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Identification of Drought Tolerant among MARDI Rice Varieties based on Morpho-Agronomic Traits and Drought Grain Yield QTLs

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ABSTRACT

The development of drought tolerant rice varieties in Malaysia is slow due to limited number of available donors for breeding program. Evaluation of the genetic resources is crucial to increase availability of the potential drought tolerant donors. This study revealed MR219, MR220, MR253, MR269, MRQ74, MRQ76, SIRAJ297, MARDI284, SEMPADAN303, SEBERNAS307 and MARDI308 showed outstanding performance under drought stress based on tiller number, panicle length, harvest index, % of filled grain and grain yield. The least square means of grain yield showed MR269 was the highest followed with MRQ76, with grain yield reduction difference between the two environments was 46.84% and 54.44%, respectively. The genotypic study observed three MARDI varieties which has more than one QTLs, namely MRQ74 ($qDTY_{3.2}$ and $qDTY_{11.1}$), MR253 ($qDTY_{2.3}$ and $qDTY_{3.2}$) and MRQ76 ($qDTY_{3.2}$, $qDTY_{2.3}$ and $qDTY_{11.1}$). The dendrogram based on morpho-agronomic traits observed MARDI284, MR269, MRQ746, MR253 and MRQ74 showed similarity with Dular. The dendrogram based on genotypic traits observed MRQ74 and MRQ76 showed similarity with Moroberekan. MRQ76 was identified as a potential drought tolerant variety as showed outstanding performance under drought stress and possessed three different QTLs namely $qDTY_{3.2}$, $qDTY_{2.3}$, and $qDTY_{11.1}$.

Introduction

Rice (*Oryza sativa*) is the foremost staple food and fulfill the nutritional requirement of about 30 million people in Malaysia. The rice production in Malaysia is mainly from rice granary areas with average production per hectare was 4.47 mt/ha (Radin Firdaus *et al.*, 2020). To date, there are 10 major rice granary areas with additions of 74 secondary granaries and 172 minor granaries (Siti Rahyla *et al.*, 2019). These granary areas are facilitated with well-developed farming system to fulfill the water requirements in the areas. Since, Malaysia is considered as a 'wet' continent as receives a rainfall at 250 centimeters a year

(Saw 2007), it is seemly shows that Malaysia is less affected with drought incidences. However, shifting in rainfall pattern during dry season and increased of earth's temperature demonstrated that Malaysia has begun experienced significant effects on climate change (Marshall 2007). The annual report of Malaysian Meteorological Department reported the warmest year was in year 2016due to severe El Niño phenomenon. Whereas, 2019 was reported as the second warmest year in the last ten years (Malaysian Meteorology Department. 2019).

Among the rice granary areas, Kemubu Agricultural Development Authority (KADA) and Muda Agricultural Development Authority (MADA) were the most facing unpredictable droughts along the planting season (Khalijah *et al.,* 2015). It has been reported that about 6 to 17% of the areas were affected by droughts from 2003 to 2012, which the highest incidences occurred in KADA(Khalijah *et al.,* 2015). Whereas, the earliest severe drought in MADA happened in year 1998 that caused RM 159.5 million losses to the rice industry (Ahmad Jamalludin and Low, 2003).

Nowadays, droughts become the major natural disasters in Malaysia. It contributes to high reductions on productivity of agriculture commodities mainly rice (Hasegawa 2009). Rosenzweig *et al.,* (1994) has stated that climate change in Malaysia affects the rice production as about 12 to 22% yield reduction. The previous study indicated that a global increase in temperature of 1°C can lead to yield reduction by 0.0308% in rice production (Khalijah *et al.,* 2015).

The serious yield reduction observed in rice plant as it is most susceptible to drought compared to other cereal crops i.e., wheat and maize, which they were showed better adaptation under less water condition. Moreover, the previous studies also discovered that rice are commonly affected to drought during reproductive stage that consequently cause serious effect on yield production (O'Toole 1982; Venuprasad *et al.,* 2007; Kumar *et al.,* 2014). Drought occurred prior to flowering time generally delayed the flowering time, decrease percentage of fertile panicle, and decrease percentage of filled grain (Pantuwan *et al.,* 2002).

According to Babu *et al.,* (2003), Lanceras *et al.,* (2004), and Gomez *et al.,* (2006) there were several considerations in identifying suitable donors for development of drought tolerant rice variety i.e., (i) selection of donors based on secondary traits that contribute to drought tolerant. There were many reported studies on usage of secondary traits for improving yield production under water limiting conditions, such as in maize (Ribaut *et al.,* 2004), wheat (Condon *et al.,* 2004) and sorghum (Sanchez 2002).Moreover, other secondary traits namely root architecture, relative water content, leaf water potential, canopy temperature, panicle exertion, leaf death and rolling had been used in identifying drought tolerant rice (Kumar 2018). As stated by Gomez *et al.,* (2006), the ideal secondary traits were easier to measure, genetically correlated with grain yield under stress, highly heritable and showed high genetic variation in the targeted species.

Another consideration was (ii) identification of quantitative trait loci (QTL). Gomez *et al.,* (2006) stated that the identification of genomiclocations linked to traits of interest and indirect selection can be done without the need of difficult phenotypic measurements. QTLs had been recognized with large and consistent effects on yield under drought stress (Venuprasad *et al.,* 2011) and had been detected in several drought tolerant component traits in rice (Li *et al.,* 2005; Kamoshita *et al.,* 2008; Swamy *et al.,* 2011;

Fen *et al.,* 2015). According to Gomez *et al.,* (2006) Even though the drought resistance traits can be detected by the locations of QTLs, however, there was still a requirement to monitor and determine the performance of the QTLs on drought grain yield under field conditions.

Currently the progress for development of drought tolerant rice variety in Malaysia is slow due to limited number of suitable donors for breeding program. To overcome this problem, there is a need to explore the available modern rice varieties and introduced as potential donors for future breeding program in MARDI. Thus, this study was conducted with specific objectives:

To evaluate the performance of fourteen MARDI rice varieties based morpho-agronomic traits under drought stress environment

To assess the genetic diversity of fourteen MARDI rice varieties based on fourteen Simple Sequence Repeat (SSR) markers linked to drought grain yield QTLs; and

To identify the drought tolerant variety among the fourteen MARDI rice varieties as potential donor variety for development the drought tolerant rice variety

Materials and Methods

Morphological Characterization under Drought Environment

Plant materials for drought stress study

Fourteen MARDI rice varieties were selected based on popularity among the farmers and newly released by MARDI (*Table 1*). The varieties were derived through breeding program by MARDI rice breeders. In addition, two introduced varieties reportedly tolerant to drought and possess drought grain yield QTLs were also used in this study as tolerant control varieties, namely Moroberekan and Dular. Whereas, IR64, was used as sensitive control variety.

Experimental and planting design

The experiment was conducted under glasshouse condition at MARDI Seberang Perai, located at the Northern part of Peninsular Malaysia. Completely Randomized Design (Gomez and Gomez 1984) was used for laying out the experiment which consisted of two replications with ten plants of each variety for both drought and control environments. Each variety was arranged randomly to allow the varieties to receive the sunlight equally. Spacing between the rows was 15.0 cm and between varieties was 20.0 cm.

Drought stress imposition

The fourteen MARDI rice varieties including two tolerant control varieties and one susceptible control variety were evaluated under drought and control environments. The seeds were sown in fiberglass troughs and transplanted into cement troughs at 20 days after sowing (DAS). Under control environment (non-stress), the cement troughs were surface flooded with standing water to a depth about 5.0 cm throughout the crop growth. Whereas, under drought stress environment, the seedlings were irrigated normally after transplanting, and thereafter drought stress was imposed by draining out water starting at late vegetative growth stage until maturity. Small canals (from the top of the soil until the bottom of the cement trough) were dug at the corner of the cement troughs for measuring the water table depth. Perforated PVC pipes were placed into the canals. The water table depth was measured daily until the water in the cement troughs totally dried. Surface irrigation was applied when most of the lines wilted and exhibited leaf drying. The irrigation is important to prevent the plants from dying during the experiment (Venuprasad *et al.,* 2007). At reproductive stage stress period, soil moisture content was gravimetrically determined on an oven dry basis. The soil samples were collected twice i.e., on 26th and 33rd days after water suspension. At each sampling time, duplicate samples were taken to a depth of 60.0 cm using an auger. The samples were immediately transferred into tightly closed aluminium cans and weighed, oven-dried at 105°C for 24 h, and reweighed after the moisture content was determined (Abd Allah *et al.,* 2010).

Morpho-agronomic characterization under drought environment

The morpho-agronomic characterization was carried when most of rice plants having the panicles. There are several morpho-agronomic traits were selected to be recorded namely leaf length (LL) = length of the topmost leaf blade below the flag leaf on the main culm second leaf, leaf width (LW) = width of the widest portion of the blade on the leaf below the flag leaf on the main culm, flag leaf length (FLL) = length of the flag leaf from the ligule to the tip of the blade, flag leaf width (FLW) = width at the widest portion of the blade on the flag leaf; tiller number (TN) = the total number of grain-bearing and non-bearing tillers of each individual plant, culm length (CL) = the distance from the ground to the tip of the panicle on the main tiller at maturity, culm diameter (CD) = the diameter at the third internode of the stem after stripping off leaves and leaf sheaths, by using digital vernier caliper; panicle length (PL) = length of main axis of panicle measured from the panicle base to the tip, grain yield (GY) = the total filled grains weight from three plants, harvest index (HI) = the grain, above ground biomass (BM) = total weight of empty grains, stalk and straw weight in grams; percent of filled grain (FG) = percentage of filled grain per plant, and maturity days (MAT) = the duration in days from seeding to the time when more than 90% of the grains on the panicles were fully ripened.

Statistical analysis for phenotypic (morpho-agronomic) data

The morphological characterization under drought and control environments were analyzed using Statistical Analysis System (SAS 9.3) to determine the mean, means square and F value. The significance levels were verified from the F values at 5% and 1% confident levels. The performance of the varieties under drought and control environments was measured with least means square and means comparison using SAS 9.3. The dendrogram for cluster analysis was performed using Multi-variate Statistical Package (MVSP).

Molecular Analysis for Drought Grain Yield QTLs

Plant material and DNA extraction

Leaf samples of each MARDI rice varieties including control varieties were collected and dried using silica gel prior to storage in freezer at -20 ^{0}C. Then for DNA extraction, the leaf samples were placed onto plate containing stainless steel bead (5mm in diameter) and immediately frozen at -80 ^{0}C for at least 24 hours. The DNA was extracted following the protocol of Mace *et al.,* (2003) with some modification in term of using semi-robotic equipment for high throughput DNA extraction. The frozen tissue was ground using Tissue Lyser (Qiagen, Germany) and immediately the Extraction buffer was added. The DNA integrity and concentration were measured using 0.8% agarose gels and

Fluoraskan Ascent (Thermo Fisher Scientific, United States), respectively.

SSR genotyping

A total of 14 SSR markers which linked to $qDTY_{3.2}$, $qDTY_{6.1}$, $qDTY_{11.1}$, $qDTY_{2.3}$ and $qDTY_{12.1}$ were determined from *GRAMENE* based on relative position reported by Dixit *et al.*, (2014) and Kumar *et al.*, (2014) (Table 2). The PCR reaction was conducted following the protocol by Schuelke (2000) by ligating the primers (either forward or reverse) with a non-fluorescent labeled M13 sequence tail (TGTAAAACGACGGCCAGT). Sequence adapter will be complimentary and ligated with fluorescent label M13 sequence adapter (FAM, NED, PET, or VIC). The PCR reaction was performed with a final volume of 10 µL, and contained 1x buffer (Invitrogen, United States), 10 µM each of forward and reverse primer, 5 µM fluorescence-labeled M13 primer, 2 µM of each dNTP (Invitrogen, United States) and 1 U of Taq polymerase (Invitrogen, United States). Target sequence amplification was done using GeneAmp® PCR System 9700 (Applied Biosystems, United States). The PCR profile was set with initial denaturation at 94°C for 2 min, followed by 36 cycles of 94 °C for 30 sec, 41–65 °C for 45 sec, and 72 °C for 45 sec, followed by final extension at 72 °C for 7 min. The PCR product was multiplexed after the amplification was done and was multiplexed up to four different fluorescent dyes. Then, the PCR products were resolved using ABI 3730 xl using GeneScan 500 LIZ as a standard ladder.

Statistical analysis for genotypic data

The raw data generated by ABI 3730 xl were scored using GeneMapper Version 5 (Thermo Fisher Scientific, United States) in order to determine the allele size. The allele calls or peaks were scored following the suggestion by Arif *et al.*, (2010). The allele size of each marker was formatted in Excel before using them as input in Power Marker program (Liu and Muse 2005). The software was used to measure major allele frequency, number of alleles, heterozygosity, gene diversity and Polymorphism Information Content (PIC) of each SSR marker. The pair wise shared-allele genetic-distance base was calculated using the same software. MEGA7 (Kumar *et al.*, 2016) was used to generate dendrogram based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) which was constructed using generated pair wise genetic distance.

Identification of rice accessions tolerant to drought stress

Strategy for selecting the promising MARDI rice variety for drought stress was determined using two dendrograms constructed based on Pearson's Correlation Coefficient using phenotypic (morpho-agronomic traits) and genotypic data (SSR markers linked to drought grain yield QTLs). The varieties which were grouped in the same cluster with tolerant control varieties in both dendrograms were considered having genetic similarities. Furthermore, the growth performance of each rice variety in the group were assessed based on the grain yield and yield related traits under drought environment. Thus, the MARDI rice variety which was grouped together with tolerant control varieties and exhibited promising growth performance under drought environment was considered as a potential MARDI rice variety for drought tolerance.

Results and Discussion

Drought imposition under drought stress environment

The fourteen MARDI rice varieties and three control varieties were evaluated under drought and normal environments. Drought stress was imposed by draining out the standing water at late vegetative stage (40 DAS). The drought was imposed at late vegetative stage to guarantee the plants be in state of drought stress condition before they reached the flowering stage. Similarly, Pantuwan *et al.,* (2002) conducted drought stress at booting stage to allow the rice plants experienced water deficit around the second week of flowering until maturity stage. The previous studies reported the effect of drought stress in rice at flowering stage, which were on yield and yield components (Abd Allah *et al.*, 2010); grain number per panicle (Boonjung 1996); dry matter production; and filled grain (Abd Allah *et al.,* 2010).

The study reached drought stress condition when the water table depths howed zero reading and soil moisture achieved below 10%. Water table depth reached zero reading on 5th days of drought imposition (*Figure 1*).While, soil moisture reached 10% at 26th days and below 10% at 33rd days of drought imposition(*Figure 2*). This study confirmed that the MARDI rice varieties were exposed to moderate and severe drought stress along the experiment (*Picture 1*). The sensitive control variety started to exhibit drought stress symptoms at 30th days of drought stress. Similarly, Zu *et al.*, (2017) also observed the top three leaves of the sensitive cultivars were tightly rolled, wilted, and drying at 10% moisture content.

Variation in morpho-agronomic traits under drought environment

The analysis onmorpho-agronomic traits under both drought and normal environments were analysed using ANOVA test (*Table 3*). There was a significant difference for all studied straits for variety and treatment (except for LW); whereas several traits namely CL, MAT, HI and GY showed significant difference for Var*Trt (*Table 4*). Table 3 showed significant difference for all studied traits under both environments, with the highly significant difference observed in LL, LW, FLL, FLW, TN, CL, BM, and MAT (*Table 3*). Dular as one of the drought tolerant control variety showed the highest mean value of LL, CL, and CD (*Table 4*). Whereas, Moroberekan is another drought tolerant control variety also showed highest mean value under drought for LW and FLW. There were four varieties exhibited more TN under drought environment compared to control varieties, namely MRQ74, MARDI284, SIRAJ297, and SEBERNAS307. The highest TN was observed in MRQ74 with the mean value of 6.70. Meanwhile, the longest PL was observed in SEBERNAS307 with the mean value of 24.00. Other MARDI varieties that also showed longer PL compared to the control varieties under drought environment are MARDI308, MR219 and SEMPADAN303.

Meanwhile, the highest mean value of BM observed in SEBERNAS307, whereas the highest mean value of HI observed in MR219; MR253; and MARDI308 with the value is 0.30. Dular showed the highest mean value of FG with the value is 61.18. Whereas the second highest of FG observed in MRQ74 followed with MRQ76. Mean value of GY observed MR269 showed the highest with the value is 4.20 followed with MRQ76 with the value is 4.06. The mean value of HI in MARDI rice varieties was higher than Dular and Moroberekan. Dular exhibited highest HI compared to the other control varieties, produced the highest GY with a value of 2.89 g. Similarly, MR219 and MARDI308 also showed higher HI among the MARDI rice varieties which produced higher GY with the value of 3.47 g and 3.55 g, respectively.

However, the highest GY was observed in MR269 followed by MRQ76 with the value of 4.20 g and 4.06 g, respectively.

In overall, the mean value under drought environment observed eleven MARDI rice varieties showed outstanding performance under drought stress environment compared to the tolerant control varieties based on GY and yield related traits, namely, TN, PL, HI, and FG. The varieties are MR219, MR220, MR253, MR269, MRQ74, MRQ76, SIRAJ 297, MARDI284, MARDI308, SEMPADAN-303, and SEBERNAS307.

Grain yield performance under drought environment

The performance of studied traits was compared based on mean between the two environments using Duncan Multiple Range Test (Table 5). All the studied traits showed reduction under drought environment. The highest percentage of reduction observed in GY with the percentage is 60.92%. HI showed about half percent reduction under drought environment with the value is 48.89%, whereas FG showed reduction at 39.51% under drought environment. Five MARDI rice varieties showed higher GY under drought environment and no significant difference with drought tolerant variety, Dular, the varieties were MARDI284, MRQ76, MR232, MR220, and MR269 (*Figure 3*). Among the varieties, MARDI284 produced the highest grain yield, followed by MRQ76.

Least square means of the GY under drought stress and normal environments was presented in *Figure 4*. This study observed the control varieties and all MARDI rice varieties showed reduction in GY under drought compared to normal environment. MR269 showed the highest least square means on GY under drought environment with the value is 4.2; followed by MRQ76 with the value is 4.1. MRQ74 showed the minimum percentage of GY reduction between the two environments with the percentage value is 10.0%. While, MR269 that showed the highest least square means on GY under drought environment; exhibited 46.84% of yield reduction difference. Whereas, MRQ76 was the second highest of least square means on GY under drought environment showed 54.44% of yield reduction difference. Meanwhile, the tolerant control variety, Dular showed the higher GY reduction compared to MR269 which was 67.05%. This study observed MR269 and MRQ76 exhibited outstanding performance in HI, GY under drought environment and also presented less grain reduction difference compared to the control varieties.

The negative effects of drought on rice traits especially on grain yield was also observed in many previous studies (Fukai and Cooper 1995; Pantuwan *et al.,* 2002; Site Noorzuraini *et al.,* 2012). Kumar *et al.,* (2015) observed the rice yield reduction difference between drought stress and non-stress ranged from 27.31% to 67.7%. Abarshahr *et al.,* (2011) stated that the increased and decreased of GY was actually due to the potential of yield related traits in the variety itself and the different responses to environmental factors. Whereas, Abd Allah *et al.,* (2010) stated the impact of drought stress upon grain yields due to reduction of productive tillers and reduction of grain number per panicle. Moreover, the outstanding traits related to grain yield under drought stress were superiority in tiller number, panicle number, low sterility and heavier in grain weight (Abd Allah *et al.,* 2010). Thus, varieties that showed prominent traits on GY and yield related traits in this study may considered as promising varieties for future breeding program in MARDI.

Genetic diversity among MARDI rice varieties

In this study, fourteen simple sequence repeat (SSR) markers were used to determine the major allele frequency, allele number, allele diversity, heterozygosity and Polymorphic

Information Content (PIC) values among the MARDI rice varieties (*Table 7*). The results showed 41 reproducible alleles were amplified with an average of 4.56 alleles per locus. The number of the alleles varied from 2 to 8 alleles per locus. A total of 6.1% of major alleles frequency were observed. Previous study reported 84 alleles per locus in 7 rice genotypes with an average of 2.89 alleles per locus (Roy *et al.,* 2015). Similarly, Singh *et al.,* (2016) detected 63 alleles with an average of 2.75 alleles per locus. The highest mean major allele frequency was observed in chromosome 3 with the value of 0.83%, while the lowest was observed in chromosome 2 and 6 with the value of 0.51. The average gene diversity over all SSR loci was 0.46, with the highest at chromosome 2 with the value of 0.67.

PIC value is a reflection of allele frequency and allele diversity which provides discriminating power of a marker locus in a given population. PIC values varied from 0.096 (RM3894) to 0.706 (RM204) with the average of 0.43. The highest mean PIC value was observed in chromosome 2 with the value of 0.628. and the lowest was observed in chromosome 3 with the value of 0.271. About 57.1% of the markers showed higher PIC values (>0.50), namely, RM530, RM14291, RM204, RM209, and RM7391. Higher PIC values indicated that the markers were highly informative, suitable for detecting more alleles and useful for distinguishing the polymorphic rate of the markers at a specific locus (DeWoody *et al.,* 1995). Previous studies observed higher PIC values (>0.50) in various rice backgrounds such as cultivars, landraces and wild relatives (Ravi *et al.,* 2003; Ram *et al.,* 2007; Site Noorzuraini *et al.,* 2013). Among the highest PIC values observed were 0.97 in 100 high yielding rice genotypes (Choudhary *et al.,* 2013); 0.93 in seven aromatic rice varieties (Roy *et al.,* 2016); and 0.95 in medicinal rice (Behera *et al.,* 2012); and 0.75 in 192 diverse rice genotypes (Nachimuthu *et al.,* 2015).

Drought grain yield QTLs in MARDI rice varieties

Previous studies had discovered several large-effect QTLs on grain yield under reproductive stage drought for both upland and lowland rice conditions(Bernier *et al.,* 2007; Kumar *et al.,* 2007; Venuprasad *et al.,* 2009; Vikram *et al.,* 2011; Ghimire *et al.,* 2012; Venuprasad *et al.,* 2012; Mishra *et al.,* 2013; Yadaw *et al.,* 2013; Kumar *et al.,* 2014; Vikram *et al.,* 2016).

Bernier *et al.,* (2007) reported the first large-effect QTL of $qDTY_{12.1}$ for grain yield under reproductive stage drought. The identified QTL is important as an ultimate used in marker-assisted backcross breeding approach and QTL introgression in popular rice varieties (Kumar *et al.,* 2014).

In this study, three QTLs were determined and linked to the selected markers, namely, $qDTY_{3.2}$ (Vikram *et al.,* 2011); $qDTY_{11.2}$; and $qDTY_{2.3}$ (Palanong *et al.,* 2014) The highest frequency of QTL was observed in $qDTY_{3.2}$, the donor variety for this QTL is Moroberekan(*Figure 5*). There were seven MARDI rice varieties that possessed this QTL namely MR219, MR220, MR253, MR269, MRQ74, MRQ76, and MARDI284. $qDTY_{3.2}$ 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 (Swamy *et al.,* 2011; Kumar *et al.,* 2014).

Table.1 List of MARDI rice varieties and control varieties for drought stress study

No.	Variety Name	Accession Number	No.	Variety Name	Accession Number
1	Moroberekan	MRGB10930	10	MRQ76	MRGB12109
2	Dular	MRGB10735	11	MR269	MRGB12120
3	IR64	MRGB10808	12	MARDI 284	MRGB12140
4	MR219	MRGB11633	13	MARDI SIRAJ 297	MRGB13019
5	MR220	MRGB11634	14	MARDI WANGI 88	MRGB13020
6	MRQ74	MRGB11787	15	SEMPADAN303	MRGB13001
7	MR232	MRGB12047	16	SEMPADAN307	MRGB13005
8	MR253	MRGB12095	17	MARDI 308	MRGB13006
9	MR263	MRGB12133			

Table.2 List of 14 specific SSR markers linked to the respective drought grain yield QTLs and the drought tolerant check varieties

Chromosome No.	QTLs	SSR Markers
3	$qDTY_{3.2}$	RM14291, RM3894, RM6013
6	$qDTY_{6.1}$	RM204, RM19367, RM549,
11	$qDTY_{11.1}$	RM3625, RM7391, RM209
2	$qDTY_{2.3}$	RM530
12	$qDTY_{12.1}$	RM573, RM1261, RM28099, RM28166

Table.3 The mean, standard deviation, variance, coefficient of variation, mean square, F value, and Pr>F of all studied traits under drought and normal environments

Variable	N	Mean	Minimum	Maximum	Std Dev	Coeff of Variation	Mean Square	F Value	Pr > F
Leaf length (LL)	68	43.60	26.44	68.10	8.54	12.91	167.93	5.30	<.0001
Leaf width (LW)	68	13.09	10.40	19.33	1.56	6.61	7.20	9.64	<.0001
Flag leaf length (FLL)	68	30.10	17.72	50.40	6.69	14.96	114.87	5.66	<.0001
Flag leaf width (FLW)	68	14.48	11.00	20.67	1.82	5.77	8.09	11.56	<.0001
Culm length (CL)	68	60.80	32.80	99.33	14.93	7.36	320.75	16.03	<.0001
Tiller number (TN)	68	4.26	2.00	7.60	1.30	16.99	3.34	6.40	<.0001
Culm diameter (CD)	68	3.15	1.63	4.50	0.61	15.79	0.66	2.65	0.0088
Panicle length (PL)	68	23.14	14.60	31.33	4.01	8.62	17.35	4.36	0.0002
Maturity (MAT)	68	126.62	97.00	145.00	13.70	2.74	131.47	10.93	<.0001
Biomass (BM)	67	13.69	5.22	25.80	4.71	20.24	46.93	6.11	<.0001
Harvest Index (HI)	67	0.34	0.08	0.59	0.13	17.10	0.01	2.12	0.0346
Filled Grain (FG)	67	60.06	23.65	92.91	19.22	16.84	224.44	2.19	0.0286
Grain yield (GY)	67	4.99	0.48	14.03	3.07	27.89	8.08	4.16	0.0003

Table.4 Statistical analysis for variety, replicate, treatment, variety*treatment for all studied traits

Source	Leaf Length	Leaf Width	Flag Leaf Length	Flag Leaf Width	Culm Length	Culm No.	Culm Diameter
Variety	<.0001**	<.0001**	<.0001**	<.0001**	<.0001**	<.0001**	0.0088**
Replicate	0.9244	0.2873	0.8304	0.9163	0.0041**	0.3966	0.667
Treatment	<.0001**	0.1433	0.001**	<.0001**	<.0001**	<.0001**	0.0052**
Var*Trt	0.9158	0.0771	0.7817	0.0845	0.0236*	0.5613	0.4417

Table.4 Cont.

Source	Panicle Length	Maturity	Biomass	Harvest Index	% Filled Grain	Grain Yield (g)
Variety	0.0002**	<.0001**	<.0001**	0.0346*	0.0286*	0.0003**
Replicate	0.8352	0.1515	0.0186*	0.0205*	0.7162	0.003**
Treatment	<.0001**	<.0001**	<.0001**	<.0001**	<.0001**	<.0001**
Var*Trt	0.5567	0.0062**	0.1916	0.0154*	0.0791	0.0034**

Table.5 Mean values of the secondary traits, phenotypic traits, and integrative traits of fourteen MARDI varieties and control varieties under drought stress

Variety Name	Leaf length (LL)	Leaf width (LW)	Flag leaf length (FLL)	Flag leaf width (FLW)	Culm length (CL)	Tiller number (TN)	Culm diameter (CD)	Panicle length (PL)	Maturity (MAT)	Biomass (BM)	Harvest index (HI)	Filled grain (FG)	Grain yield (GY)
Moroberekan	42.54	15.17	35.29	16.00	56.00	2.67	3.60	19.34	144	5.22	0.09	23.65	0.48
Dular	60.28	12.90	35.00	14.90	67.10	3.50	3.84	22.00	132	11.38	0.26	61.18	2.89
IR64	43.70	10.50	29.66	13.10	35.40	5.60	2.46	18.40	138	8.48	0.16	35.55	1.35
MR219	38.87	13.60	25.98	13.70	50.30	4.30	2.87	22.20	141	11.65	0.30	50.48	3.47
MR220	37.29	14.20	28.19	14.30	52.30	4.40	2.66	21.90	137	12.31	0.28	50.01	3.38
MR232	43.81	13.40	33.98	14.40	44.90	5.30	3.06	20.20	144	12.94	0.21	47.04	2.67
MR253	32.23	11.90	19.51	12.00	41.20	5.00	2.34	16.80	132	8.88	0.30	51.15	2.65
MR263	30.64	11.40	21.14	13.90	45.40	4.00	2.01	17.70	138	7.15	0.29	41.76	2.04
MR269	33.98	12.70	25.16	12.00	54.80	5.20	2.56	21.10	137	14.60	0.29	49.43	4.20
MRQ74	38.99	12.20	24.00	12.20	40.20	6.70	2.84	17.70	135	10.39	0.28	58.38	2.73
MRQ76	36.56	13.50	26.55	14.34	57.20	4.67	3.54	17.54	138	14.33	0.27	54.40	4.06
SIRAJ297	35.41	12.90	26.15	14.20	48.20	6.10	2.71	19.90	139	14.43	0.18	30.40	2.66
WANGI88	41.63	12.20	26.93	12.15	40.25	4.85	2.97	16.90	144	8.74	0.14	34.11	1.26
MARDI284	42.74	12.65	27.90	14.00	51.43	6.23	3.47	21.78	141	15.87	0.19	49.14	3.07
SEMPADAN303	42.42	13.50	35.96	13.60	50.90	4.70	2.69	22.10	137	11.21	0.21	38.75	2.37
SEBERNAS307	41.33	14.20	31.84	13.90	57.15	5.85	3.78	24.00	144	17.02	0.20	38.76	3.46
MARDI308	37.35	12.90	24.85	13.00	53.80	5.40	3.09	23.00	135	12.19	0.30	41.96	3.55

Table.6 Mean comparison between two environments using Duncan's Multiple Range Tested

Studied Traits	Duncan's Multiple Range Test		Percentage of reduction (%)
	drought	control	
Leaf length (LL)	39.99	47.22	15.31
Leaf width (LW)	12.93	13.24	2.34
Flag leaf length (FLL)	28.12	32.07	12.32
Flag leaf width (FLW)	13.63	15.34	11.15
Culm length (CL)	49.80	71.80	30.64
Tiller number (TN)	3.54	4.97	28.77
Culm diameter (CD)	2.97	3.33	10.81
Panicle length (PL)	20.15	26.13	22.89
Maturity (MAT)	114.79	138.44	17.08
Biomass (BM)	11.77	15.56	24.36
Harvest Index (HI)	0.23	0.45	48.89
Filled Grain (FG)	45.11	74.58	39.51
Grain yield (GY)	2.79	7.14	60.92

Table.7 Major allele frequency, allele number, gene diversity, heterozygosity, and polymorphism information content (PIC) for 14 simple sequence markers (SSRs) in 14 MARDI varieties

Chromosome No.	*qDTY*	Marker	Major Allele Frequency	Allele No	Gene Diversity	Heterozygosity	PIC
2	2.3	RM530	0.512	6	0.668	0.023	0.628
3	3.2	RM3894	0.946	2	0.101	0.012	0.096
	3.2	RM6013	0.857	3	0.251	0.260	0.230
	3.2	RM14291	0.674	5	0.516	0.011	0.488
6	6.1	RM204	0.460	8	0.732	0.161	0.706
	6.1	RM19367	0.561	5	0.565	0.167	0.384
	6.1	RM549	0.438	4	0.418	0.266	0.458
11	11.1	RM3625	0.768	4	0.375	0.083	0.332
	11.1	RM209	0.611	4	0.554	0.000	0.499
	11.1	RM7391	0.711	4	0.412	0.09	0.552
12	12.1	RM573	0.573	4	0.528	0.188	0.442
	12.1	RM1261	0.622	3	0.248	0.218	0.284
	12.1	RM28099	0.587	5	0.588	0.308	0.506
	12.1	RM28166	0.418	4	0.482	0.214	0.468

Fig.1 The graph shows the level of water table depth in five days at drought stress environment

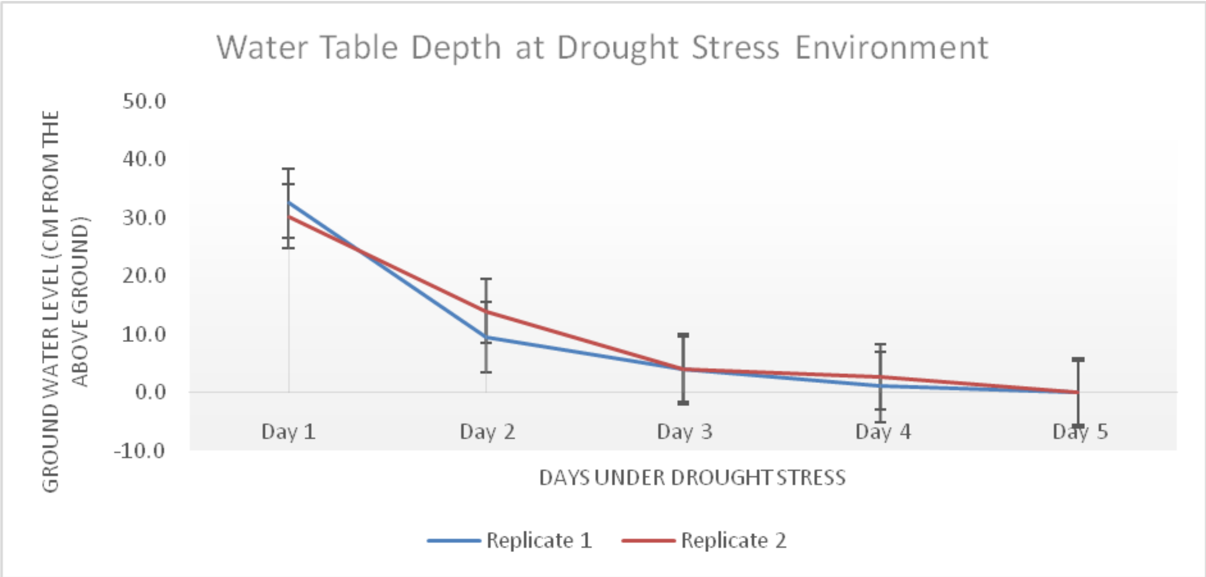


Fig.2 The graph shows the soil moisture under drought stress at day 26 and 33

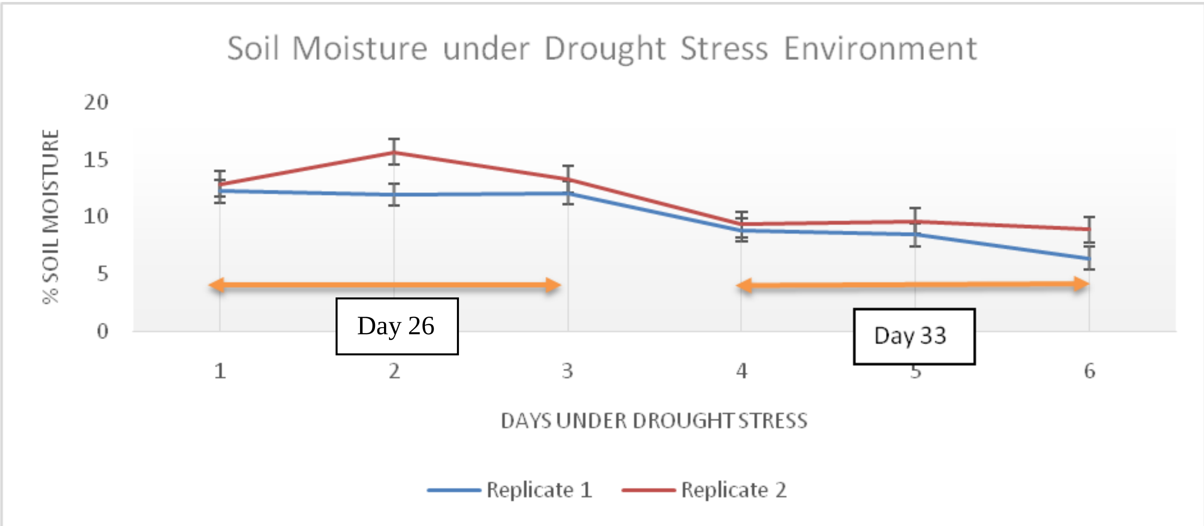


Fig.3 The grain yield of 14 MARDI rice varieties under drought stress environment.

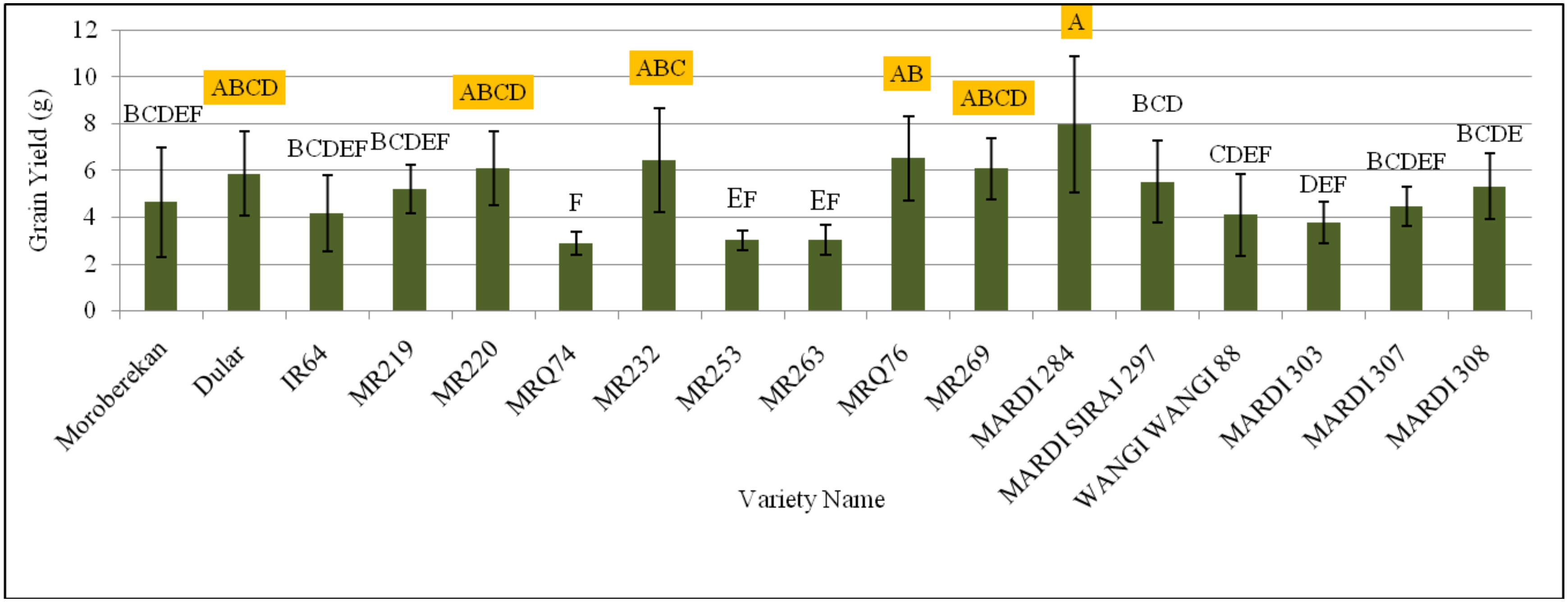


Fig.4 The least mean squares of grain yield under drought and control environments

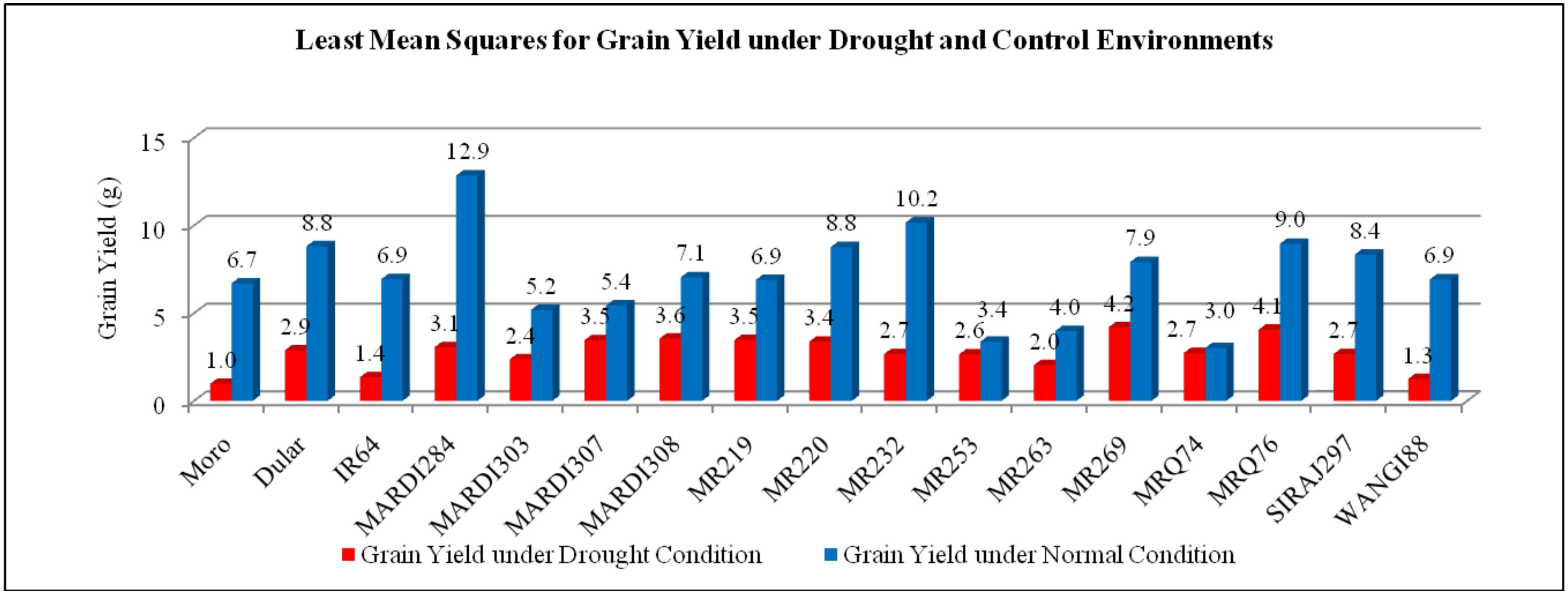


Fig.5 Frequency of SSR markers of each drought tolerance QTLs in MARDI varieties

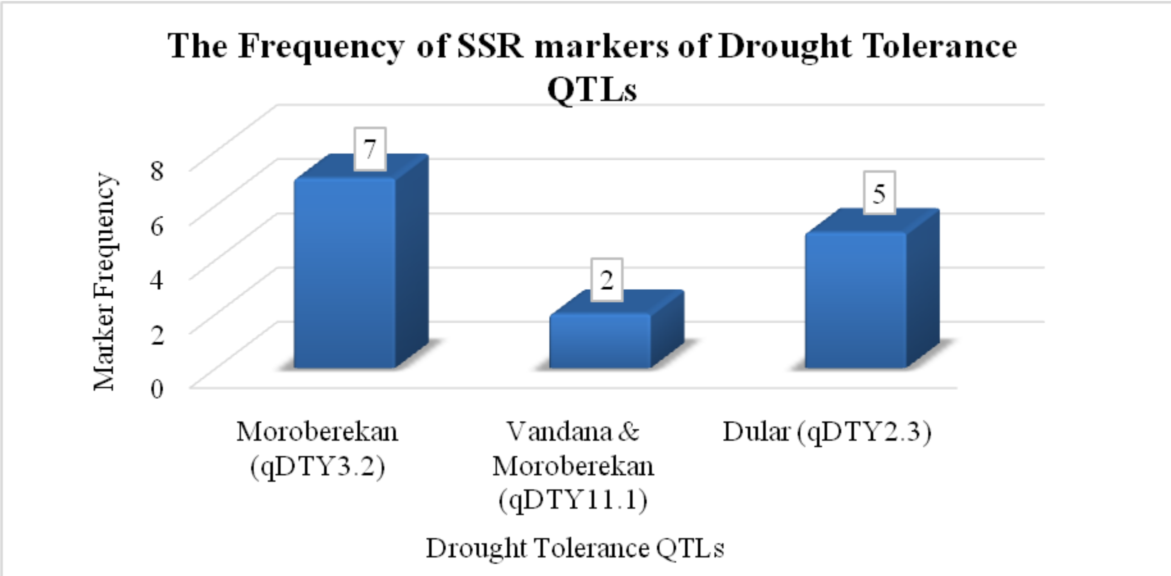


Fig.6 Dendrogram based on morpho-agronomic traits of fourteen MARDI rice varieties under drought stress environment

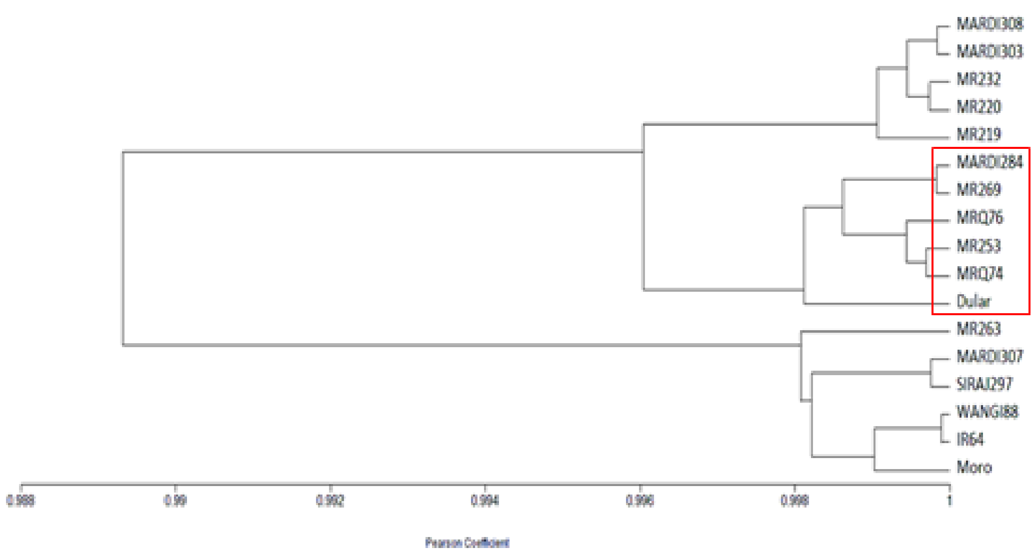
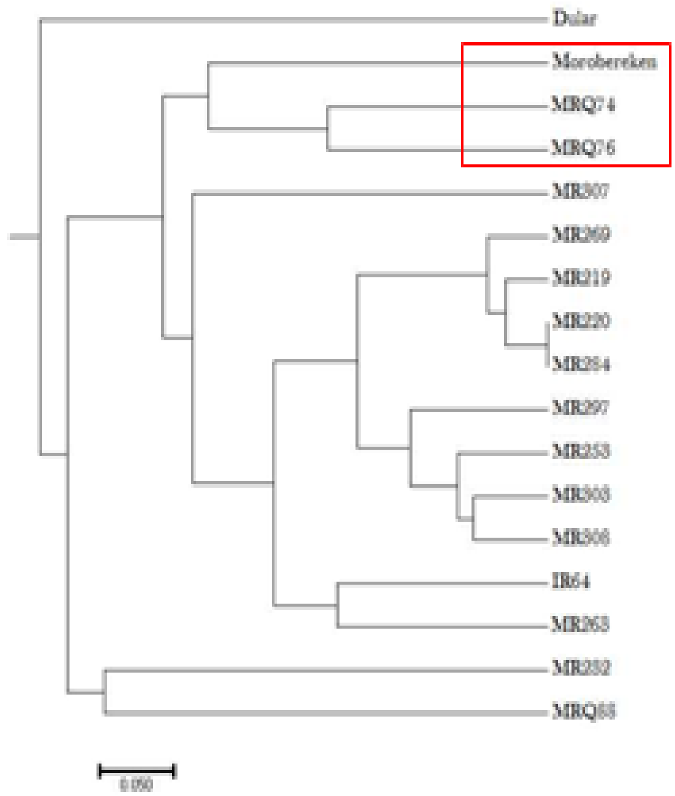


Fig.7 Dendrogram for fourteen MARDI varieties based on fourteen Simple Squence Repeat (SSR) markers linked to drought grain yield QTLs



Picture.1 Rice plants under drought stress environment

The marker also reported exhibit consistent effects on grain yield in two or more genetic backgrounds and ecosystems (Ghimire *et al.*, 2012; Yadaw *et al.*, 2013).

The second highest frequency of QTL was observed in $qDTY_{2.3}$. The donor variety for this QTL is Dular. This QTL was amplified in five varieties, namely, MR253, MRQ76, SIRAJ297, WANGI88, and SEBERNAS307.This QTL had been reported had an effect on grain yield in cross multiple backgrounds (Kumar *et al.,* 2014). Whereas, the lowest frequency of QTL was observed in $qDTY_{11.1}$. The QTL was found in two MARDI rice varieties, namely, MRQ74 and MRQ76.$qDTY_{11.1}$also had been reported produce higher yield at reproductive stage under drought stress conditions (Sandhu *et al.*, 2018).

This finding also discovered three MARDI rice varieties possess more than one QTLs. There wereMRQ74 possess $qDTY_{3.2}$ and $qDTY_{11.1}$; MR253 possess $qDTY_{2.3}$ and $qDTY_{3.2}$; whereas MRQ76 possess $qDTY_{3.2}$, $qDTY_{2.3}$ and $qDTY_{11.1}$. As stated by Swamy *et al.,* (2011) the presence of at least one grain yield QTL will provide major effect in drought panel lines. Whereas, varieties with combinations of several QTLs allow more positive interactions between the different QTLs and provide more possibilities for breeders to capture a high yield advantage under reproductive-stage drought (Kumar *et al.,* 2014).

Identification of drought tolerant variety among MARDI rice varieties

Two dendrograms for cluster analysis were constructed based on morpho-agronomic traits and genotypic traits using fourteen SSR markers linked to drought grain yield QTLs. Pearson's Correlation Coefficient used to measure statistical relationship based on morpho-agronomic traits divided MARDI rice varieties into three major groups(*Figure 6*). There are five MARDI rice varieties that were grouped together with Dular namely MARDI284, MR269, MRQ746, MR253, and MRQ74. While, genetic similarity coefficient based on genotypic traits showed most of the MARDI rice varieties were positioned into two major groups(*Figure 7*). Where, MRQ74 and MRQ76, were grouped together with Moroberekan.

MARDI284, MR269, MRQ76, MR253, and MRQ74which were grouped with Dular showed higher mean value in culm number, harvest index, % of filled grain, and grain yield. These varieties showed outstanding growth performance under drought environment almost similar to Dular. Three varieties, namely, MR269, MRQ76 and MARDI284 also showed no significant difference with Dular on grain yield under drought stress environment.

Based on the genotypic traits, MRQ74 and MRQ76 which were grouped together with Moroberekan were identified as carrier of more than one drought grain yield QTLs. $qDTY_{3.2}$ and $qDTY_{11.1}$ were identified in MRQ74, meanwhile $qDTY_{3.2}$, $qDTY_{2.3}$, and $qDTY_{11.1}$ were identified in MRQ76. As an overall finding, the study showed thatMRQ76 is the prominent variety based on morpho-agronomic traits and presence of QTLs under drought environment. The variety among the highest in GY production, FG, HI, less yield reduction difference under drought environment; and also possess three drought grain yield QTLs namely $qDTY_{3.2}$, $qDTY_{2.3}$, and $qDTY_{11.1}$that allowed the variety to perform well under drought stress environment.

Evaluation and assessment of genetic diversity in identifying the prominent MARDI rice variety that is suitable and adaptable to drought is considered as a primary step for designing effective breeding program in MARDI. This study reveals sufficient genetic diversity among the MARDI rice varieties and successfully identified MRQ76asthe most outstanding variety as exhibited great performance on morpho-agronomic traits (i.e., GY, FG, and HI, and yield reduction difference) under drought environment. In addition, MRQ76 was identified possess the highest numbers of grain yield QTLs compared to other MARDI rice varieties. The QTLs observed in MRQ76 were $qDTY_{3.2}$, $qDTY_{2.3}$, and $qDTY_{11.1}$. This variety can be recommended for planting in drought prone areas and introduced as a promising donor variety for development of drought tolerant rice variety in MARDI.

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