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Diversity assessment of Malaysian rice germplasm accessions for drought tolerant grain yield QTLs

(Penilaian kepelbagaian germplasma asesi padi Malaysia untuk QTLs hasil padi tahan kemarau)

A.R. Site Noorzuraini*, T.H. Borromeo**, N.C. Nestor**, G.M. Diaz*** and K. Arvind****

Keywords: SSR markers, PIC, similarity coefficient, cluster analysis

Abstract

Diversity among 80 Malaysian rice accessions was assessed based on 119 Simple Sequence Repeat (SSR) markers. The Polymorphic Information Content (PIC) values of 89% of the SSR markers were above 0.50 indicating that those markers were highly informative. The presence of Quantitative Trait Loci (QTLs) was assessed based on 45 markers linked to drought grain yield QTLs. Five drought grain yield QTLs were present in the 80 Malaysian rice accessions. The highest frequency was 10.6% found in $qDTY_{12.1}$ present in Way Rarem, Boewani, Pulut Malaysia 1, CI–9534, IR1561–243–5–6, CICA4, IR2797–156–3, Chianung Sen Yu, MR 142 and Huma Wangi Lenggong. Second highest was $qDTY_{3.1}$ present in 8.5% of the germplasm, observed in Apo, Mokwoo, Way Rarem, CICA 4, Biris, MR 185, Tainan and Q70. Two UPGMA cluster analysis were performed based on 119 SSR markers and 45 specific SSR markers which linked to QTLs for grain yield under drought. The rice accessions were grouped at 77% similarity coefficient and produced 7 clusters using 119 SSR markers and 9 clusters using 45 specific SSR markers.

Introduction

Plant breeders normally define drought as 'a shortfall of water availability sufficient to cause loss in yield', or 'a period of no rainfall or irrigation that affects crop growth' (Fukai and Cooper 1995). According to Blum (1988) and Zhang et al. (1999), drought is a complex phenomenon, and the severity of drought varies with locations and years (Cooper and Somrith 1997; Cooper 1999; Pantuwan et al. 2002). Plants can tolerate drought through three mechanisms, namely, drought tolerance, drought escape and drought avoidance (Blum 1988; Zhang et al. 1999). Drought tolerance differs from drought escape based on drought response mechanisms in plants such as stomatal closure, leaf rolling, enhanced root growth and enhanced ABA production (Levitt 1980). Drought escape is defined as the ability of a plant to complete its life cycle before drought occurs (Levitt 1980).

*Strategic Resources Research Centre, MARDI Seberang Perai, P.O. Box No. 203, 13200 Kepala Batas, Pulau Pinang, Malaysia
**Crop Science Cluster, University of the Philippines Los Baños, College, Laguna 4031, Philippines
***Institute Biosciences, University of the Philippines Los Baños, College, Laguna 4031, Philippines
****N.C. Brady Laboratory Building, International Rice Research Institute, DAPO Box 7777, Metro Manila 1301, Philippines
Authors' full names: Site Noorzuraini Abd Rahman, Teresita H. Borromeo, Nestor C. Altoveros, Genaleen M. Diaz and Arvind Kumar
E-mail: zuraini@mardi.gov.my

Whereas, mechanism of drought avoidance depends on the type of stress, occurring on the whole plant, the organ or the cellular levels (Blum 2005). Drought avoidance in plants is achieved by maintaining cellular hydration through osmotic adjustment, in spite of reduction in whole-plant water potential (Blum 2005).

Drought is the most important abiotic stress in plants and a major yield-limiting factor for agricultural production worldwide. In Malaysia, rice is one of the major agricultural crops grown in an area of about 1.8 million ha (AQUASTAT 2011) with an annual production of about 2.4 million tonnes (FAO 2009). The rice production systems in Malaysia are classified mainly into 3 types, namely, irrigated lowland, rainfed lowland and rainfed upland rice. Irrigated rice contributes the highest to the total rice production in Malaysia (Malaysian National Committee of ICID). Recently, due to the effect of climate change, *El Niño* phenomenon, widespread drought in many rice growing areas in Malaysia is common. The most severe drought occurred in MADA areas in the year 1998 with RM159.5 million losses to the rice industry (Ahmad Jamalludin and Low 2003).

Under rainfed lowland rice, drought can be classified into vegetative stage drought, intermittent drought and reproductive stage drought (Chang et al. 1979). The reproductive stage drought occurs during the flowering and grain filling stages thus causing significant yield losses in many rainfed lowland rice areas (Pandey et al. 2007). Selection of drought resistant rice for rainfed lowlands is not easy. There are many factors influencing the selections such as incomplete understanding of the mechanisms of drought resistance, large genotype by environment interaction (G x E) for yield. Nevertheless, some traits have been studied for their use in breeding for drought tolerance and the results showed promising correlation of the traits for improving yield under drought conditions (Lafitte et al. 2003). As reported by Fukai (1999), high potential yield and ability to maintain high leaf water potential were appropriate phenology in breeding for drought tolerance in rice because they were associated directly with higher grain yield under drought.

Nowadays, many studies were carried out for Quantitative Trait Loci (QTLs) mapping of grain yield under drought. At the International Rice Research Institute (IRRI), many drought grain yield QTLs have been identified and successfully used in breeding programmes such as $qDTY_{12.1}$ (QTL for upland environment), $qDTY_{2.1}$, $qDTY_{2.2}$, $qDTY_{4.1}$, $qDTY_{9.1}$ and $qDTY_{10.1}$ (QTLs for lowland environment). Bernier et al. (2009) reported the $qDTY_{12.1}$ on chromosome 12 can improve grain yield under severe to moderate reproductive stage drought stress and has a large and consistent effect on grain yield under upland drought stress environments. While $qDTY_{1.1}$, identified on chromosome 1 in Nagina 22 (N22), was derived from the population in the backgrounds of Swarna, IR64 and MTU1010 with an additive effect of 30.2, 25.8 and 16.9% respectively. The QTL was first reported as a major and consistent effect in multiple elite genetic backgrounds under both stress and non-stress environments (Vikram et al. 2011).

Recently, MARDI has screened rice accessions through drought field screening (unpublished). The study observed that several rice accessions have better ability to tolerate drought. Since the study was only based on field screening, the information gathered was not sufficient to identify the drought tolerant accessions. Therefore, this study was carried out to assess the diversity of 80 Malaysian rice accessions based on 119 random Simple Sequence Repeat (SSR) markers which include 45 specific SSR markers that are linked to drought grain yield QTLs. The study was also to identify the accessions which might carry drought grain yield QTLs. The identified rice

accessions might be useful as donor parents for future breeding programmes, especially for developing drought tolerant rice variety.

Materials and methods

Malaysian rice germplasm accessions

A total of 80 rice accessions were selected from the previous drought field screening studies done at Stesen MARDI, Seberang Perai. The materials consisted of Malaysian landraces, breeding lines, varieties, cultivars and introduced accessions. This study also used 12 foreign varieties reportedly tolerant to droughts, possessing QTLs related to drought traits as reference check varieties. They are Vandana, Apo, PSBRC-82, UPLRi7, Mokwoo, Aday Sel, N22, IR81896-B-195, IR81896-B-142, IR77298-5-6-18, IR77298-14-1-2-10 and Way Rarem, and two drought susceptible checked varieties namely IR64 and MTU1010 were included in the experiment.

The DNA of the respective accessions was extracted from young leaves using the Modified CTAB method (Murray and Thompson 1980). This method was a standard procedure used by the Gene Array and Molecular Marker Analysis (GAMMA) Laboratory, Plant Breeding, Genetics and Biotechnology (PBGB) division, IRRI. A total of 10 fresh leaves were collected from each accession. The leaves were freeze dried and ground in liquid nitrogen. The powdered leaves were mixed with 800 µl 2X warmed CTAB (cetyl-trimethylammonium bromide) extraction buffer and 2 µl of 20% SDS (sodium dodecyl sulfate). The samples were incubated at 65 °C for 30 – 60 min and were cooled briefly. Then, 800 µl chloroform: isomyl alcohol (24:1) was added to the mixture. The mixture was centrifuged at 12,000 rpm for 10 min and the upper phase was transferred into a new 2.0 ml microcentrifuge tube. DNA was precipitated with isopropanol (1:1 vol), precooled to a temperature of –20 °C. The samples were placed in a –20 °C freezer overnight. The DNA pellets were rinsed with 500 µl of 70% ethanol and re-centrifuged. The ethanol was drained off and the DNA pellets were left to dry. DNA was dissolved in 200 µl of 1X TE (Tris-EDTA) buffer and treated with 2 µl RNase (10 g/ml). The mixture was incubated at 37 °C for 30 min. Samples were stored in a –20 °C freezer as stock solution or as working solution at 4 °C.

Genotyping of the Malaysian rice accessions

A total of 80 Malaysian rice accessions as well as 14 check varieties were genotyped. From the original 125 markers evaluated, only 119 markers were polymorphic and subsequently used in the analysis (*Table 1*). These 119 microsatellites markers included a total of 45 markers that were reportedly linked to various QTLs related to various drought tolerant traits (*Table 2*). The 45 specific markers were observed located closest to logarithm of the odds ratio (LOD) peak of grain yield under stress such as RM1261, RM315, RM28130, RM431, etc. (Bernier et al. 2007). The LOD value is important to detect the putative grain yield QTLs in each chromosome. The QTLs observed were $qDTY_{1.1}$, $qDTY_{1.2}$, $qDTY_{2.1}$, $qDTY_{2.2}$, $qDTY_{2.3}$, $qDTY_{3.1}$, $qDTY_{4.1}$, $qDTY_{9.1}$, $qDTY_{10.1}$ and $qDTY_{12.1}$. The accessions (genotypes) were analysed based on the presence/absence of the amplified PCR products by using the previously extracted DNAs as the templates.

The PCR amplification was done with 15 µl reaction mixture having 2 µl (10 – 20 ng) of DNA, 1.5 µl of 10X PCR buffer, 1.5 µl of 1mM dNTPs, 1.0 µl of 5 µM each of forward and reverse primers, 1.0 µl of 1:10 *Taq* polymerase and 7.0 µl of sterile water. Preparation of the reaction mixture was done according to the standard procedure by Gene Array and Molecular Marker Analysis (GAMMA) Laboratory, Plant Breeding, Genetics and Biotechnology (PBGB) division, IRRI. The mix was prepared on 96-well polycarbonate plates and centrifuged at 3,000 rpm for 1 min. The procedure on thermo cycler was followed as described by Panaud et al. (1996). After

Table 1. List of random and specific SSR markers grouped according to the chromosome number

	MARKER NAME	MOTIF
Chromosome 1	RM315	(AT)4(GT)10
	RM104	(GA)9
	RM113	(CA)8
	RM129	(CGG)8
	RM522	(AAT)6
	RM212	(CT)24
	RM3825	(GA)21
	RM582	(TC)20
	RM12091	–
	RM431	(AG)16
	RM11943	(GA)11
	RM513	(TC)11
	RM12182	(AG)11
	RM12146	(AG)11
	RM12233	(TCC)8
	RM10488	(AG)12
	RM12023	(TCG)8
Chromosome 2	RM71	(ATT)10T(ATT)4
	RM12979	(TTTC)6
	RM12992	(CGC)7
	OSR17	(AATT)n
	RM262	(CT)16
	RM263	(CT)34
	RM327	CAT)11 (CTT)5
	RM3549	(GA)12
	RM6374	(GAA)16
	RM521	(TC)14
	RM573	(GA)11
	RM12460	(TC)16
	RM12569	(AG)10
	RM555	(AG)11
	RM12727	(CTC)7
	RM324	(CAT)21
	RM279	(GA)16
	RM236	(CT)18
	RM13211	(AGGC)5
	RM109	(AG)16

	MARKER NAME	MOTIF
Chromosome 3	RM523	(TC)14
	RM520	(AG)10
	RM251	(CT)29
	RM15983	(AT)17
	RM6817	(TCT)9
	RM416	(GA)9
	RM16030	(AG)11
	RM517	(CT)15
Chromosome 4	RM16672	(GGA)7
	RM142	(CGG)7
	RM255	(AGG)5(AG)2-(GA)16
	RM551	(AG)18
	RM518	(TC)15
	RM335	(CTT)25
	RM17524	(AGCC)5
Chromosome 5	RM334	(CTT)20
	RM26	(GA)15
	RM249	(AG)5A2(AG)14
	RM87	(CTT)3T(CTT)11
	RM13	(GA)6-(GA)16
	RM440	(CTT)22
Chromosome 6	RM469	(AG)15
	RM589	(GT)24
	RM400	(ATA)63
	RM541	(TC)16
	RM510	(GA)15
	RM19637	(CT)10
	RM20300	(GCT)7
Chromosome 7	RM180	(ATT)10
	RM248	(CT)25
	RM295	(GA)2A(GA)3G2(GA)9
	RM21388	(GGC)7
	RM542	(CT)22
	RM455	(TTCT)5
	RM20776	(GCC)8
	RM320	(AT)11GTAT(GT)13
Chromosome 8	RM210	(CT)23
	RM408	(CT)13
	RM88	(TCT)11
	RM330	(CAT)5
	RM223	(CT)25

	MARKER NAME	MOTIF
Chromosome 9	RM553	(CT)10
	RM296	(GA)10
	RM219	(CT)17
	RM23680	(GA)10
	RM108	(GGT)10
	RM524	(AT)11
	RM24421	–
	RM566	(AG)15
	RM24390	(CT)11
	RM24350	–
	RM444	(AT)12
Chromosome 10	RM222	(CT)18
	RM311	(GT)3(GTAT)8(GT)5
	RM258	(GA)21 (GGA)3
	RM25694	(CT)11
	RM171	(GATG) 5
	RM25185	(CGC)7
	RM271	(GA)15
	RM304	(GT)2(AT)10(GT)33
	RM24932	(CT)11
Chromosome 11	RM552	(TAT)13
	RM287	(GA)21
	RM229	(TC)11 (CT)5C3(CT)5
	RM286	(GA)16
	RM224	(AAG)8(AG)13
	RM21	(GA)18
	RM441	(AG)13
Chromosome 12	RM28166	(CT)12
	RM28076	(CT)13
	RM1261	(AG)16
	RM491	(AT)14
	CG29430	Source from IRRI
	RM28130	(GAG)7
	RM511	(GAC)7
	RM28099	(GCG)7
	RM28048	(CGC)8
	RM519	(AAG)8
	RM28083	(AT)17
	RM28089	(ATC)12
	Indel8	Source from IRRI

Table 2. List of 45 specific SSR markers linked to the respective drought grain yield QTLs and the drought tolerant check varieties

QTLs	SSR Markers	Drought tolerant check variety
$qDTY_{1.1}$	RM431, RM11943, RM12023, RM12091, RM12146, RM12233	N22
$qDTY_{1.2}$	RM212, RM3825	N22
$qDTY_{2.1}$	RM13211, RM6374, RM3549, RM324, RM521, RM327	Apo
$qDTY_{2.2}$	RM109, RM236, RM279	Aday Sel
$qDTY_{2.3}$	RM263, RM573	Apo
$qDTY_{3.1}$	RM520, RM416, RM16030	Apo / IR77298-14-1-2-10 / IR81896-B-195 / IR81896-B-142
$qDTY_{4.1}$	RM551, RM335, RM518	Way Rarem
$qDTY_{9.1}$	RM556, RM24350, RM24390, RM24421	Aday Sel / IR77298-5-6-18
$qDTY_{10.1}$	RM258, RM25694, RM171, RM590	Aday Sel
$qDTY_{12.1}$	RM28166, RM573, RM28048, Indel 8, RM28076, RM28089,RM28099, RM28130, RM1261, RM511, CG29430, RM523	Way Rarem

the PCR reaction was completed, 4 µl of 6X loading dye was added to each well. The PCR products with sizes lower that 180 bp were run on an 8% polyacrylamide gel using 100 volt for about 1 – 2 h. While the PCR products with sizes more than 180 bp were run on a 6% polyacrylamide gel using 90 volt for about 1 – 1½ h. The gel was stained using a Sybrsafe solution and amplified DNA product bands were visualized under a UV trans-illuminator.

Molecular markers and varietal diversity analysis

The emerged bands on the gels were scored. The allele frequency, genetic diversity, Polymorphic Information Content (PIC) and molecular diversity were assessed using Power Marker (v3.25). Dendrograms and diversity between accessions were produced using the Unweighted Pair-Group Method based on Arithmetic Average (UPGMA) using NTSYS-pc software (v2.02). Relationships between the accessions were depicted based on the Jaccard's similarity coefficient. Clustering was based on the grouping of the accessions at 77% similarity coefficient. The linkage disequilibrium between marker pairs was tested at 1% significance level by Power Marker (v3.25).

Results and discussion

Genetic diversity

The Polymorphic Information Content (PIC) values, a reflection of allele frequency and allele diversity among the accessions varied from one locus to another (*Table 3*). These values ranged from 0.12 (Indel 8) to 0.89 (RM335), with a mean of 0.68. Within the chromosomes, chromosome 5 had the highest mean PIC value of 0.75, followed by chromosome 9 and 10 (0.74), while chromosome 7 had the lowest mean PIC value of 0.57. About 89% of the SSR markers genotyped showed PIC values of 0.50 or more. The markers which showed an average PIC value of more than 0.5 indicated that the SSR markers were highly informative and extremely useful for detection of more alleles in the germplasm accessions and distinguished the polymorphic rate of a marker at a specific

Table 3. Allele frequency, genetic diversity and PIC for each SSR marker in Malaysian rice germplasm

Chro no.	Maker name	Allele freq	Gene div	PIC	Chro no.	Maker name	Allele freq	Gene div	PIC
1	RM315	0.38	0.73	0.69		RM518	0.21	0.85	0.83
	RM104	0.29	0.79	0.76		RM335	0.16	0.90	0.89
	RM113	0.45	0.72	0.68		RM17524	0.23	0.83	0.81
	RM129	0.57	0.63	0.60	5	RM334	0.39	0.78	0.76
	RM522	0.48	0.63	0.56		RM26	0.37	0.74	0.70
	RM212	0.26	0.82	0.80		RM249	0.38	0.74	0.70
	RM3825	0.22	0.85	0.84		RM87	0.32	0.76	0.72
	RM582	0.72	0.45	0.43		RM13	0.36	0.80	0.78
	RM12091	0.45	0.75	0.72		RM440	0.34	0.82	0.81
	RM431	0.34	0.77	0.74	6	RM469	0.60	0.59	0.56
	RM11943	0.39	0.73	0.69		RM589	0.34	0.80	0.77
	RM513	0.39	0.70	0.65		RM400	0.37	0.78	0.75
	RM12182	0.24	0.84	0.82		RM541	0.48	0.69	0.65
	RM12146	0.38	0.75	0.72		RM510	0.37	0.73	0.69
	RM12233	0.44	0.71	0.67		RM19637	0.53	0.63	0.58
	RM10488	0.46	0.74	0.72		RM20300	0.32	0.80	0.77
	RM12023	0.81	0.33	0.30	7	RM180	0.55	0.67	0.65
2	RM71	0.31	0.76	0.72		RM248	0.43	0.76	0.74
	RM12979	0.30	0.79	0.76		RM295	0.36	0.73	0.68
	RM12992	0.34	0.82	0.80		RM21388	0.49	0.67	0.62
	OSR17	0.37	0.77	0.74		RM542	0.81	0.34	0.33
	RM262	0.36	0.77	0.74		RM455	0.81	0.31	0.26
	RM263	0.23	0.85	0.84		RM20776	0.52	0.63	0.57
	RM327	0.37	0.75	0.71		RM320	0.40	0.75	0.72
	RM3549	0.21	0.84	0.82	8	RM210	0.34	0.78	0.75
	RM6374	0.34	0.81	0.79		RM408	0.53	0.63	0.57
	RM521	0.32	0.78	0.75		RM88	0.44	0.63	0.55
	RM573	0.31	0.80	0.78		RM330	0.37	0.70	0.65
	RM12460	0.22	0.85	0.83		RM223	0.61	0.59	0.55
	RM12569	0.37	0.74	0.70	9	RM553	0.22	0.83	0.81
	RM555	0.30	0.77	0.74		RM296	0.29	0.80	0.77
	RM12727	0.87	0.23	0.20		RM219	0.22	0.84	0.81
	RM324	0.28	0.83	0.81		RM23680	0.34	0.80	0.77
	RM279	0.36	0.78	0.75		RM108	0.32	0.82	0.79
	RM236	0.66	0.51	0.47		RM524	0.45	0.69	0.64
	RM13211	0.40	0.73	0.68		RM24421	0.43	0.72	0.69
	RM109	0.40	0.69	0.63		RM566	0.22	0.86	0.84
3	RM523	0.70	0.47	0.44		RM24390	0.62	0.57	0.53
	RM520	0.47	0.68	0.63		RM24350	0.38	0.74	0.69
	RM251	0.31	0.79	0.76		RM444	0.35	0.78	0.76
	RM15983	0.34	0.75	0.70		RM222	0.31	0.82	0.79
	RM6817	0.36	0.80	0.78		RM311	0.29	0.83	0.81
	RM416	0.55	0.54	0.44		RM258	0.32	0.76	0.72
	RM16030	0.22	0.83	0.81		RM590	0.27	0.85	0.83
	RM517	0.54	0.67	0.64	10	RM25694	0.56	0.63	0.60
4	RM16672	0.71	0.46	0.43		RM171	0.38	0.76	0.72
	RM142	0.37	0.77	0.74		RM25185	0.23	0.81	0.79
	RM255	0.59	0.56	0.49		RM271	0.30	0.80	0.77
	RM551	0.32	0.77	0.73		RM304	0.41	0.71	0.67

(cont.)

Table 3. (*cont*.)

Chro no.	Maker name	Allele freq	Gene div	PIC	Chro no.	Maker name	Allele freq	Gene div	PIC
	RM24932	0.32	0.78	0.75		RM491	0.24	0.86	0.85
11	RM552	0.76	0.39	0.35		CG29430	0.45	0.60	0.52
	RM287	0.47	0.66	0.60		RM28130	0.41	0.71	0.66
	RM229	0.45	0.71	0.68		RM511	0.43	0.72	0.68
	RM286	0.34	0.75	0.71		RM28099	0.85	0.27	0.25
	RM224	0.30	0.77	0.73		RM28048	0.50	0.66	0.62
	RM21	0.21	0.86	0.84		RM519	0.33	0.74	0.69
	RM441	0.27	0.82	0.79		RM28083	0.65	0.53	0.50
12	RM28166	0.35	0.79	0.76		RM28089	0.56	0.63	0.60
	RM28076	0.59	0.60	0.56		Indel8	0.94	0.12	0.12
	RM1261	0.28	0.81	0.79					

locus (DeWoody et al. 1995). Similarly, earlier studies on genetic diversity in rice also observed higher PIC values among various rice backgrounds such as cultivars, landraces and wild relatives (Ram et al. 2007; Ravi et al. 2003). These findings indicated high genetic diversity due to differences in origin, ecotype and speciation (Ram et al. 2007). Moreover, SSR markers may exhibit high PIC values because of codominant expression, multiallelism (Ferreira and Grattapaglia 1998; Ram et al. 2007) and mostly monolocus (Gracia et al. 2004).

Highest mean allele frequency was observed in Chromosome 7 with a value of 0.55 followed by chromosome 12 with 0.47, while the lowest was 0.34 on chromosome 10 (*Table 3*). The mean allele frequency for 12 chromosomes was 0.41. The average gene diversity over all SSR loci was 0.71, ranging as low as 0.12 (Indel 8) to as high as 0.90 (RM335). Gene diversity refers to the level of biodiversity. It is important to identify the total number of genetic characteristics (heterozygosity) in the genetic makeup of a species in a population. In this study, the results indicated that the germplasm contained relatively high genetic diversity, primarily because they were represented by landraces, breeding lines and also introduced varieties. Besides that, these rice accessions have a wide genetics background because the landraces were gathered from different geographical regions in Malaysia i.e. upland and lowland areas, while the introduced varieties were gathered from exchange programmes with many countries.

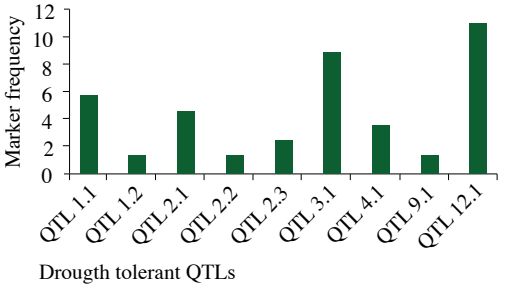

Figure 1. Frequency of SSR markers for each QTL for grain yield under drought in 80 Malaysian rice accessions

Drought grain yield QTLs in Malaysian rice germplasm

Out of 10 QTLs screened for grain yield, 9 were linked to the markers and amplified, namely, $qDTY_{1.1}$, $qDTY_{1.2}$, $qDTY_{2.1}$, $qDTY_{2.2}$, $qDTY_{2.3}$, $qDTY_{3.1}$, $qDTY_{4.1}$, $qDTY_{9.1}$ and $qDTY_{12.1}$ (*Figure 1*). Only 5 QTLs for grain yield were observed to be present among the Malaysian rice accessions. The $qDTY_{12.1}$ was the most common, found in 10.6% of the germplasm. The peak marker for this QTL was between markers RM511 and RM28048. This QTL was present in Way Rarem, Boewani, Pulut Malaysia 1, CI-9534, IR1561-243-5-6,

CICA4, IR2797-156-3, Chianung Sen Yu, MR142 and Huma Wangi Lenggong. Second highest was *qDTY*$_{3.1}$ present in 8.5% of the germplasm. The peak marker for this QTL was between markers RM416 and RM520 present in Apo, IR81896-B-195, IR81896-B-142, Mokwoo, Way Rarem, CICA 4, Biris, MR185, Tainan 5 and Q70. The *qDTY*$_{1.1}$ was observed in 5.3% of the germplasm. The peak marker for this QTL was between markers RM11943 and RM12023 present in Vandana, N22, Bangkok, Boewani and IR1561-243-5-6; *qDTY*$_{2.1}$ was present in Apo, Siam Pilihan, Merah Wangi and Y755; *qDTY*$_{4.1}$ was present in Vandana, Biris and Kashmir Basmati. The remaining QTLs namely *qDTY*$_{2.3}$, *qDTY*$_{1.2}$, *qDTY*$_{2.2}$ and *qDTY*$_{9.1}$ were present in drought tolerant check varieties namely IR77298-14-1-2-10, Apo, N22 and Aday Sel.

The results obtained were in agreement with an earlier report by Swamy et al. (2011) on the validation of major effect on grain yield QTLs in a panel of 96 drought lines. They observed that *qDTY*$_{12.1}$ was highest, present in 85% of the lines. The study also identified several QTLs for grain yield as Mega-QTLs (MQTLs) which were most precise and consistent across the environments and genetic backgrounds that are useful in Marker-assisted Selection (MAS), fine mapping, candidate gene identification and functional analysis. The MQTLs were MQTL$_{1.2}$, MQTL$_{1.3}$, MQTL$_{1.4}$, MQTL$_{12.1}$, MQTL$_{3.2}$, MQTL$_{4.1}$ and MQTL$_{2.1}$. These MQTLs were important for MAS because of their small genetic region (less than 1.3 Mb) and physical intervals (around 6 cM) and also a phenotypic variance of more than 10% (Swamy et al. 2011).

Genetic similarity among the Malaysian rice accessions

Two UPGMA cluster analysis were performed and dendrograms were constructed, using all the 119 SSR markers and 45 specific SSR markers linked to drought grain yield QTLs respectively. The clustering for 119 SSR markers was based on grouping of the accessions at 77% similarity coefficient. The UPGMA using all the 119 marker genotypes grouped the 94 genotypes (80 rice accessions and 14 check varieties) into 7 clusters (*Figure 2*). Cluster 1 consisted of 10 drought tolerant check varieties namely UPLRi7, IR77298-14-1-2-10, Apo, Vandana, IR81896-B-195, IR81896-B-142, Way Rarem, IR77298-5-6-18, Mokwoo, PSBRC64 and 2 susceptible check varieties, MTU1010 and IR64. Aday Sel as one of the drought tolerant check variety, was grouped together with Basmati 370 in Cluster 6. While, the remaining Malaysian rice accessions were separately grouped in 4 clusters (Cluster 2 - Cluster 5).

The clustering for the 45 specific SSR markers was also based on grouping of the accessions at 77% similarity coefficient. The 94 genotypes were grouped into 9 clusters (*Figure 3*). Cluster 1 consisted of 4 drought check varieties namely IR64, MTU1010 (susceptible check variety), UPLRi7 and IR77298-141-1-2-10 (tolerant check variety). Cluster 2 consisted of 6 drought tolerant check varieties namely Apo, Vandana, PSBRC64, Way Rarem, Mokwoo and IR77298-5-6-18. Cluster 3 showed several rice accessions, namely, Q31, Maswangi MRQ74, New Rex, IR20, Pusa Basmathi, IR2061-213-2-16 and Q72 grouped together with N22. Cluster 6 and 7 also showed several rice accessions grouped together with drought tolerant check varieties. Cluster 6 consisted of Bangkok, CICA4 and Kurau Wangi, while the check varieties were IR81896-B-195 and IR81896-B-142. Cluster 7 consisted of Q74 with Aday Sel as check variety. The remaining rice accessions were separately grouped into 4 clusters, namely, cluster 4, 5, 8 and 9.

Based on the pattern of these 2 dendrograms, the clustering patterns showed precise similarities in terms of genetic background of each variety. The 2 IRRI lines, namely, IR81896-B-195 and IR81896-B-142, which were Near Isogenic

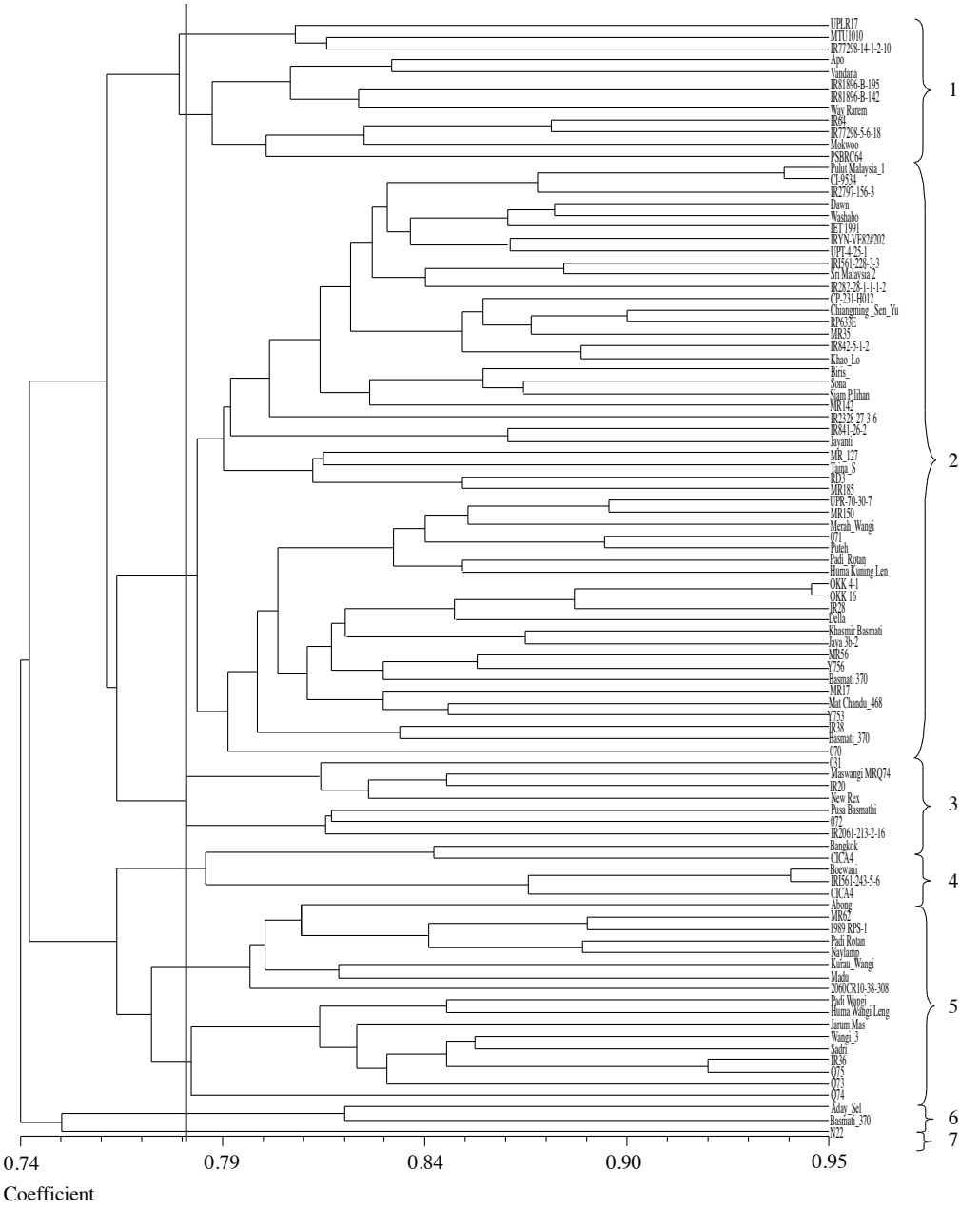

Figure 2. Dendrogram resulting from the analysis of all 94 rice accessions using 119 SSR markers

Lines (NILs) developed for lowland drought tolerant variety (Kumar 2012), showed close genetic similarities in both types of SSR markers. Besides that, 2 improved Malaysian rice varieties, namely, QKK 4-1 and QKK-16 also showed closest genetic similarities in both SSR markers. This indicated that both SSR markers can successfully group the Malaysian rice germplasm according to their genetic similarities.

Drought tolerant check variety, N22, was developed for highly risk prone irrigated environment which possessed QTLs for grain yield under drought on chromosome 1,

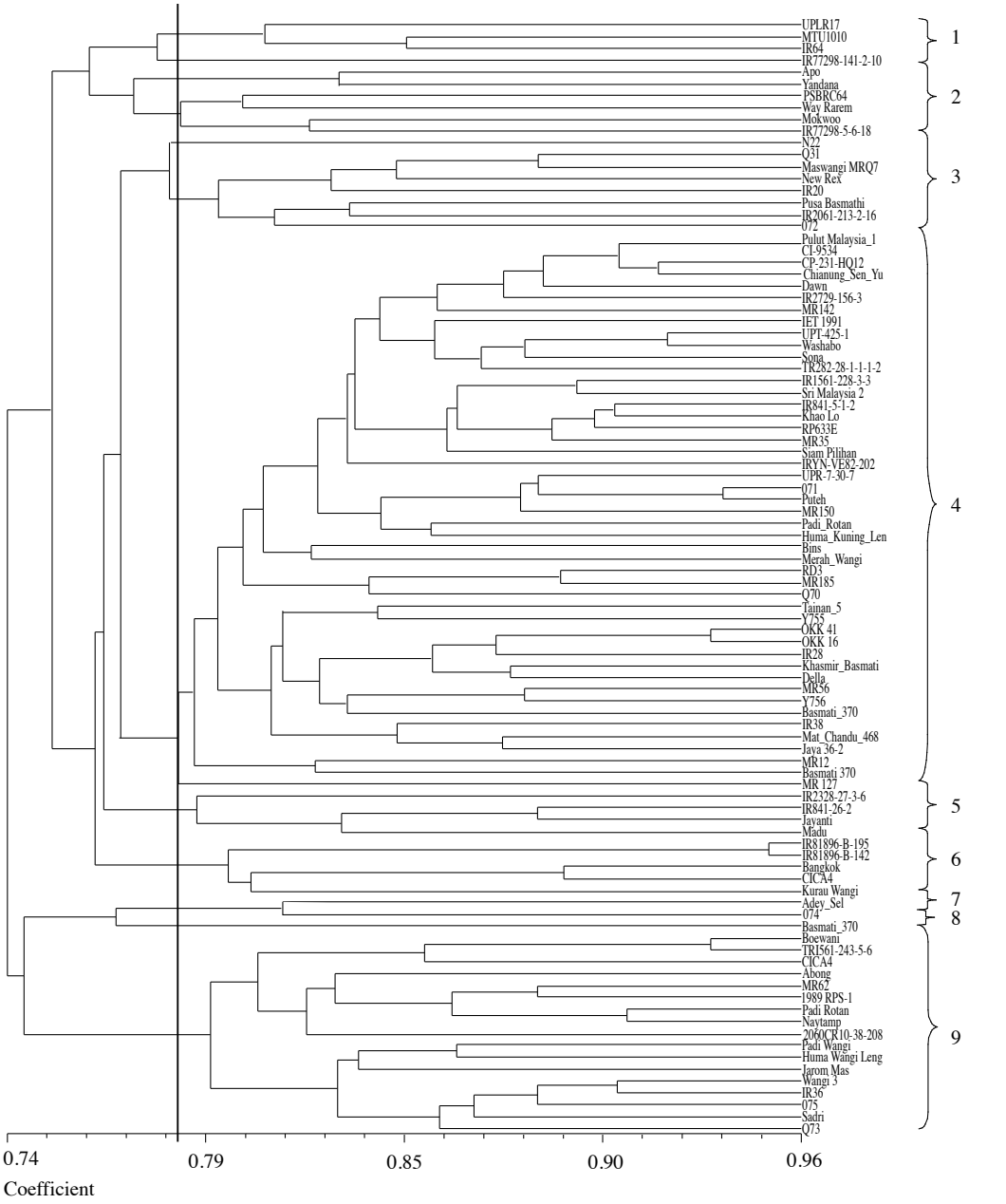

Figure 3. Dendrogram resulting from the analysis of all 94 rice accessions using 45 SSR markers linked to grain yield QTL

namely, $qDTY_{1.1}$ and $qDTY_{1.2}$. Another 2 drought tolerant check varieties, namely, IR81896-B-195 and IR81896-B-142, also possessed drought grain yield QTLs, namely, $qDTY_{3.1}$. The Malaysian rice accessions which carried these QTLs and grouped together with these check varieties were CICA4 and Bangkok. As reported by Vikram et al. (2011), several QTLs may be present and showed large and consistent effect in one population. The study observed that several QTLs, namely, $qDTY_{1.1}$,

qDTY$_{2.3}$ and *qDTY*$_{3.2}$, from N22 were important for increasing grain yield under drought stress present in 3 $F_{3:4}$ populations. Since, several QTLs may be present in one variety, this explained the difficulties in clustering these Malaysian rice accessions based on each QTLs. Fine mapping, the creation of a genetic map assigning DNA fragments to chromosomes, maybe the best approach to identify the specific QTLs in these Malaysian rice accessions.

Linkage disequilibrium of Malaysian rice germplasm

The pair-wise linkage disequilibrium (LD) observed at interchromosomal, intrachromosomal and over all the 12 chromosomes is presented in *Table 4*. The results showed that 5 pairs of markers were present on chromosome 2, 3 pairs on chromosome 1, 2 pairs on chromosome 6 and 1 pair each on chromosomes 3, 4, 9, 10, 11 and 12. There were no marker pairings observed on chromosomes 5, 7 and 8.

The correlation coefficient of the frequencies, r^2 value, showed ranges between 0.008 (chromosome 4) and 0.098 (chromosome 12). It was estimated that the values of $r^2 > 0.0703$ were probably due to genetic linkage according to Breseghello and Sorrells (2006). The intra-chromosomal marker pairs showed a significant level of LD ($p < 0.01$), while the marker pairs in LD were observed on chromosome 2, chromosome 6 and chromosome 9. The pair-wise r^2 of the markers on chromosome 12 were higher than 0.0703, however, the LD level was not significant ($p > 0.01$). The pair-wise r^2 from this study did not reach the baseline of 0.0703 except for chromosome 12. This may indicate that the marker density in this study was not sufficient to detect consistent LD (Yu et al. 2012).

Conclusion

The study observed that the Malaysian rice accessions contained relatively high genetic diversity and presence of important QTLs

Table 4. Proportion, χ^2 and r^2 values of SSR marker pairs in linkage disequilibrium in 80 Malaysian rice accessions

Marker pairs	Number of pairs	P	Mean r^2 for marker pairs	$\chi 2$
Inter–chromosomal	102 (4356)	0.02	0.020	0.24
Intra-chromosomal	16 (847)	0.01	0.024	0.28
Chromosome 1	3 (108)	0.02	0.020	0.24
Chromosome 2	5 (337)	0.01	0.022	0.26
Chromosome 3	1 (21)	0.04	0.013	0.15
Chromosome 4	1 (17)	0.05	0.008	0.09
Chromosome 5	0 (0)	–	–	–
Chromosome 6	2 (112)	0.01	0.020	0.24
Chromosome 7	0 (0)	–	–	–
Chromosome 8	0 (0)	–	–	–
Chromosome 9	1 (64)	0.01	0.025	0.30
Chromosome 10	1 (48)	0.02	0.018	0.21
Chromosome 11	1 (24)	1.14	0.014	0.16
Chromosome 12	1 (16)	1.16	0.098	1.17
Total	134 (5950)	0.18	0.020	

( ) = Total number of unpaired markers for each chromosome
χ^2 = Test statistics for the marker pairs in each chromosome
P = Proportion of marker pairs in LD
r^2 = Correlation coefficient between a pair of the alleles

for grain yield under drought stress. The germplasm which carried the QTLs were Huma Wangi Lenggong, Siam Pilihan, Merah Wangi, Biris, Bangkok, CI-9534, CICA 4, Chianung Sen Yu, Tainan 5, Boewani, Kashmir Basmati, IR1561-243-5-6, IR2797-156-3, IR1561-243-5-6, Y755, Q70, Pulut Malaysia 1, MR 142 and MR 185. Rice accessions identified in this study could be useful for future breeding programmes especially for developing drought tolerant rice variety.

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Abstrak

Kepelbagaian 80 asesi padi Malaysia dinilai berdasarkan 119 penanda berulang jujukan mudah (SSR). Nilai kandungan maklumat polimorf (PIC) menunjukkan 89% daripada penanda SSR melebihi 0.50 membuktikan bahawa penanda molekular yang digunakan adalah sangat bermaklumat. Kehadiran ciri lokus kuantitatif (QTLs) dinilai berdasarkan 45 penanda molekular yang berhubung kepada QTLs hasil padi semasa keadaan kemarau. Terdapat sebanyak 5 QTLs hadir pada 80 asesi padi Malaysia. Frekuensi tertinggi ialah 10.6% pada $qDTY_{12.1}$ hadir dalam Way Rarem, Boewani, Pulut Malaysia 1, CI-9534, IR1561-243-5-6, CICA4, IR2797-156-3, Chianung Sen Yu, MR142, dan Huma Wangi Lenggong. Frekuensi kedua tertinggi ialah $qDTY_{3.1}$ sebanyak 8.5% terdapat dalam germplasma hadir dalam Apo, Mokwoo, Way Rarem, CICA 4, Biris, MR185, Tainan 5 dan Q70. Dua analisis kluster UPGMA dibina berdasarkan 119 penanda SSR dan 45 penanda SSR spesifik yang berhubung dengan QTLs hasil padi dalam keadaan kemarau. Asesi padi Malaysia telah dikelaskan pada 77% pekali keserupaan dan masing-masing menghasilkan tujuh kluster menggunakan 119 penanda SSR dan 9 kluster menggunakan penanda SSR spesifik.

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