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EXPLOITING WILD ACCESSIONS FOR DEVELOPMENT OF HIGH YIELDING NEW RICE GENOTYPES

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ABSTRACT

Eight transgressive variants were selected from a cross between *Oryza rufipogon* Griff. (IRGC105491) and MR219, a Malaysian high yielding rice cultivar. The field trials revealed the yield potentiality of the variants and showed significantly ($p<0.05$) higher yield than the control, MR219. Quantitative trait loci (QTLs) for agronomic traits were validated in the selected variants in BC_2F_5 generation. The yield of these variants was influenced by several QTLs related to days to maturity, tillers per plant, panicles per plant, spikelets per panicle and thousand grain weight. Chromosome segment analysis confirmed the introgression of wild alleles for yield and yield related traits. Registration process for eight variants has been initiated under the National Plant Variety (NPV) Act of Malaysia with the preferred name of UKMRC1 to UKMRC8. The DUS test was conducted in 2011 following UPOV guidelines in collaboration with Department of Agriculture, Malaysia to confirm distinctness, uniformity and stability of these variants. Steps will be taken for some of the promising registered variants to be released as new varieties.

Key words: *Oryza rufipogon,* inter-specific hybridization, introgression, yield potentiality, transgressive variants, DUS test

INTRODUCTION

An inter-specific hybridization between genetically diverse parents offers a way of widening the gene pool of cultivated rice. It also broadening the range of genetic variation for plant improvement by selection and thus, enhancing the possibility of identifying transgressive variants that outperform the parents by a substantial amount. Wide hybridization has been used for many years to introduce qualitative characters such as disease and insect resistance and male sterility (Brar & Khush 1997; Dalmacio *et al.,* 1995) and pericarp colour (Bhuiyan *et al.,* 2011) from wild species into the elite breeding materials. More recently quantitative traits have been targeted in inter-specific crossing programs which introduce the genes from exotic sources for further improvement of yield and its related traits which have been successful in many crop species (McCarty *et al.,* 2004; Sackes *et al.,* 2003).

For most of the quantitative traits, a phenotype is conditioned by several genes having either trait-enhancing or trait-depressing alleles (Xiao *et al.,* 1998). Introgression of diverse alleles for yield improvement may increase the rate of yield (Reyna & Sneller, 2001). Currently, rice breeding faces the problem of yield plateaus, caused by narrow genetic base of parent materials (Rangel *et al.,* 1996; Tanksley & McCouch, 1997). Exploitation and utilization of the favourable alleles of wild rice might be able to overcome the yield plateaus. Xiao *et al.* (1998) reported that even though *O. rufipogon* is phenotypically inferior for all of important traits, transgressive segregants outperformed over the *O. sativa* background cultivar. The AB-QTL strategy can identify hidden alleles from the wild species that can increase the yield potential of commercial cultivars (Tanksley & McCouch 1997). This method has been applied to rice with promising results where trait improving QTL alleles from an inferior phenotype, namely the wild rice relative *O. rufipogon*, were identified in the backgrounds of the elite Chinese hybrid V20/Ce64 (Xiao *et al.,* 1998), the upland Brazilian rice variety Caiapo (Moncada *et al.,* 2001), the U.S. tropical *japonica* cultivar Jefferson (Thomson *et al.,* 2003) and the Malaysian *indica* cultivar MR219 (Sabu *et al.,* 2006; Wickneswari *et al.,* 2012).

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Initially 26 BC_2F_5 variants were evaluated through multi-location trials and eight BC_2F_8 transgressive variants with high yield and early maturity were filed for registration under the NPV Act of Malaysia. The DUS test was used to confirm the distinctness, uniformity and stability of the new plant varieties. The objectives of this scientific paper are to report the findings on i) performance of transgressive variance on yield and its related traits using multi-environment replicated field trials, ii) validation of QTLs in advanced breeding lines and iii) assessment of recurrent parent genetic background along with the introgressed chromosome segments conferring different yield related QTLs from the wild parent and iv) distinctness, uniformity and stability of the promising transgressive variants.

MATERIALS AND METHODS

Plant Materials

Twenty six transgressive variants (Table 1) were evaluated in multi-location field trail under three different locations in Malaysia. Eight variants were selected on the basis of field performance in BC_2F_5 and BC_2F_6 generation for QTL validation and introgressed chromosome segment analysis.

Experimental field and design

The seedlings of BC_2F_5 generation were grown in the experimental field of Malaysian Agricultural Research and Development Institute (MARDI) Seberang Perai, Penang and transplanted to the fields at three different locations at the age of twenty-four-days under irrigated condition. Two locations were selected from farmer's field from two rice growing areas (Sungai Besar, Selangor and Gurun, Kedah) and the other location was selected from the research field of MARDI and Department of Agriculture, Malaysia to evaluate field trait value under different environments. Crop growth periods were in the off season (March, 2007 to August, 2007) and main season (September, 2007 to February, 2008). Thus, the experiments were conducted under six different environments. The variants and the cultivar, MR219 (used as control) were considered as treatments and assigned in the randomized complete block design (RCBD) with three replications. A total of 81 experimental plots were used with an area of 4m x 4m in each environment. Fertilizer application and cultural operations were done following the local farmer's practice (N:P:K=100:80:60 kg/ha).

Table 1. Designation and code of the evaluated transgressive variants in BC_2F_5 generation

Designation	Code	Designation	Code
R1-1-1-1	G1	R14-9-69-4	G15
R1-2-2-1	G2	R14-9-69-5	G16
R2-7-15-5	G3	R16-8-80-2	G17
R2-10-18-2	G4	R17-1-83-2	G18
R4-9-27-1	G5	R17-1-83-3	G19
R4-9-27-4	G6	R19-4-95-3	G20
R6-2-31-2	G7	R19-4-96-3	G21
R7-6-38-2	G8	R19-5-96-4	G22
R7-7-39-4	G9	R19-9-98-5	G23
R8-9-49-10	G10	R24-10-106-1	G24
R9-5-55-3	G11	R26-2-108-1	G25
R9-9-58-2	G12	R26-6-113-1	G26
R14-9-69-2	G13	MR219 (Control)	G27
R14-9-69-3	G14		

Yield trait evaluation and analysis

The procedures for field trait evaluation are described in Bhuiyan *et al*. (2011). The replicated data for different agronomic traits were analyzed using different statistical approaches. Duncan's Multiple Range Test ($\alpha = 0.05$) was used for mean separation using PROC MEANS of SAS version 9.1.3.

QTL validation in the selected advanced breeding lines

Eight BC_2F_5 breeding lines were selected for QTL validation. The methods of field evaluation and QTL validation of the materials are described in Wickneswari *et al*. (2012).

Introgressed chromosome segment analysis for selected variants

Genotyping was done for the eight selected variants and the procedures for genotyping are described in Bhuiyan *et al*. (2011). Graphical genotype GGT 2.0 (van Berloo, 2008) was used for recurrent parent genetic background analysis and detection of chromosome segment introgression from the wild parent.

Distinctness, Uniformity and Stability (DUS) test

DUS test for new varieties is based on visual assessments, scores and measuring of certain characteristics of a variety. The test was conducted at Bumbung Lima, Seberang Perai based on the UPOV Test Guidelines (Jabatan Pertanian Malaysia, 2009) under the supervision of Department of Agriculture, Malaysia. The trial was established following RCBD with two replications. Each replication consisted of 800 plants and 10 plants were selected randomly for data collection for quantitative traits. A total of 58 out of 60 recommended characters were assessed of which 13 were quantitative and 45 were qualitative. Two characters, kneeing ability and male sterility were not assessed as these characters were not relevant for the new varieties being tested.

Distinctness: The difference observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not much that more than a single growing cycle is required to provide assurance that the differences observed between varieties are significantly consistent. Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the character being examined, i.e. whether it is expressed in a qualitative or quantitative manner.

Uniformity: For the assessment of uniformity of the characters on the plot as a whole, the acceptance probability of 95% was applied. In case of the experiment (sample size of 1500 plants), the maximum number of off-types allowed was 4.

Stability: In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. When a variety showed uniformity, it is considered as stable.

RESULTS AND DISCUSSION

Performance of the variants for yield traits

Table 2 shows the mean performance of the individual variants for different yield traits. Variant G7 showed the highest yield value among the variants. The mean yield of variant G7 across the environments was 7% higher than the recurrent parent, MR219; contributed by yield enhancing alleles from *O. rufipogon* (Table 3). Variants G6, G7, G8, G15 and G25 produced high number of panicles per plant across the environments. Variant G6 produced higher number of spikelet per plant followed by G11 and G7 compared to other variants across the environments but was not significantly different from MR219. Ram *et al*. (2007) found similar results from a study using *O. rufipogon* as a donor parent with *indica* background in an advanced breeding line. They reported that eight lines derived from wide crosses were superior in yield than both of the *indica* parents by 10.2-21.4%. Variants G15 and G26 showed short growth duration compared to MR219 which could be due to introgression of the

Table 2. Mean value for yield traits across the environments

Variants	DTM	TPL	PPL	SPL	TGW	YLD
G1	123	20.0	18.9	2303	26.3	5.45
G2	122	18.5	17.5	1881	28.4	5.81
G3	121	17.4	16.7	1941	27.3	5.66
G4	121	17.5	17.2	2004	27.5	5.89
G5	124	19.0	18.2	2345	26.2	5.90
G6	124	20.1	19.6	2462	25.5	5.96
G7	122	19.1	18.3	2400	25.8	6.47
G8	121	20.2	19.1	2032	29.6	6.21
G9	121	17.3	16.8	2279	25.5	5.70
G10	122	18.1	17.7	2382	24.5	5.63
G11	123	17.3	16.8	2453	24.8	6.03
G12	122	16.9	16.4	1925	28.6	5.70
G13	121	17.3	17.0	2119	26.3	6.06
G14	121	18.3	17.7	2074	26.5	6.00
G15	118	20.5	19.2	1868	28.2	5.94
G16	121	17.7	16.7	2103	26.3	6.06
G17	122	17.4	16.7	2210	26.0	5.92
G18	121	18.9	18.2	2122	26.6	5.60
G19	122	18.8	18.0	2206	27.1	6.09
G20	122	18.3	17.2	2170	26.1	5.59
G21	123	20.0	19.2	2226	24.9	5.81
G22	121	19.8	18.9	2098	23.7	5.50
G23	121	18.6	17.7	2075	26.4	5.35
G24	121	19.3	18.1	2185	26.7	5.46
G25	123	19.3	19.3	2281	27.4	5.80
G26	118	16.5	15.6	1864	26.9	5.57
G27	125	17.3	17.1	2314	25.5	6.05
CV	0.74	11.1	12.0	12.15	2.7	6.1
$LSD_{0.05}$	0.59	1.37	1.40	172	0.47	0.23

Abbreviations used: days to maturity (DTM), tillers per plant (TPL), panicles per plant (PPL), spikelet per plant (SPL), 1000 grain weight, gm (TGW), yield, t/ha (YLD)

Table 3. QTLs validated in the variants of BC_2F_5 generation

Variant	QTLs for different traits
G5	qPPL-6, qTPL-6, qDTM-11,qDTM-4
G7	qPPL-6, qTPL-6
G8	qTPL-2, qPPL-2, qSPL-1-1, qPPL-6, qTPL-6
G13	qDTM-1-2, qDTM-6, qGW-6
G15	qSPL-8
G16	qSPL-8, qDTM-1-2
G19	–
G26	qSPL-1-1,qDTM-1-1

QTLs for earliness from *O. rufipogon*. Xiao *et al*. (1996, 1998) and Septiningsih *et al*. (2003) reported similar results from their studies with the same wild donor parent. Variant G8 showed significantly higher value for thousand grain weight followed by G12 across the environments.

Validation of QTLs for different agronomic traits of the variants

A total of 12 QTLs (Table 3) were identified in the selected population comprising of eight transgressive variants in BC_2F_5 generation. The QTL details are given in Wickneswari *et al*. (2012). The QTLs identified in the BC_2F_5 generation were controlled the traits for yield component and growth period. All QTLs detected in the earlier generation (BC_2F_2) were found in the advanced generation. One QTL for grain weight (qGW6) was newly detected in the evaluated breeding lines. The QTL was gained in the population during the advancement of the generation as they were in heterozygous condition. Cho *et al*. (2007) found similar results from an investigation of QTL evaluation in rice. Two QTLs (qTPL-2, and qTPL-6) for tillers per plant and two QTLs (qPPL-2, qPPL-6) for panicles per plant were detected as the regulator of the traits. The *O. rufipogon* allele showed the increased effect for tillers per plant and panicles per plant at all the loci. The same QTL was identified in the BC_2F_2 generation. Thomson *et al*. (2003) found the other QTLs located on chromosome 3 and 7 which controlled panicles per plant. Septiningsih *et al.* (2003) observed in a study, that the QTLs qPPL-1-1, qPPL-1-2, qPPL-11-1 and qPPL-11-2 for panicles per plant had the increased effect by IR64. Xiao *et al*. (1998) found two genomic region on chromosomes 1 and 2 for the number of panicle per plant using a hybrid parent with *O. rufipogon*. Tian *et al.* (2006) also found two QTLs qPPL-1 and qPPL-2 which had the positive effect and increased the panicle number in Guichao 2, a high yielding commercial *indica* cultivar. The QTLs qSPL-1-1 and qSPL-8 were detected for spikelets per plant located on chromosomes 1 and 8. The increased effect was contributed by *Oryza rufipogon* allele for spikelets per plant at one locus on chromosome 1 and this QTL significantly increased spikelets per plant in the population. Both of these two QTLs were found in the earler generation (BC_2F_2). Five QTLs for days to maturity were detected on five chromosomes namely chromosomes 1, 4, 6 and 11. The *O. rufipogon* allele caused earliness at two loci (qDTM-1-1 and qDTM-11) and late maturity at two loci viz., qDTM-4 and qDTM-6. Among the variants, G26 showed the significant earliness in the phenotypic evaluation indicating that the QTLs which were carried by the lines is stable. Some of the lines had the QTLs for both early and late maturity but phenotypically showed their late maturity. This result might be occured due to the superiority of the QTLs responsible for the late maturity. Septiningsih *et al.* (2003) found the the *O. rufipogon* alleles on chromosome 1 was associated with earliness of heading and was also associated with earliness of maturity.

Genetic background and introgressed segment analysis

The graphical genotyping of the promising variants was done through the software GGT 2.0 (van Berloo 2008). The background and introgressed segment analysis is summarised in Table 4.

Background analysis of the variants showed an average recovery of 84.5% of the MR219 genome while that of *Oryza rufipogon* was 8.9% with residual heterozygosity of 2.0%. The similar result was found by Gopalkrishnan *et al*. (2008) and reported from a study of background analysis using 31 BC_1F_5 lines that the selected variants (recombinants) carried the recurrent parent genome to the extent of 86.3%. Xiao *et al.* (1998) found in a study using BC_2 test cross population that the introgressed families contained 1.5 to 11.5% of wild genome. A large heterozygous genome was found in variant G8 having 5 heterozygous segments. Homozygosity might be appeared in this variant after selfing in further generation.

DUS test

Table 5 shows the distinctness among the variants for some traits. The variants were grouped for the specific traits. Significant differences indicate the distinctness of the traits. The distinctness was controlled by the genes of the individual.

Table 6 shows the differences between the replication averages of the individual variant for specific traits. The non-significant differences indicated the uniformity of the specific trait, and thus implying stability of that trait.

Table 4. Summary on background and introgressed segment analysis

Variant	Preferred name	A (%)	B (%)	H (%)	U (%)	H-segments
G5	UKMRC1	90.2	3.1	2-0	4.7	1
G7	UKMRC2	88.3	6.8	0.5	4.4	1
G8	UKMRC3	83.4	8.3	5.5	2.7	5
G13	UKMRC4	78.3	11.1	3.4	7.2	3
G15	UKMRC5	79.4	11.4	2.8	6.4	2
G16	UKMRC6	81.0	10.3	0.0	8.7	0
G19	UKMRC7	85.9	12.2	0.6	1.4	1
G26	UKMRC8	89.4	8.7	1.9	0	2
Ave.		84.5	8.9	2.0	4.4	

A = MR219 genome, B = *O.rufipogon* genome, H = Heterozygous region, U = Undetected region

Table 5. Distinctness among the variants for some traits in DUS test

Variants	Mean value of the traits							
	Grain length	Geed width	Panicle length	Panicle number	Culm thickness	Culm length	Leaf length	Leaf width
UKMRC1	10.05^{cde}	2.30^{bc}	26.94^{a}	23.7^{abcd}	7.40^{a}	98.67^{a}	54.25^{a}	14.35^{ab}
UKMRC2	10.10^{bcde}	2.44^{abc}	26.68^{a}	22.7^{abcde}	7.10^{ab}	98.90^{a}	51.81^{abcd}	14.76^{a}
UKMRC3	10.37^{bc}	2.48^{abc}	26.12^{ab}	25.4^{ab}	7.25^{ab}	94.65^{abc}	50.27^{bcd}	12.95^{ab}
UKMRC4	10.31^{bcd}	2.29^{bcd}	26.82^{a}	21.9^{bcde}	6.88^{ab}	93.63^{bc}	50.13^{bcd}	13.31^{ab}
UKMRC5	10.74^{a}	2.36^{abc}	26.56^{a}	22.1^{bcde}	6.33^{b}	87.85^{def}	48.06^{d}	14.23^{ab}
UKMRC6	10.43^{ab}	2.25^{cde}	27.32^{a}	20.1^{de}	6.85^{ab}	96.50^{ab}	49.38^{cd}	14.03^{ab}
UKMRC7	9.92^{e}	2.61^{a}	24.74^{c}	26.0^{a}	6.85^{ab}	92.65^{bcd}	52.46^{abc}	14.10^{ab}
UKMRC8	9.55^{f}	2.29^{bcd}	26.50^{a}	20.5^{cde}	7.13^{ab}	91.00^{cde}	52.57^{abc}	14.46^{ab}

Means with the same letter are not significantly different at $p<0.05$

Table 6. Uniformity of the variants for some traits in DUS test

Variants	Differences between two replications							
	Grain length	Grain width	Panicle length	Panicle number	Culm thickness	Culm length	Leaf length	Leaf width
UKMRC1	0.06^{ns}	0.16^{ns}	0.17^{ns}	1.10^{ns}	0.40^{ns}	3.85^{ns}	0.70^{ns}	2.21*
UKMRC2	0.08^{ns}	0.01^{ns}	0.23^{ns}	1.80^{ns}	0.60^{ns}	2.20^{ns}	3.04^{ns}	1.84*
UKMRC3	0.51^{ns}	0.02^{ns}	1.55^{ns}	0.80^{ns}	0.60^{ns}	2.90^{ns}	2.08^{ns}	0.76^{ns}
UKMRC4	0.01^{ns}	0.46^{ns}	1.00^{ns}	3.30^{ns}	0.95^{ns}	1.95^{ns}	0.34^{ns}	1.22^{ns}
UKMRC5	0.39^{ns}	0.19^{ns}	0.48^{ns}	1.40^{ns}	0.45^{ns}	4.70*	1.67^{ns}	3.02*
UKMRC6	0.33^{ns}	0.06^{ns}	1.08^{ns}	3.10^{ns}	0.60^{ns}	5.40*	1.96^{ns}	0.33^{ns}
UKMRC7	0.02^{ns}	0.24^{ns}	1.40^{ns}	3.80^{ns}	0.60^{ns}	3.50	4.89^{ns}	0.07^{ns}
UKMRC8	0.00^{ns}	0.14^{ns}	0.37^{ns}	1.30^{ns}	0.10^{ns}	2.30^{ns}	3.84^{ns}	0.00^{ns}

^{ns}no significant different, * significant different at $p<0.05$

CONCLUSIONS

The results indicated that the wild relatives of cultivated rice, *O. rufipogon*, contain alleles that can help to improve agronomic traits, including yield. This discovery implies that the world's reservoir of wild and unadapted germplasm may hold the key to increase of future productivity in rice. Among the evaluated variants, G5, G7, G8, G13, G16 and G19 are promising for high yield; variants G15 and G26 are promising for short growth duration along with high yield. These transgressive variants were filed for new plant variety status under the National Plant Variety Act with preferred name UKMRC 1 (G5), UKMRC 2 (G7), UKMRC3 (G8), UKMRC4 (G13), UKMRC 5 (G15), UKMRC 6 (G16), UKMRC 7 (G19) and UKMRC8 (G26).

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