected genotypes (IURON 6, IURON 18, MR219-4, and DULAR) as donor parents of drought tolerance. The objectives of the present study are to screen and identify the most potentially tolerant rice genotypes and identify link foreground markers that respond to drought among parental plants. The sensitivity of rice genotypes to drought stress was observed, where DULAR recorded the highest score of leaf rolling, which was grouped in the most sensitive genotypes, followed by MR219 as susceptible genotypes; meanwhile, MR219-4 and IURON6 were intermediate ranking, while IURON 18 was less sensitive (moderately resistant) genotypes according to leaf rolling score which was negatively correlated with stomatal conductance. It was found that out of 21 linked/functional markers that were screened for polymorphism, five (RM201, RM28148, RM511, RM3392, and RM520) showed polymorphism between the two parents. The RM511 functional marker linked to IURON18 was tested and polymorphic between the two parents. The rice variety IURON 18 was identified as a valuable source for creating drought-resistant rice through marker-assisted backcrossing.

Keywords: Drought, drought-tolerant, rice (*Oryza sativa*), genotypes, morphology, physio-biochemical attributes, polymorphism

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INTRODUCTION

Rice is a major cereal crop, and Asia dominates the global rice production and consumption (Shah et al., 2024). Droughts are a significant cause of crop yield reduction in rainfed farming lands across South Asia, impacting an estimated 23 million hectares of cultivated fields. The prediction that 15 million hectares of flooded-irrigated rice crops in Asia will become drought-prone by the end of 2025 (Islam et al, 2023). The pre-flowering and grain filling stages are considered critical periods for rice, during which the plant is particularly susceptible to drought stress, which can significantly impact yield and quality. Rice is a water-intensive crop that requires ample water throughout its lifecycle (Yadav et al., 2023).

Adopting drought-tolerant rice varieties is crucial for stabilising food grain supply and improving agriculture in areas facing water scarcity (Hassan et al, 2023). The selection of crop varieties for drought-prone areas focuses on identifying genotypes that exhibit both high yields and drought tolerance. It is challenging to solely rely on yield as the sole measure of drought tolerance due to the complex nature of drought tolerance itself, including its heritability (Site Noorzuraini et al., 2021). Alternatively, some morphophysiological parameters like stomatal conductance, leaf water potential, tiller number, and leaf rolling score. These indices, and others, help breeders assess the performance of genotypes under both stress and optimal conditions, allowing them to select lines that maintain yield even in drought-prone environments (Fen et al., 2015). Further, screening with drought-linked SSR (Simple Sequence Repeats) markers was selected for parental survey for the identification of polymorphic foreground. SSR (Simple Sequence Repeats) markers are DNA sequences that are easily identified in a genome, making them relatively straightforward to use in genetic studies (Das et al., 2024; Dixit et al., 2015; Vikram et al., 2016).

Molecular markers have been regarded as an important tool in genetic variability studies and supersede morphological markers in the sense that their analyses are not influenced by the environment (Akos et al., 2019b). Various DNA or molecular markers are available for genetic studies in rice; however, microsatellite or simplesequence repeat (SSR) markers have been employed widely because they exhibit highallelic variability and can be easily magnified by PCR. In addition, microsatellite markers are numerous, broadly spread throughout the rice genome, species-specific, codominant, and naturally polymorphic in contrast with other DNA markers (Chukwu et al., 2020; Miah et al., 2013).

These studies used a long-established elite rice variety in Malaysia, MR219, which was once commonly widely cultivated by the local rice farming community due to its high yield potential but possessed drought sensitivity as a recurrent parent. The rice variety was screened together with four other genotypes (IURON 6, 18, MR219-4 and DULAR) to act as donor parents for drought tolerance. Therefore, the present studies were designed with the following objectives:

1. To screen and identify potentially drought-tolerant rice genotypes based on morpho-physiological responses under drought stress; and

2. To identify polymorphic SSR markers associated with drought tolerance for potential donor selection.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted in the greenhouse condition with average temperature ranges from 30-to 35 °C and 22 °C to 24 °C during day and night, respectively. The average relative humidity ranges from 60% to 92%. The location of the experiment was in Ladang 16 (Latitude 2°59'31" N, longitude 101°43'59" E) at the Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The experiment was conducted under greenhouse conditions to ensure controlled environmental variables and uniform drought stress imposition.

Planting Materials

The selected rice genotypes in this study consisted of an elite variety (MR219), two lines that were collected from the International Upland Rice Observational Nursery (IURON): IURON6 and IURON18, one local advanced mutant line (MR219-4) from Nuclear Agency Malaysia, and one drought-tolerant control variety (DULAR) obtained from Rice Germplasm Centre MARDI Malaysia. Table 1 shows the five rice genotypes and their origin that were examined in this study. All genotypes were planted in a small plastic pot with a diameter of 30 cm and a height of 30 cm. Three seedlings at 21 days old were transplanted in each pot filled with the third quarter of Tok Yong soil series, that having a clay loam texture containing 20% sand, 47% silt and 33% clay with a pH of 5.3 and 2.2% organic carbon. The soil was obtained from the rice-growing area of Kemubu Agricultural Development Authority (KADA), Kelantan, Malaysia. Soil texture was determined using the hydrometer method, while soil pH and organic carbon content were analysed following standard laboratory procedures.

Table 1
Designation of rice genotypes was used for screening

Code	Rice Genotype	Designation
V1	IURON 6	BP1976B-2-3-7-TB-1-1
V2	IURON 18	CT 15679-17-1-1-2-3-M
V3	MR219 mutant	MR219-4
V4	DULAR	IRGC32561
V5	MR219	MR219-Recurrent parent

Basal fertilisation application was applied following Kamarudin et al. (2018) at an equivalent rate of N:P:K (150:60:50 kg ha^{-1}) proportionally adjusted for pot culture conditions. Weed control can be achieved through various methods, including manual weeding for narrow-leaf weeds and herbicide application for broadleaf weeds. Integrated Pest Management (IPM) practices, including targeted chemical control, were employed when necessary.

Drought Stress Treatments

Drought stress was imposed by withholding irrigation at the active tillering stage (25 DAT) until plants reached a leaf rolling score of 4 based on the IRRI Standard Evaluation System (SES). Soil moisture tension ranged between -40 and -60 kPa, measured using tensiometers (Takemura DM 8, Japan) installed at a depth of 30 cm (Table 2). After the beginning of the re-irrigation, the plants were kept to normal irrigation until harvest at 45 DAT.

Table 2
Description of leaf rolling score

Scale	Leaf Rolling Score	Rate
0	Leaves healthy	Highly resistant
1	Leaves start to fold	Resistant
2	Leaves folding (deep V-shaped)	Moderately resistant
3	Leaves fully cupped (U-shaped)	Moderately susceptible
4	Leaves margins touching (O-shaped)	Susceptible
5	Leaves tightly rolled	Highly susceptible

*Source: International Rice Research Institute (2014)

Soil Moisture Measurement

Soil moisture was measured by using a soil moisture meter (HH2) every day (11 days) while drought stress treatment was started at 25 DAT until 36 DAT.

Morphophysiological Parameter

Leaf Area

Leaf area of rice plant was sampled once at harvest, 45 DAT. The samples were taken and separated into two parts: culm and leaf. Leaf area was measured using a LI-COR 3100 area metre. Before the leaf area was measured, the rice leaves were ensured not to roll to avoid invalid readings. The measurement unit was expressed in centimetre square (cm^2).

Leaf Elongation Rate

Leaf elongation rate (LER, mm day^{-1}) of individual third leaves was calculated as the slope of the linear regression line through the data points within the phase of linear increase in leaf length, where L_f (mm) is the final leaf length and LER (mm day^{-1}) is leaf elongation rate. Elongation of the third leaf was measured.

Tiller Number

The number of tillers was manually counted on 45 days after transplanting. Observation and calculation of tiller numbers were continuously conducted until the paddy reached 45 days after transplant (DAT).

Leaf Rolling Score

The leaf rolling score was recorded at mid-day, 11 days after stress inducement using the scales by referring to SES for rice of IRRI (2014) (Table 2) from 0 (flat) to 5 (tightly rolled).

Shoot Dry Weight

The rice plant was harvested 45 days after transplant and was partitioned into two parts, namely leaf and culm. Samples were labelled according to the treatment and replication. Then, the separated parts of culm and leaf were put inside a brown paper bag and were dried in the oven at 60 °C for three days. After 3 days, shoot dry weight of the rice sample was taken with an electronic balance. The measurement unit was in grammes (g).

Stomatal Conductance

The fully developed leaf located at the third from the top was selected for the measurement of stomatal conductance using a porometer. Each treatment was taken at 11 days after the stress treatment. The readings taken were recorded manually, which were expressed in $mmolm^{-2}s^{-1}$.

Abscisic Acid (ABA) Concentration

After approximately four weeks of growth, segments of leaf and root tissue (~200 mg dry weight each) were harvested, rinsed to remove soil residues, flash-frozen in liquid nitrogen, then freeze-dried and stored in a -80 °C freezer. The samples were subsequently sent to Lancaster University for the quantification of abscisic acid (ABA) concentrations in leaves (on day 9 and at harvest) and roots.

Following the protocol described by McAdam et al. (2016), the leaf and root samples were bead-milled (Qiagen, Hilden, Germany) using 3 mm beads at 25 Hz/s for 3 minutes.

In short, 200 mg of tissue was placed into a 2 ml microcentrifuge tube, to which 400 µl of ethyl acetate was added, and the mixture was thoroughly homogenised. The homogenate was then centrifuged at 13,000 X g for 10 minutes at 4 °C to isolate the supernatant.

The samples were analysed using high-performance liquid chromatography coupled with electrospray ionisation mass spectrometry (HPLC-ESI/MS). An Agilent 100 HPLC system (Agilent Technologies, Santa Clara, CA, USA) was interfaced with an Applied Biosystems Q-TRAP 2000 mass spectrometer (Applied Biosystems, Foster City, CA, USA). Chromatographic separation was conducted at 35 °C using a 3 µm C18 column (100 X 2 mm). The mobile phase system consisted of two solvents: Solvent A and Solvent B, comprising acetonitrile and 0.1% formic acid, respectively. The gradient elution profile was programmed as follows: 5-60% Solvent B from 0 to 7.5 minutes, followed by 60-95% B from 7.5 to 10 minutes. The column was then flushed with 95% B for 3 minutes and subsequently re-equilibrated with 100% Solvent A for 10 minutes. The injection volume was 2 µl, and the flow rate was maintained at 4 ml/min.

Mass spectrometric detection was carried out using negative-mode electrospray ionisation (ESI). Instrument parameters were optimised using the Quantitative Optimisation tool in Analyst software by simultaneously infusing MS calibration standards via syringe pump and injecting standards into a 200 µl/min stream of a 1:1 mixture of Solvents A and B. The optimised settings were as follows: cone voltage, 40 V; capillary voltage, 3 kV; source temperature, 400 °C; desolvation gas flow rate, 900 L/h; and cone gas flow rate, 50 L/h.

Molecular Screening Method

DNA Extraction, PCR Conditions, and Electrophoresis Procedures

Healthy leaves were harvested from 14-day-old healthy seedlings. Immediately after collection, the leaves were carefully wrapped in aluminium foil, appropriately labelled, and then stored at -80 °C until DNA extraction. Total genomic DNA was extracted from leaf tissues using the method described by Doyle and Doyle (1990) with minor modification: Approximately 250 mg of leaf tissue was ground using a mortar and pestle as well as liquid nitrogen to a very fine powder. The powdered leaf samples were transferred into 2 ml micro-centrifuge tubes, and 1000 µl CTAB buffer and 3 µl of mercapethanol were incorporated into the tubes, respectively, and then the samples were shaken at 65 °C and at 500 rpm for 1 hour usingan Eppendorf thermo shaker. Subsequently, the samples were centrifuged at 500 rpm for 5 minutes with the aid of a refrigerated centrifuge machine. Thereafter, the total supernatants were moved into a new 1.5 ml tube and then 600 µl of chloroform: isomyl alcohol was incorporated and mixed by inverting. The samples were then centrifuged at 13,000 rpm for 5 mins in order to get a phase separation and then the upper phase was moved into a new 1.5 ml tube again. In addition, 600 µl of isopropanol

at 4 °C was incorporated and mixed carefully by inverting the tubes at least 50 times, and no vortex was used. The samples were then incubated for 30 minutes at -20 °C and centrifuged at 13,000 rpmfor 10 minutes. The supernatant was poured out, and the pellets were air-dried and rinsed by the addition of 600 µl of 75% ethanol 1 to 2 times until the white DNA fibre was seen. Finally, the DNA samples were subjected to 50 µl of TE buffer and 1 µl of RNase, and kept at -40 °C for future analysis. The quality of DNA was tested using NanoDrop2000 (Thermo Fisher Scientific Inc., USA), and the majority of the DNA samples were within the acceptable range of 1.8-2.0 for 260/280 and 2.0-2.22 for 260/230 (Jun-Yan et al., 2006).

The polymerase chain reaction (PCR) amplification was accomplished using thermal cycler (T100TM, Bio-Rad, UK) in 15 µl reaction and each individual component final concentrations were 7.5 µl Power Taq Master Mix (2x) (Bioteke Corporation, China), 4.5 µl nuclease-free water, 1 µl of 10 µM of forward and reverse primer and 1 µl and 1 µl of 70 ng genomic DNA, respectively. A touchdown PCR protocol was adopted as described by Shamsudin et al. (2016) for PCR amplification as follows: 94 °C for 5 minutes; followed by 10 cycles of 94 °C for 15 sec, 62 °C for 15 sec (decreasing 0.5 °C per cycle), then 72 °C for 15 seconds, followed by 30 cycles of 94 °C for 15 seconds, 52 °C for 15 seconds, then 72 °C for 15 seconds and, a final extension for 10 min at 72 °C followed by rapid cooling to 4 °C before analysis for foreground and background markers each. The entire process took a period of 1 hr 24 minutes.

The magnified PCR products were settled in a 3% w/v Metaphor Agarose gel with 10 µl gel dye (Gel view, 10 µl per 100 ml 1×TBE) (Bioteke Corporation, China) to determine amplicons in a 1×TBE buffered electrophoresis. The gel was carried out at 80 V for 1 hour. A 50 base pair ladder (GeneDireX, Inc., Taiwan, ROC) (reference ladder) appropriate for use in agarose gel electrophoresis as a standard molecular weight was adopted to detect the size of alleles of the magnified PCR products. Eventually, the bands were observed under ultraviolet light employing digital Molecular Imager® (GelDocTM XR, Bio-Rad Laboratories Inc., USA).

Genotyping of SSR Markers

SSR markers were selected based on previous reports of association with drought tolerance in rice. PCR amplification was performed following standard protocols and polymorphism information content (PIC) was calculated to evaluate marker effectiveness. The linked microsatellite markers, are employed to monitor the target locus which are polymorphic between the two parents, were used to determine the tightness of linkage (Hasan et al., 2015). Different alleles of the genotypes were scored using software (Image Lab 3.1) viz A and B. Allele scoring was performed using Image Lab 3.1 software with reference to a 50 bp molecular weight ladder to ensure accurate size determination, while the polymorphic

bands were only retained if they showed clear, reproducible differentiation between parental lines. Loci exhibiting inconsistent amplification or suspected null alleles across replicates were excluded from analysis to ensure scoring reliability.

Experimental Design and Statistical Analysis

The experiment was arranged in a completely randomised design (RCBD) with three replications, where each replication consisted of one pot containing three seedlings. In each replication, pots were arranged side by side with a spacing distance of 15 x 20 cm. Analysis of variance (ANOVA) was conducted for each parameter under investigation to assess the extent of variation attributable to genotypes, stress treatments, and their interaction. This analysis was performed using Statistical Analysis Software (SAS) version 9.4 to evaluate the degree of variability. The average, data span, relative standard deviation, and variability index were also determined for each parameter. Tukey's test was employed for mean comparisons, and correlations among traits were analysed using the CORR procedure in SAS.

RESULTS AND DISCUSSION

Soil Moisture

Figure 1 shows the changes in soil moisture over the period of soil drying. Drought stress conditions were observed once the soil moisture fell below 20% at day 11, after standing water had drained out. Meanwhile, during the treatment period, soil moisture in well-irrigated conditions was kept close to full volumetric saturation (100%) at all times. Plants were re-irrigated when check varieties reached the score of 4 for leaf rolling, and the soil moisture achieved below 20% showed that the genotypes experienced moderate drought stress during the experiment.

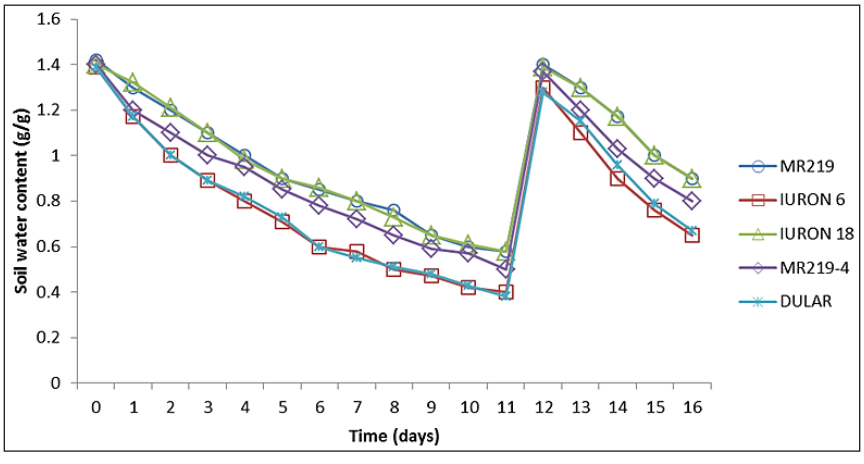

Figure 1. Development of soil water deficit and differences in soil moisture depletion rates among genotypes

Morphophysiological Traits

For the leaf rolling score, there was a statistically significant effect ($P<0.05$) of genotype, drought stress treatment, and their interaction. Similarly, data were recorded for leaf elongation rate, shoot dry weight, leaf area, stomatal conductance and leaf ABA concentration at day 9 drought stress was imposed. There was a significant difference for drought stress treatment ($P\leq0.05$) for leaf ABA concentration at harvest. Meanwhile, data were recorded with no significance of genotypes and their interaction of ABA concentration after re-irrigation. There were significantly different of genotype and drought stress treatment for root ABA concentration at day 9 drought stress was imposed; however, there was no interaction between genotype and treatment, similarly occurred for tiller number.

The reduction of tiller number, leaf elongation rate, leaf area, and shoot dry weight was detected under drought stress conditions compared to well-watered plants (Table 3 and 4). Leaf ABA concentration at day 9 after drought stress was imposed showed slightly higher values compared with well-watered plants. However, little increment was recorded in leaf ABA concentration at harvest, showing plants were not affected after re-irrigation. The value of stomatal conductance decreased until 90% under drought stress conditions, showing severe affected by drought stress (Table 3).

Percentage of Leaf ABA Accumulation Increment

Drought increases leaf ABA concentration by 183% at day 9 after drought stress was imposed (averaged across genotypes). IURON 6 and DULAR show the highest percentage of increment between well-watered and drought stress, meanwhile, IURON 18 showed the lowest increment percentage. On rewetting, leaf ABA concentrations were similar for both drought-stressed and well-watered plants. However, the percentage increment showed that IURON 6 and DULAR were significantly higher than IURON 18, MR219-4, and MR219. There is almost half as much (33%) increase in ABA concentration in the leaves of well-watered plants within a 4-day period between the drought cycle and harvest (Figure 2).

Leaf Rolling Score in Drought Stress

Leaf movement in rice was significantly affected by water stress. Leaf rolling reaction to drought stress was shown in Figure 3. According to the research, IURON 18 exhibited the least leaf rolling among the varieties, whereas DULAR recorded the highest leaf rolling rating on day 11 following the onset of drought stress. Leaf rolling scores were gradually increased at day 7 of drought stress imposition. Marked variations were observed among the genotypes and leaf rolling scores under drought stress conditions on day 8 after drought stress was applied. Plants were re-irrigated when a plant reached the score of 4 for leaf rolling, which was found at day 11.

Table 3
Means of leaf rolling score (for last day of drying cycle - day 9); leaf elongation rate; shoot dry weight; leaf area; stomatal conductance (day 9); leaf ABA concentration (day 9); leaf ABA concentration (at harvest); root ABA concentration; and tiller number under well-watered (WW) and drought stress (DS) conditions. Data are means ± SE for all irrigation treatments and all cultivars

Variable		IURON 6	IURON 18	MR219-4	DULAR	MR219
Leaf Rolling Score	WW	0.0±0.0	1.0±0.5	0.0±0.0	0.0±0.0	1.3±0.3*
	DS	3.5±0.6*	2.0±0.9	3.1±0.6*	5.0±0.0	3.4±0.4*
Leaf Elongation Rate (cm day^{-1})	WW	5.2±0.2	5.0±0.2	5.5±0.2	4.7±0.1	3.8±0.3*
	DS	3.0±0.4*	2.4±0.6*	2.8±0.4*	2.2±0.2*	2.6±0.2*
Shoot Dry Weight (g)	WW	5.9±0.3	3.8±0.2	5.5±0.3	6.1±0.5	3.8±0.2
	DS	3.9±0.4*	3.1±0.2*	3.7±0.4*	4.3±0.3	3.8±0.2
Leaf Area (cm^2)	WW	885.5±46.0	509.0±25.6*	763.5±46.0	1008.3±51.6	525.7±22.6*
	DS	512.9±10.5*	361.3±20.2*	451.9±10.5*	631.5±11.6*	415.5±26.2
Stomatal Conductance ($mmolm^{-2}s^{-1}$)	WW	216.6±17	114.9±24.8*	186.6±17	246.1±12.8	129.5±25.1*
	DS	54.6±17.4*	68.4±8.1*	61.6±17.4*	14.8±2.0*	60.6±12.2*
Leaf ABA Concentration - Day 9 ($ngg^{-1}dwt$)	WW	376.8±36.4.	332.1±38.7	439.0±33.5	251.2±24.5	439.0±32.6*
	DS	928.7±70.6	576.8±76.4*	1104.7±75.1	1028.7±150.6	921.0±97.8
Leaf ABA Concentration - at harvest ($ngg^{-1}dwt$)	WW	747.0±152.7	644.1±66.4	774.0±127.5	347.0±30.0	792.6±44.7
	DS	821.1±58.6	807.4±63.9*	802.1±86.5	562.1±53.9	844.3±34.6*
Root ABA Concentration - Day 9 ($ngg^{-1}dwt$)	WW	75.1±19.2	112.6±26.0	66.8±16.1*	117.0±16.7	55.1±12.8*
	DS	172.7±31.3*	155.1±30.0	131.8±30.0	278.0±32.1	128.7±13.3*
Tiller Number	WW	11±0.49	8.00±0.50*	11±0.49	19.00±1.17	10.00±0.3*
	DS	8.00±0.44	9.00±0.53	8.00±0.44	15.00±0.63*	7.00±0.74

Table 4
Drought tolerance ranking of genotypes

Scale	Rate	Leaf Rolling	Genotype
0	Highly Resistant	No symptoms of stress	
1	Resistant	No rolling	
2	Moderately Resistant	Partially rolled, unrolled in the evening	IURON18
3	Moderately Susceptible	Partially, unrolling in the late evening and early morning	IURON6, MR219-4
4	Susceptible	Complete, unrolling in the morning	MR219
5	Highly Susceptible	Like a tube; no unrolling in the morning	DULAR

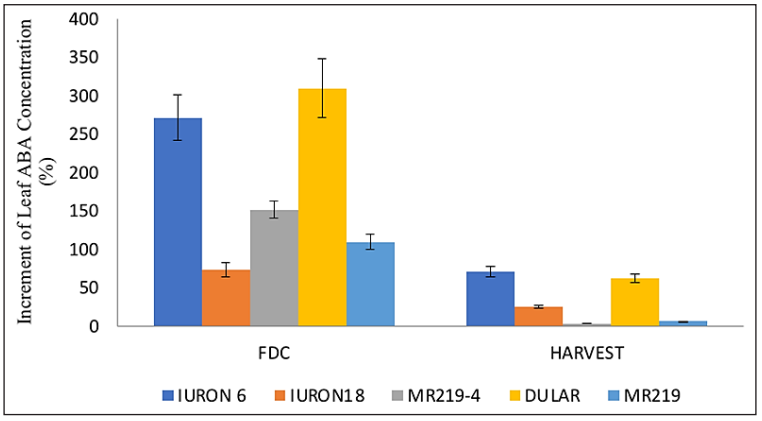

Figure 2. Percentage of leaf ABA accumulation increment between well-watered and drought stress at day 9, first drying cycle (FDC) and at harvest (after rewetting). Bars represent means ±SE of 3 replicates

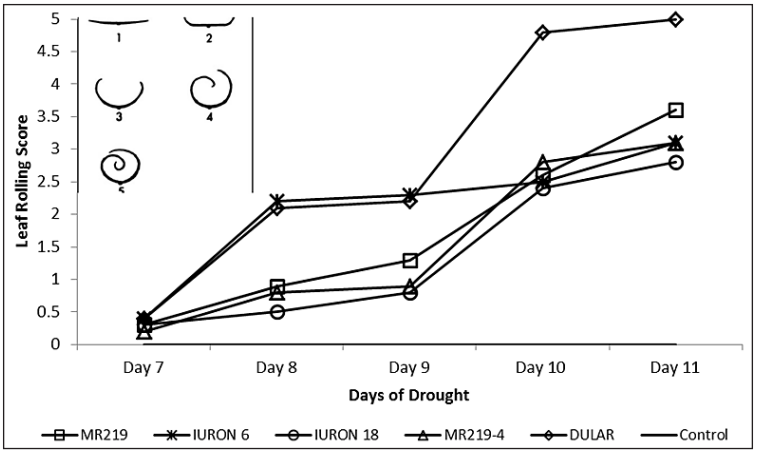

Figure 3. Leaf rolling measurements of all genotypes under drought conditions. Symbols represent means ±SE of leaf rolling scores

On the other hand, IURON 18 had the highest performance in drought tolerance ranking by moderately resistant, followed by IURON 6, and MR219-4 are moderately susceptible, and MR219 is susceptible rice to drought stress; meanwhile, DULAR is highly susceptible to drought stress. This indicated that IURON 18 had drought-tolerant properties which could be used as a donor for breeding purposes. A key indicator of drought in plants is leaf rolling, which is regarded as a crucial characteristic in rice breeding. Intense leaf rolling adversely affects plant growth, development, and grain production. However, moderate leaf rolling helps minimise water loss by decreasing the transpiration rate, thereby supporting plant survival under drought stress. Hence, regulating leaf rolling is a vital approach for enhancing rice yield during drought stress (Li et al., 2019). Because of its agricultural significance, leaf rolling has attracted considerable interest from plant researchers.

Relationship between Leaf Rolling and Stomatal Conductance

A negative linear relationship occurred between stomatal conductance and leaf rolling score, as shown in Figure 4. When the leaf rolling score increased, the stomatal conductance linearly decreased. Stomatal conductance decreased drastically with the increase in the degree of drought stress. The increase in leaf rolling score showed an increase in drought stress level. The equation for the negative linear relationship is shown below in Equation 1:

$$\text{Stomatal conductance} = -14.885x + 83.915 \text{ with } R^2 = 0.7067 \ (n = 15) \qquad [1]$$

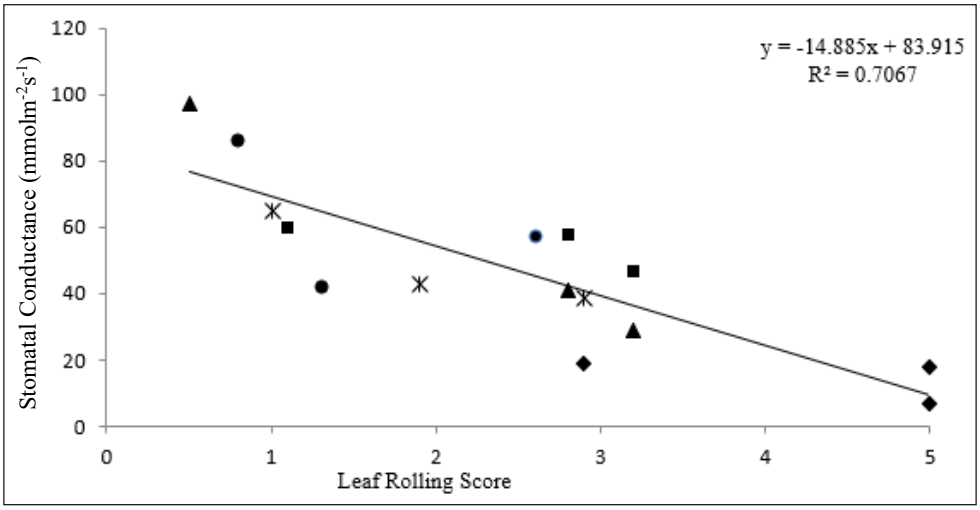

Figure 4. Relationship between leaf rolling and stomatal conductance (daily averages for three days. Star = IURON6; circle = IURON18; triangle = MR219-4; diamond = DULAR; square = MR219. Error bars omitted for clarity

Correlation between Morphological Variables in Response to Drought Stress

Leaf area showed a positive association with leaf elongation rate (r = 0.44) and tiller number (r = 0.86). At the same time, leaf elongation exhibited a positive relationship with leaf area (r = 0.44) and dry biomass (r = 0.36). However, leaf elongation rate is a poor predictor of important yield components (leaf area and dry mass). Meanwhile, leaf area is a better predictor of these yield components under drought stress. Tiller number was positively correlated with leaf area (r = 0.86) and dry biomass (r = 0.82). There was a positive correlation of dry biomass with leaf area (r = 0.94), leaf elongation rate (r = 0.36) and tiller number (r = 0.82) as shown in Table 5.

Table 5
Correlation table of morphological characteristics following drought exposure

	Correlations			
	Leaf Area (cm^2)	**LER ($cmday^{-1}$)**	**Tiller Number**	**Dry Biomass (g)**
Leaf area (cm^2)		0.44**	0.86**	0.94**
LER ($cmday^{-1}$)	0.44**		0.11	0.36**
Tiller number	0.86*	0.11		0.82**
Dry biomass (g)	0.94	0.36**	0.82**	

Note. * Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

Molecular Screening

In this study, five simple sequence repeat marker (SSR) were used to determine the polymorphism between MR219 as the recipient and other genotypes (IURON 6, IURON 18, MR219-4, DULAR) that will be chosen as the donor (Figure 5). Based on the gel images, the results showed IURON 18 distinguishing the polymorphism for five SSR foreground markers, namely RM201, RM28048, RM520, RM3392, and RM511. IURON18 will be selected as a donor parent because of carrying the character of interest. Foreground molecular markers showing polymorphism between the two parents, MR219 and IURON18, for the various target genes: RM511 ($qDTY_{12.1}$) is linked to increased harvest index, biomass yield, plant height and number of days to flowering, and RM520 ($qDTY_{3.1}$, $qDTY_{3.2}$) is associated with leaf dry weight, stem dry weight, total shoot dry weight, and leaf rolling score.

Another factor in selecting appropriate donors for developing drought-tolerant rice varieties was the identification of quantitative trait loci (QTL). According to Site Noorzuraini et al. (2021), pinpointing genomic regions associated with desired traits and performing indirect selection can be achieved without relying on complex phenotypic assessment. QTLs have been identified with significant and stable impacts on yield under drought conditions (Venuprasad et al., 2012) and have been found in various drought-tolerance related traits in rice (Akos et al., 2019a; Mohd Ikmal et al., 2019). Although drought resistance traits can be identified through QTL mapping, it remains necessary to evaluate and validate the effectiveness of these QTLs on grain yield under drought conditions in the field. For molecular screening, it was found that out of 21 linked/functional markers that were screened for polymorphism, five (RM201, RM28148, RM511, RM3392, and RM520) showed polymorphism between the two parents. The RM511 functional marker (Dixit et al. 2014) linked to IURON18 was tested and polymorphic between two parents. Although the use of functional/QTL-linked markers (RM511, RM520) may reduce null allele frequency due to conservation of primer-binding regions in drought-related genomic regions. This result corroborates the finding of Akos et al. (2019b), where their

improved varieties were developed and confirmed genotypically with drought tolerance QTLs ($qDTY_{12.1}$). Meanwhile, drought-tolerant alleles for QTL ($qDTY_{3.1}$ and $qDTY_{3.2}$) were confirmed using the functional marker RM520 (Singh et al. 2016) reported demonstrates a substantial positive contribution to grain yield under harsh drought stress in upland and lowland environments. $qDTY_{3.2}$ is one of the known Mega-QTLs (MQTL) and one of the large-effect QTLs was identified as the most precise and consistent across the environments and useful for Marker Assisted Selection (MAS), candidate gene identification and functional analysis (Kumar et al., 2014). This study reveals IURON 18 as the most promising genotype, showing superior performance in morphophysiological traits under drought environment in relation to parental polymorphism.

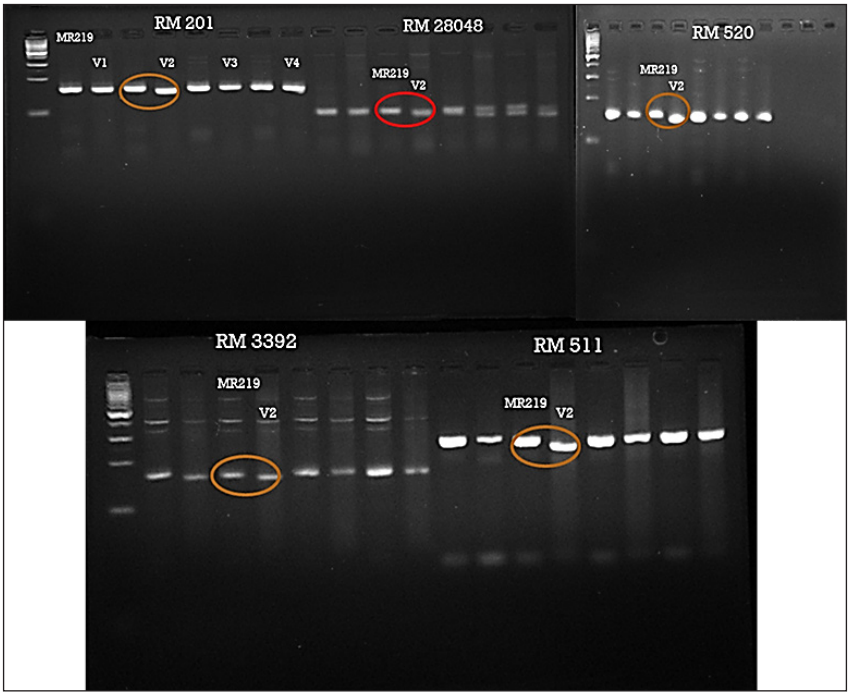

Figure 5. Screening of parental lines, IURON 6 (V1), IURON18 (V2), MR219-4 (V3), DULAR (V4), and recipient parent MR219, for polymorphism using some of the SSR markers linked with drought tolerance genes. Running on 3% metaphor agarose gel stained with midori green. M: 50bp Ladder

CONCLUSION

The study revealed substantial differences among the selected rice genotypes for the majority of the traits evaluated. In general, drought stress imposed significantly reduced leaf area, leaf elongation rate, shoot dry weight, and tiller number. In contrast, drought increases leaf ABA concentration by 183% at day 9 after drought stress was imposed

(averaged across genotypes). IURON 6 and DULAR show the highest percentage of increment between well-watered and drought stress, meanwhile, IURON 18 showed the lowest increment percentage. Leaf movement in rice plants was markedly influenced by drought stress. The present study showed that IURON 18 exhibited the least leaf rolling among the genotypes, whereas DULAR recorded the highest leaf rolling score at day 11 after drought stress was imposed. Moreover, IURON 18 exhibited the greatest performance on morphophysiological traits under drought environment in relation to parental polymorphism. Based on the results, we concluded that IURON18 was moderately resistant in drought tolerance ranking, followed by IURON6, MR219-4, and MR219, and DULAR were highly susceptible to drought stress.

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