



# Identification of introgressed alien chromosome segments associated with grain quality in *Oryza rufipogon* x MR219 advanced breeding lines using SSR markers

P. Fasahat<sup>1</sup>, K. Muhammad<sup>2</sup>, A. Abdullah<sup>3</sup> and R. Wickneswari<sup>1</sup>

<sup>1</sup>School of Environmental and Natural Resource Sciences, National University of Malaysia, Kuala Lumpur, Malaysia

<sup>2</sup>Department of Food Science, University Putra Malaysia, Kuala Lumpur, Malaysia

<sup>3</sup>School of Chemical Science and Food Technology, National University of Malaysia, Kuala Lumpur, Malaysia

Corresponding author: R. Wickneswari

E-mail: wicki@ukm.my

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**ABSTRACT.** A limited backcross procedure was utilized to introgress genes associated with grain quality traits from *Oryza rufipogon* (Accession No. IRGC 105491), a wild rice from Malaysia, to the cultivated rice *O. sativa* cv. MR219, a popular high yielding Malaysian rice cultivar. A set of 10 BC<sub>2</sub>F<sub>7</sub> progenies were selected based on the field performance and phenotypic appearance in BC<sub>2</sub>F<sub>5</sub> and BC<sub>2</sub>F<sub>6</sub> generations, which initially started with 266 progenies in the BC<sub>2</sub>F<sub>2</sub> generation. These 10 advanced breeding lines are similar to each other but differ in several important grain quality traits, which can be traced to *O. rufipogon* introgressions. Phenotyping and genotyping of BC<sub>2</sub>F<sub>7</sub> variants were considered for QTL analysis. The introgressed lines did not show any significant changes compared to the recurrent parent MR219 for the traits grain density and milled rice percentage. All 10 progenies showed significantly higher head rice percentages (70-88%) than the recurrent parent

MR219. Variants G13 and G15 had higher amylose contents than MR219. All variants were analyzed using polymorphic SSR markers. Of the 34 SSR markers, only 18 showed introgression from *O. rufipogon* for chromosomes 1, 2, 3, 5, 6, 8, 10, and 11. Graphical genotypes were prepared for each variant, and association between the introgression regions and the traits that increased grain quality was visualized. Based on marker trait association, some of the QTLs are stable across environments and genetic backgrounds and could be used universally.

**Key words:** Advanced breeding lines; Grain quality; Molecular analysis

## INTRODUCTION

Nearly two-thirds of the world's population consumes rice (*Oryza sativa* L.) as the staple food. It has been reported that as much as 75% of the daily calorie intake of the people in some Asian countries is derived from rice. The genus *Oryza* comprises more than 20 wild and two cultivated species. The wild species of *Oryzae* are an important source of genetic variation for agronomic traits such as resistance to various biotic and abiotic stresses and for improved yield potential (Brar and Khush, 1997). Since the wild germplasm has not been thoroughly exploited yet, there is still a great potential to develop new modern rice varieties by incorporating new useful genes from wild races. The common wild rice *O. rufipogon* is the most important genetic resource for rice improvement in terms of its accessibility from gene transfer through sexual means (Oka, 1988). It is widely distributed in South and Southeast Asian countries, including Malaysia where *O. sativa* is a major staple food and extensively cultivated.

Discovery of the productivity-increasing potential of alien genes came primarily from the study of Frey et al. (1983) in oats. Tanksley et al. (1996) conceived the synergistic association of QTL mapping and alien gene introgression and suggested the AB-QTL strategy. Next, mining of productivity QTLs from wild and exotic donors (usually having the same genome completion as the recipient) under parallel backcrossing and evaluation, together with molecular marker analysis, appeared to be a powerful plant breeding strategy. The first successful utilization of this method in rice (Xiao et al., 1996, 1998) increased interest considerably. In that study, chromosome segments from a Malaysian accession of *O. rufipogon* (IRGC 105491) resulted in a yield increase in the BC<sub>3</sub> testcross progeny, using parental lines of an elite hybrid variety from China as recurrent parent. The present study used the Malaysian accession of *O. rufipogon* (IRGC 105491) in context of a pure line recipient (MR219), which is known for its high yield. Two other studies with the same *O. rufipogon* accession but different recipient genotypes for productivity genes were carried out in previous studies (Thomson et al., 2003; Septiningsih et al., 2003). All these studies evaluated alien gene introgression and its phenotypic expression in the segregating phase.

Studies showing grain quality improvement in the homozygous final product are important from a plant breeding viewpoint, and the present study attempted to provide this information. However, the AB-QTL strategy of molecular mapping did not accompany this study, with molecular analysis being limited to the identification of alien chromatin regions in stable homozygous lines. However, utilization of an *O. rufipogon* accession that has been used in other studies, with different recipients, also allowed beneficial comparisons particularly regarding the adaptability of specific QTL under different environments and backgrounds.

## MATERIAL AND METHODS

### Population development

In the year 2003, an advanced backcross program using a wide hybridization between *O. rufipogon* and MR219, focusing on the development of a high-yielding rice cultivar was started by Wickneswari et al. (2003). Twenty-six transgressive variants were identified as promising for cultivar development after a successful pedigree selection in the BC<sub>2</sub>F<sub>3</sub> and BC<sub>2</sub>F<sub>4</sub> generations. The variants (Table 1) used in the present study were selected based on field performance and pericarp color in the BC<sub>2</sub>F<sub>5</sub> and BC<sub>2</sub>F<sub>6</sub> generations in three different environments over two seasons (off season and main season). Based on the yield per hectare parameter, variants G7, G13 and G16 out-yielded the check cultivar, MR219. The code for the variants was used for easy reference in future studies. The selected variants were considered as treatments and assigned in the completely randomized design with two replications. These 10 variants were further evaluated for grain quality and analyzed for *O. rufipogon* introgression using SSR markers as presented in following sections.

### Physicochemical properties

Grain density is a measure of weight per volume, in this case measured as the weight of rough rice grains per liter. Filled rough rice ratio was calculated as the weight of filled rough rice grains and taken as a percentage; to estimate filled grains ratio, two random sets of 100 grains each from harvested grains were taken. Percentage of de-husked rice grains was the percentage of the weight of de-husked grains compared to the weight of the sample of rough rice. Husks were removed from 100 g grains in duplicate to obtain brown rice (Septiningsih et al., 2003). Percentage of milled rice grains was calculated as the weight of milled rice grains divided by the weight of the sample of rough rice (using a sample of total de-husked rice of a 300-g sample of rough rice) and taken as a percentage. Percentage of head rice was calculated as the weight of whole grain or part of grain with a length greater than or equal to three-quarters of the average length of the test sample kernels divided by the weight of the milled rice sample and stated as a percentage (Septiningsih et al., 2003). Broken rice grains were divided into large broken grains, medium broken grains, small broken grains, and chips (Septiningsih et al., 2003). The sum of all data obtained divided by the weight of milled rice sample was stated as a percentage of broken rice grains.

The length, width and thickness of brown rice (50 grains per sample) were measured with the help of a digital micrometer (Steinmeyer, Germany). Size and shape were determined according to the RTWG (1997). Rice samples were analyzed for protein [by standard Kjeldahl procedure (MS1194, 1991)] and amylose content. Amylose was measured following the International Standard for determination in rice (ISO/DIS 6647-1 and 6647-2, 2005) using the FIAFOSS Sampler 5027 autoanalyzer (FOSS, Hoganas, Sweden).

### Marker analysis

DNA was extracted from the leaves at the age of 14 days following the modified chloroform-based DNA extraction protocol by Murray and Thompson (1980) and using the DNeasy

Plant Mini kit (Qiagen, Germany). PCR conditions were as described by Panaud et al. (1996). Primer sets of 69 microsatellites were used for analyzing the introgression regions and selected from the Gramene database ([www.gramene.org](http://www.gramene.org)) related to grain quality; it has been mapped by Septiningsih et al. (2003) and Aluko et al. (2004). Markers were initially used for parental polymorphism survey using a 2% high-resolution agarose gel. The SSR markers that were monomorphic were replaced by SSR markers located nearby ([www.gramene.org](http://www.gramene.org)). Additional markers were analyzed around the region that showed introgression. Of 69 SSR markers, a total of 34 polymorphic SSR markers were used for analyzing the introgression lines. The forward markers were fluorescent-labeled at the 5'-end with FAM (blue), HEX (green), NED (yellow), or PET (red) and synthesized by the 1stBase Company. Six panels of fluorescent-labeled microsatellite markers were created for this study. PCR products were sent to 1stBase Company for fragment detection. Fragment lengths (allele size) were estimated and scored with the GeneMapper version 4.0 software. Fragment size of the variants in the BC<sub>2</sub>F<sub>7</sub> generation was determined following the procedure used by Bhuiyan et al. (2011) with the graphical genotyping GGT 2.0 software (van Berloo, 2008). The variants that showed the size of both alleles to be similar to the recurrent parent (MR219) were scored as A and similar to the donor parent (*O. rufipogon*) as B. The variants that showed the allele size of both parents were scored as H.

The significance of differences between treatment means was determined by analysis of variance (ANOVA) using SAS version 9.1 (SAS Institute, Cary, NC, USA) with a significant level of 0.05. The Duncan multiple range test ( $\alpha = 0.05$ ) was determined for mean separation using the SAS software.

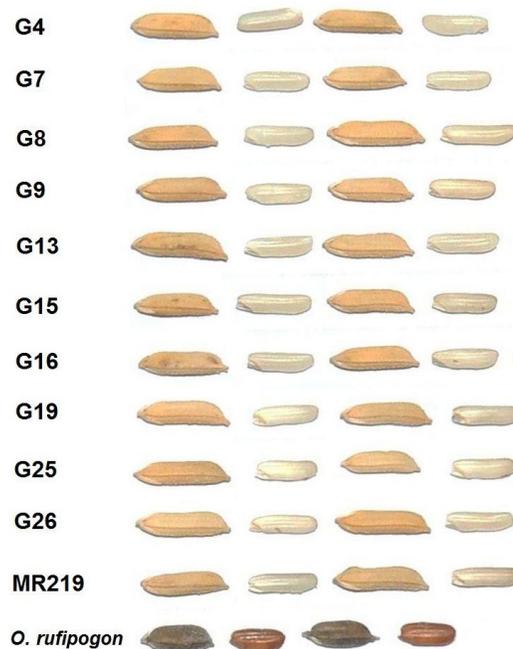
## RESULTS

### Physicochemical performance of introgression lines

Based on field performance and pericarp color, a set of 10 introgression lines (Table 1) was selected for further grain quality evaluation and molecular analysis. On the basis of rough rice hull color, all samples were the “straw hull” type and awnless, and based on the length and length/breadth ratio, all samples could be classified as the long and slender variety (Figure 1). A strict selection for improving the grain type of MR219 was conducted in each generation, and the variants selected for grain quality evaluation had a physical appearance of a grain type resembling the recurrent parent MR219 (Figure 1).

**Table 1.** List of the transgressive variants evaluated in this study.

Sl. No.	MARDI rice gene bank designation (BC <sub>2</sub> F <sub>7</sub> )	Variant code
1	R2-10-18-2-B-B	G4
2	R6-2-31-2-B-B	G7
3	R7-6-38-2-B-B	G8
4	R7-7-39-4-B-B	G9
5	R14-9-69-2-B-B	G13
6	R14-9-69-4-B-B	G15
7	R14-9-69-5-B-B	G16
8	R17-1-83-3-B-B	G19
9	R26-2-108-1-B-B	G25
10	R26-6-113-1-B-B	G26
11	MR219 (control)	



**Figure 1.** Grain type of parents and selected transgressive variants.

Analysis of variance revealed significant genotypic differences for filled rough rice ratio, de-husked rice percentage, head rice percentage, broken rice percentage, and protein and amylose content. Actual values for significant traits are presented in Table 2.

**Table 2.** Physicochemical properties of BC<sub>2</sub>F<sub>7</sub> MR219/*Oryza rufipogon* introgression lines averaged over replications.

Line No.	Grain density (g/L)	Filled rough rice ratio (%)	De-husked rice (%)	Milled rice (%)	Head rice (%)	Broken rice (%)	Protein content (%)	Amylose content (%)
G4	583.8 <sup>ns</sup>	98.0 <sup>c</sup>	79.6 <sup>bcd</sup>	77.0 <sup>ns</sup>	77.2 <sup>de</sup>	22.5 <sup>cd</sup>	9.4 <sup>a</sup>	22.6 <sup>c</sup>
G7	634.8	98.0 <sup>c</sup>	80.1 <sup>abcd</sup>	77.6	88.5 <sup>a</sup>	11.2 <sup>f</sup>	7.9 <sup>c</sup>	19.2 <sup>c</sup>
G8	601.6	99.0 <sup>ab</sup>	79.8 <sup>bcd</sup>	76.5	70.9 <sup>f</sup>	28.5 <sup>b</sup>	8.8 <sup>b</sup>	20.7 <sup>d</sup>
G9	587.2	96.5 <sup>d</sup>	80.1 <sup>abc</sup>	77.1	77.3 <sup>de</sup>	22.3 <sup>cd</sup>	8.1 <sup>c</sup>	25.1 <sup>b</sup>
G13	584.9	99.0 <sup>ab</sup>	80.4 <sup>ab</sup>	77.4	81.2 <sup>bc</sup>	18.3 <sup>c</sup>	8.1 <sup>c</sup>	25.7 <sup>a</sup>
G15	600.0	99.5 <sup>a</sup>	79.3 <sup>d</sup>	75.5	72.9 <sup>f</sup>	26.6 <sup>b</sup>	9.0 <sup>ab</sup>	25.7 <sup>a</sup>
G16	600.3	98.0 <sup>c</sup>	79.6 <sup>bcd</sup>	77.0	82.7 <sup>b</sup>	17.0 <sup>e</sup>	9.2 <sup>ab</sup>	20.6 <sup>d</sup>
G19	617.0	98.0 <sup>c</sup>	80.0 <sup>abcd</sup>	76.6	74.1 <sup>ef</sup>	25.2 <sup>bc</sup>	9.0 <sup>ab</sup>	22.7 <sup>c</sup>
G25	579.1	98.0 <sup>c</sup>	80.6 <sup>a</sup>	77.1	81.6 <sup>bc</sup>	18.1 <sup>c</sup>	9.2 <sup>ab</sup>	18.4 <sup>f</sup>
G26	590.1	98.5 <sup>bc</sup>	80.0 <sup>abcd</sup>	77.4	78.4 <sup>cd</sup>	20.8 <sup>de</sup>	9.5 <sup>a</sup>	20.8 <sup>d</sup>
MR219	608.8	99.0 <sup>ab</sup>	79.5 <sup>cd</sup>	72.7	60.2 <sup>s</sup>	39.2 <sup>a</sup>	9.2 <sup>ab</sup>	25.1 <sup>b</sup>
CV(%)	2.4	0.3	0.4	1.6	2.1	7.5	2.3	0.4

Means with the same letter are not significantly different. ns = not significant; CV = coefficient of variation.

Grain density was not significantly different among variants. Filled rough/total rough rice ratio was slightly skewed toward higher values, suggesting that most of the variants had good filled rough counts. As for grain quality components, de-husked rice percentage showed a distinct increase in all lines. However, variation for milled rice percentage turned out to be

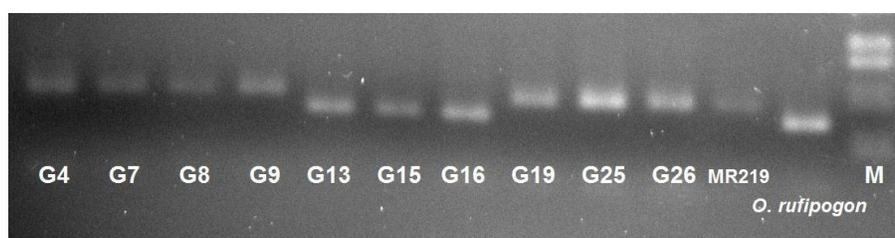
nonsignificant. All variants gave appreciably higher milling percentages (above 70%). A 50% milling out turn or less is undesirable since it means that 50% of the rice are discarded as husk and bran after milling. All the evaluated lines showed significantly higher head rice percentage compared to MR219, and the increase over MR219 in these variants ranged from 10.9% (G8) to 28.4% (G7) (Table 2). Broken rice percentage showed improvement in all variants with lower levels than in MR219. The statistical results revealed that the protein content was significantly affected by the rice variants and ranged from 7.9 to 9.5%. Two of the 10 variants evaluated showed significantly ( $P < 0.01$ ) higher amylose content relative to MR219. Genotype G9 was statistically at par with MR219 for amylose content (Table 2).

### Marker analysis and development of graphical genotypes

Of the 34 polymorphic markers spanning 10 chromosomes, only 18 showed *O. rufipogon* introgression in 10 selected  $BC_2F_7$  progenies (Table 3). The banding patterns of microsatellite primer pairs for polymorphic marker RM13345 linked with head rice and broken rice percentage genes for 10 samples along with two parents are shown in Figure 2. The majority of markers were skewed towards MR219, which may be explained by the two backcrosses and subsequent selection during population development.

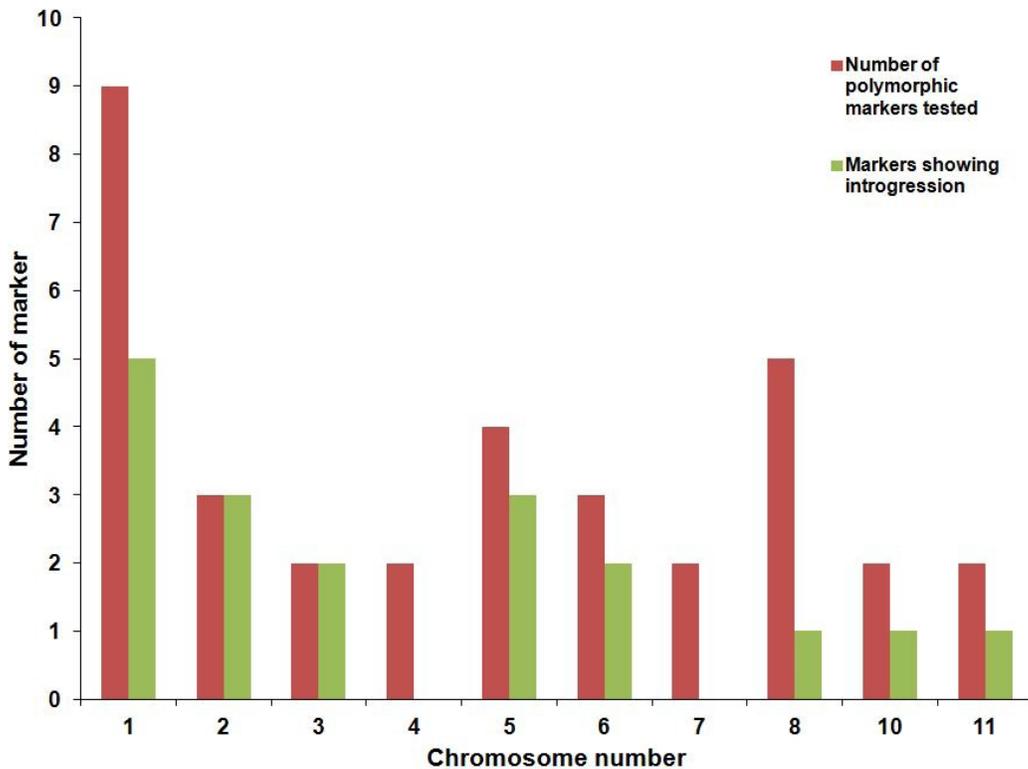
**Table 3.** Information on the polymorphic microsatellite markers used for introgression analysis in rice.

SSR markers	Primer sequences (5'-3')		Chromosome	Repeat motif	Expected PCR product size (bp)
	F: sequence of forward primer	R: sequence of reverse primer			
RM5	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG	1	(GA) <sub>14</sub>	113
RM23	CATTGGAGTGGAGGCTGG	GTCAGGCTTGCATTCTC	1	(GA) <sub>15</sub>	145
RM297	TCTTTGGAGGCGAGCTGAG	CGAAGGGTACATCTGCTTAG	1	(GA) <sub>13</sub>	148
RM493	TAGCTCCAACAGGATCGACC	GTACGTAAACGGGAAGGTG	1	(CTT) <sub>9</sub>	211
RM572	CGGTTAATGTCATCTGATTGG	TTCGAGATCCAAGACTGACC	1	(TC) <sub>14</sub>	159
RM263	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	2	(CT) <sub>34</sub>	199
RM240	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCTCCATCC	2	(CT) <sub>21</sub>	132
RM13345	CTTCTCCTGCTCCTCCGATCC	CTTCCATGGCTGTCCTTACC	2	(CT) <sub>10</sub>	167
RM7	TTCGCCATGAAGTCTCTCG	CCTCCCATCATTTCTGTTGTT	3	(GA) <sub>19</sub>	180
RM251	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTTCGATC	3	(CT) <sub>29</sub>	147
RM26	GAGTCGACGAGCGGCAGA	CTGCGAGCGACGGTAACA	5	(GA) <sub>15</sub>	112
RM178	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCTGC	5	(GA) <sub>5</sub> (AG) <sub>8</sub>	117
RM334	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG	5	(CTT) <sub>20</sub>	182
RM253	TCCTTCAAGAGTGCAAACC	GCATTGTCATGTCGAAGCC	6	(GA) <sub>25</sub>	141
RM540	GCCTTCTGGCTCATTTATGC	CTAGGCCTGCCAGATTGAAC	6	(AG) <sub>16</sub>	172
RM264	GTTGCGTCTACTGCTACTTC	GATCCGTGTCGATGATTAGC	8	(GA) <sub>17</sub>	178
RM239	TACAAAATGCTGGGTACCCC	ACATATGGGACCCACCTGTC	10	(AG) <sub>5</sub> TG(AG) <sub>2</sub>	144
RM229	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTAATGT	11	(TC) <sub>11</sub> (CT) <sub>2</sub> C <sub>3</sub> (CT) <sub>5</sub>	116



**Figure 2.** Marker banding patterns in  $BC_2F_7$  population for SSR marker RM13345. Lane M = molecular marker.

The number of markers tested on each chromosome along with the number showing *O. rufipogon* introgression are given in Figure 3. In this material, no introgression was observed for chromosomes 4 and 7.



**Figure 3.** Proportion of microsatellite markers that showed introgression from *Oryza rufipogon* in all the 10 selected variants.

For all chromosomes, introgression varied from one marker (chromosomes 8, 10 and 11) to five markers (chromosome 1) in 10 variants. Alien segments were identified for all the variants. While G4 carried only two *O. rufipogon* markers, G8 carried eight markers. The level of heterozygous segments varied from 3 segments in G25 to 0 in G7 (Table 4). Based on the marker profile, the graphical genotypes marking the chromosome segments from the two parental species were generated for each of the introgression lines (Figure 4). Graphical GenoTypes (GGT) analysis results of the BC<sub>2</sub>F<sub>7</sub> variants showed an average recovery of 83.3% of the MR219 genome while that of *O. rufipogon* was 11.4% with a residual heterozygosity of 2.8% (Table 4).

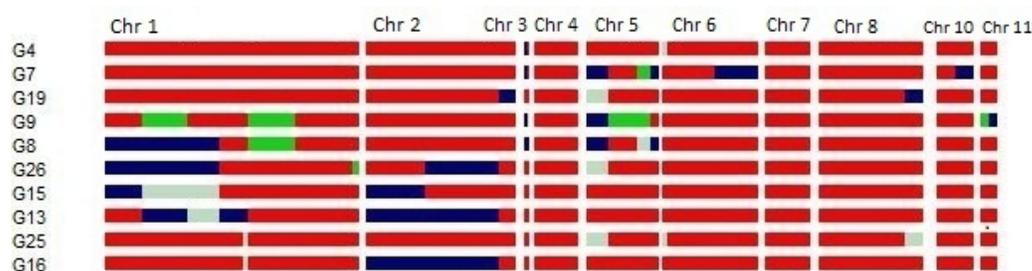
### Physicochemical properties in relation to alien introgressed regions

As described in the previous section, not only did grain density show a nonsignificant difference between the variants, but also no introgression could be found for this trait

among variants, which was the same for the filled rough rice ratio trait. Although milled rice percentage was not significant, introgressed segments could be detected for G7, G8, G9, G19, G25, and G26 at RM26 on chromosome 5. G7 had additional introgression in the region marked by RM239 on chromosome 10. Variant G13 showed significantly higher de-husked rice percentage relative to MR219 (Table 2), and one introgressed segment was found on chromosome 1 at the RM297 locus. One of the variants (G7) carried the *O. rufipogon* allele at RM239 on chromosome 10 but failed to show differences compared to the recurrent parent for de-husked rice percentage. All samples had higher head rice percentage relative to MR219. Although no chromosome segment could be detected for G4 and G19, they showed higher head rice percentage compared to MR219. The common introgressed segments in lines G13 and G16 were marked by RM5 on chromosome 1 and RM263 and RM13345 on chromosome 2. Variants G7, G9, G15, G25, and G26 had one *O. rufipogon* allele at different loci. A great number of introgression segments were detected on chromosome 1 for broken rice (Figure 4). All variants showed a lower broken grain percentage (<30%) compared to MR219 (39.2%). Variants G8, G13, G15, and G26 shared a common introgression pattern with *O. rufipogon* alleles at RM23 and RM493 for broken rice percentage. Additional introgression at locus RM572 (chromosome 1) could be detected for lines G8, G15 and G26 while G13, lacking the *O. rufipogon* allele in this region. Variants G13 and G16 shared common introgressed segments, which are marked

**Table 4.** Summary of results for introgressed segment analysis.

Variant	A (%)	B (%)	H (%)	U (%)	Total (cM)	H-segments
G4	99.0	0.4	0.6	0	539.0	1
G7	86.8	11.4	0	1.8	539.0	0
G8	74.6	18.1	1.8	5.6	539.0	1
G9	78.4	3.9	0.4	17.3	539.0	1
G13	70.9	25.4	3.7	0	539.0	1
G15	79.0	11.7	9.3	0	539.0	1
G16	83.1	16.2	0.7	0	539.0	1
G19	93.2	4.2	2.6	0	539.0	1
G25	93.8	0	6.2	0	539.0	3
G26	73.7	22.9	2.6	0.9	539.0	1
Average	83.3	11.4	2.8	2.6		



**Figure 4.** Graphical genotypes of the 10 introgression lines showing the 10 rice chromosomes. Red squares represent the recurrent parent (*Oryza sativa*) proportion of the genome. Blue squares represent the wild (*Oryza rufipogon*) homozygous introgressions, gray squares are the heterogeneous introgressions, and green squares represent missing data.

by RM263 and RM13345 on chromosome 2. G8 had two introgressed segments at loci RM178 and RM334 (chromosome 5). Variants G7, G15 and G26 had introgressed segments at RM334, RM13345 and RM263, respectively. Variants G7, G9 and G13 had lower protein content relative to the recurrent parent. In these lines, the introgressed segments were marked by RM253 on chromosome 6, RM229 on chromosome 11 and RM297 on chromosome 1, respectively. G19 had one segment on chromosome 2 by RM240 and similar protein content as MR219. The same trend as protein content was evident for amylose content. Lines G4, G7, G8, G19, and G25 had introgressed segments from *O. rufipogon* but showed lower amylose content, but not G9. The set of 10 lines reported here may not show an obvious illustration of grain quality effects of individual markers or QTL, but an indirect assumption can be made for the three markers RM263, RM13345, and RM178. These markers were used simultaneously for genotyping percentage of head rice and broken rice grains, probably responding to selection for samples.

## DISCUSSION

Selection of parental lines having the desirable traits that will face the objective of the breeder is the first step in crop improvement. This is followed by making crosses between the parents to produce a segregating population. Selection of progeny indicating the desirable traits then initiates and continues as the population is advanced from one generation to the next. Introgression of one or more genes from a donor into the background of an elite variety (recurrent or recipient parent) and to improve the recurrent parent genome as quickly as possible is one of the main objectives of plant breeding. There are several ways to introgress useful genes from a wild relative to a cultivated variety, generally on the basis of backcrosses and selection for the trait phenotype. The *O. rufipogon* segments identified in 10 introgression lines also need to be viewed in light of information from related studies. Differences in the quantity of the fibrous husk, which encloses the rice grain, were also pronounced between rice variants. The lemma and palea of the rice husk are maternal tissues (Shobha Rani et al., 2008). Normally the husk content is 20-22% of the rough rice, although a variation of 18 to 26% has been recorded (Shobha Rani et al., 2008). The variants tested showed significant difference in the percentage of de-husked rice grains. Recently, Bhonsle and Sellappan (2010) reported that the de-husking percentage for traditionally cultivated scented and basmati rice varieties ranged from 72 to 82. Rice husks are considered an economically interesting source of natural antioxidants, prompting many studies to explain their biochemical mechanisms in protection against oxidative stress-caused damages (Butsat and Siriamornpun, 2010). Variant G13 carried the *O. rufipogon* allele for this trait (Figure 4). De-husked rice QTL (QTL-*br1*) in this region has also been mapped in the interval RM297-RM315 (Aluko et al., 2004). RM239 on chromosome 10 is another marker for which introgression was identified in G7 in this study. Septiningsih et al. (2003) detected one QTL for brown rice in the interval RM474-RM239 in an *O. sativa* IR64 x *O. rufipogon* cross. Graphical genotyping software programs, such as GGT (van Berloo, 2008), are very useful tools for selecting preferable backcross progeny on the basis of their genotypic content.

Head rice percentage is an inherited trait, even though environmental factors such as temperature and humidity during ripening and postharvest stages are known to affect grain breakage during milling (Shobha Rani et al. 2008). The head rice grain percentage showed

the greatest variation ranging from 60.2 to 88.5. A similar result was obtained by Shobha Rani et al. (2008). Li et al. (2008) reported that head rice yield for *indica* rice (*O. sativa* L., cv. Liangyou 2186) as a control was 63.8%. Unexpectedly, the effect of the wild alleles was beneficial, suggesting that they are associated with more stability of grains during milling. Aluko et al. (2004) found three chromosomal regions associated with QTLs for head rice percentage, which were in the same region as in this study. Septiningsih et al. (2003) found 2 QTLs by RM263 on chromosome 2 and RM178 on chromosome 5 with the beneficial alleles coming from *O. sativa* in both cases.

Many grains are lost as a result of shattering and are cracked throughout threshing if the harvest is too late, which results in grain breakage during milling (<http://www.knowledgebank.irri.org/rkb/index.php/quality-characteristics-of-paddy>). The percentage of broken rice grains ranged from 11.1 to 39.2. Sarker and Farouk (1989) reported that long grain varieties are more vulnerable to breakage during milling compared to short grain varieties, and this may also account for the lower head rice yield. It has also been reported that the mass loss and breakage are influenced by the cultivar, kernel shape, and thickness of the aleurone layer (Juliano, 1972). Diako et al. (2011) reported that broken rice percentage for four commercial Ghanaian rice varieties was 13.60-32.70%. As expected, the amount of broken grains was negatively correlated with head rice percentages ( $r = -0.99$ ,  $P < 0.01$ ). A similar correlation was also reported by Septiningsih et al. (2003). Introgression lines with *O. rufipogon* segments at different loci showed a decreasing effect on broken grains. Broken grain QTL in these regions has also been mapped in cultivated germplasm (Septiningsih et al., 2003). The main advantages of the Kjeldahl method for food protein analysis are its high level of precision, its compatibility with nearly all food matrices, its wide recognition in the food analytical and regulatory communities, and its options for automation (Moore et al., 2010). Protein, an important factor affecting the eating quality of rice, was considerably high (>7%) for all variants (Table 2). Significant differences ( $P < 0.05$ ) existed in the protein contents among all variants. Eggum and Juliano (1975) reported that high levels of fertilizer increase total protein content. Other factors such as short growth periods, soil salinity or alkalinity may also increase the protein content of grain. A large proportion of the total variability in protein content is attributable to environment (Shobha Rani et al., 2006). The range of protein content values obtained in the present study (Table 2) was slightly higher than that reported by Juliano and FAO (1993) (7.3-8.6%), Yadav et al. (2007) (5.46-7.02%), and Eggum et al. (1993) (7.1-8.7%). The ranges obtained for the samples fall within that for polished rice (6.78-10.5%) (Adu-Kwarten et al., 2003).

Increasing the protein content values in this cereal as a result raises protein intake, particularly in countries where rice is consumed as the staple food (Perez et al., 1996). On the basis of the differences found between variants in different environments and to the above-mentioned views, the hypothesis is raised that some of the rice variants could be used to contribute to higher or lower protein intake, based on certain needs of each individual (i.e., higher protein intake to contribute to growth; smaller protein intake to control kidney problems). RM253 on chromosome 6 and RM229 on chromosome 11 are markers for which introgression was identified in this study and whose wild parent allele has been previously shown by Aluko et al. (2004) to have a negative effect on protein content. In fact, three introgression lines G7, G9 and G13, which carried the *O. rufipogon* segment, showed lower protein content relative to MR219. However, not all the lines carrying the *O. rufipogon* segment showed this negative effect, probably due to other introgressed regions providing a neutralizing or negative influ-

ence. One of the progenies (G19) carried the *O. rufipogon* allele at RM240 but failed to show any significant increase in protein content over the recurrent parent.

The amylose content of the rice starch is an important eating quality factor. It is directly associated with volume expansion and water absorption during cooking and with hardness, whiteness and dullness of cooked rice. Genetic studies have indicated that the nonwaxy trait dominates over the waxy trait (Kumar and Khush, 1987). Among nonwaxy parents, high amylose is completely dominant over low or intermediate amylose, and intermediate is dominant over low (Kumar and Khush, 1987). The statistical results pertaining to amylose content of different rice variants are presented in Table 2. The amylose content ranged from 18.4 to 25.7% among the rice variants. This range is narrower than that reported by Kennedy and Burlingame (2003) (0.5-33%), who described the amylose content of 2000 rice varieties. González et al. (2004) reported amylose values of 20.5-26.7% for eight milled rice varieties, obtained by the DSC method. The amylose content was found to be higher in G13 followed by G15, and the lowest amylose content was observed in G25 across all samples. In the present study, the average amylose content for all samples except G7 and G25 was intermediate, which correlates with extreme elongation during cooking and soft texture of cooked rice. Aluko et al. (2004) detected three QTLs, amy3, amy6 and amy8 on chromosomes 3, 6 and 8 in the intervals RM7-RM251, RM190-RM253 and RM230-RM264, respectively, to enhance amylose content in the same region as in this study. Other researchers have also detected a QTL for amylose content in the same region of the interval RM190-RM253 near the waxy gene on chromosome 6 (He et al., 1999; Tan et al., 1999; Lanceras et al., 2000; Septiningsih et al., 2003). Thus, the lack of an increase in amylose content in variants G4, G7, G8, G19, and G25 might have been due to a neutralizing effect of both positive and negative alleles from *O. rufipogon*. Introgression line G9, which carried *O. rufipogon* alleles at RM7 and RM251, failed to show variation in amylose content with respect to the recurrent parent. Previous studies based on mapping populations derived from *O. sativa* x *O. sativa* crosses detected QTLs controlling amylose content on chromosomes other than chromosome 6 (He et al., 1999; Lanceras et al., 2000). These results support the idea that both the major waxy gene and modifying genes control amylose content, as previously reported by McKenzie and Rutger (1983).

Grain quality characteristics of new varieties are always compared to those of comparably grown leading commercial varieties of appropriate grain type. If, after a number of years or at several locations, characteristics of new varieties resemble or are superior to those of standard varieties, they are assessed as having acceptable or distinguished cooking and processing quality; if not, they are judged undesirable or of unknown quality (Owens, 2001). On the whole, the majority of *O. rufipogon* alleles that were common with other studies seemed to be effective across recipient genotypes. The evaluation of test cross progeny as attempted by Xiao et al. (1998) may identify some QTL that may have positive effects in heterozygous conditions and not in homozygous conditions as illustrated by Semel et al. (2006). These alleles seem novel and are not common in cultivated germplasm. The idea of segmental introgression lines has been developed in tomato to improve rate of progress of breeding on the basis of wild species resources. Superiority or positive effects that are retained after the alien segments become homozygous are the ones that can be quickly achieved in the form of improved pure line variety. In the present study, the introgressed segments of *O. rufipogon* increased the head rice percentage and showed a positive effect on decreasing the amount of broken rice percentage in all variants, while remaining at the MR219 level for other traits. The situation clearly propagates

the likelihood of incorporating the improvements for the two components in a single genotype.

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