



Identification of medium-grain rice based on GS3, a gene linked to rice grain size

Bui Phuoc Tam^{1,4}, Pham Thi Be Tu^{2,*}, Nguyen Thi Pha³

¹PhD student of Can Tho University, Can Tho City 94000, Vietnam

²College of Agriculture, Can Tho University, Can Tho City 94000, Vietnam

³Institute of Food and Biotechnology, Can Tho University, Can Tho City 94000, Vietnam

⁴Genetics and Rice Breeding Department, Loc Troi Agricultural Research Institute, An Giang province 90000, Vietnam

*Corresponding author: ptbtu@ctu.edu.vn

SUBMITTED 30 September 2023 REVISED 22 January 2024 ACCEPTED 12 March 2024

ABSTRACT Previous studies have used molecular markers associated with the GS3 gene to differentiate between short and long rice. However, there are three classifications of grain size: long, short, and medium. The identification of medium-grain rice using these markers linked to the GS3 gene is yet to be confirmed. Hence, this study aimed to identify medium-grain rice through phenotyping and genotyping. Grain characteristics including grain length (GL), grain width (GW), and the length-to-width ratio (GL/GW) were measured using SmartGrain software. The genotype was then amplified with the GS3 gene-linked DRR-GL (double round-robin for grain length) molecular marker. The results revealed that medium-grain rice, as identified by the DRR-GL marker, exhibited DNA bands at the position of 150 bp. These bands differed from those observed in long-grain rice, but they were consistent with those found in short-grain rice. The genotypic results further indicated that PCR products obtained with the DRR-GL marker in medium-grain rice accounted for 86.8% of the phenotypic variation in grain size. This study provides fundamental genetic insights into the identification of medium-grain rice and contributes to optimizing effects on rice breeding related to grain size.

KEYWORDS Grain size; GS3 gene; Medium-grain rice; PCR (polymerase chain reaction); SmartGrain software

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops and is a staple food for over half of the world's population (Hori and Sun 2022). Grain quality, besides yield, is attended by rice consumers, food industries, farmers, and seed producers. Grain size is an important factor in grain quality and crop market value (Huang et al. 2013; Ngangkham et al. 2018; Hori and Sun 2022). Grain length (GL), grain width (GW), and the length-to-width ratio (GL/GW) define grain size. These data are measured with rulers or other types of calipers, however, this task requires numerous attempts. In order to the optimum measurement, the SmartGrain software is developed by Tanabata et al. (2012) for high-throughput measurement of seed size and shape. This software employs a novel image analysis technique to reduce the time required for seed preparation and image capture. The rice grain size was categorized by International Rice Research Institute (2014) as extra-long (GL > 7.5 mm), long (GL = 6.60-7.50 mm), medium (GL = 5.51-6.60 mm), and short (GL 5.5 mm). There are slender (GL/GW > 3.0), medium (GL/GW = 2.1-3.0), robust (GL/GW = 1.1-2.0), and round (GL/GW < 1.1)

rice grain shapes.

Rice grain size and shape are the appearance of the grain that is of interest to consumers. People in different regions have needs and preferences for each appearance of rice grains. Medium-grain rice has an advantage over long-grain rice since it has a higher recovery rate due to fewer broken milled grains (Badi 2013). Moreover, medium rice is usually longer and heavier than short-grain rice. This is considered to contribute to the improvement of yield in medium rice varieties compared to short rice (Xing and Zhang 2010). Furthermore, the world average grain rice price is forecast to remain relatively higher than other grain size rice (Wailles and Chavez 2015). Thus, research on traits on medium grain rice, including quality traits, helps build a complete genetic foundation for grain quality as well as research on breeding medium grain rice varieties.

Grain size is one of the genetically complex traits controlled by polygenes and interacting pathways (Ramkumar et al. 2010; Ngangkham et al. 2018; Jiang et al. 2022). Over 8500 quantitative trait loci (QTL) related to grain size trait in rice have been mapped using diverse segregating populations derived from diverse parents (Ngangkham

et al. 2018). Several major QTLs affecting the rice grain size have been cloned and characterized in recent years, such as *GS3*, *GW2*, *GS5* (Li et al. 2018; Hori and Sun 2022), *GS2* (Hu et al. 2015), *TGW6* (Ishimaru et al. 2013; Li et al. 2018), *GL7/GW7* (Wang et al. 2015b,a), and *GS9* (Zhao et al. 2018).

GS3 which located on chromosome 3's pericentromeric region encodes a protein with a putative PEP-Blike domain, a transmembrane region, a putative TNFR/NGFR cysteine-rich domain, and a VWFC module (Kim et al. 2014; Ngangkham et al. 2018). At the protein level, Exon 2 of *GS3* contains a single nucleotide polymorphism (SNP) that converts a cysteine codon (TGC) to a termination codon (TGA) (Xue et al. 2014). Ramkumar et al. (2010) developed the *GS3* gene-based marker, named as the DRR-GL which accounts for 80–90% of the phenotypic variation in grain size. In this study, the experiment was carried out to identify medium grain rice among varieties based on the DRR-GL molecular marker linked to the *GS3* gene.

In this study, the experiment was carried out to identify medium grain rice among varieties based on the DRR-GL molecular marker linked to the *GS3* gene.

2. Materials and Methods

2.1. Materials

A total of 342 varieties of the 44k-SNP Rice Diversity Panel (RDP) were provided by the Genetic Resources Center, International Rice Research Institute (IRRI). The RDP, which is gathered from 82 countries, represents a broad range of genetic variation with phenotypic and/or SSR data. The DRR-GL marker system consists of primers and their

sequences as follows: EFP: 5'-aggctaaacatgcccattctc-3', ERP: 5'-cccaacgttcagaaataatgtgctg-3', IRSP: 5'-aacagcaggctggcttactctctg-3', IFLP: 5'-acgtgctctccagatgctga-3') (Ramkumar et al. 2010).

2.2. Measuring the rice grain size

Each rice sample was randomly selected with 90 grains and each variety was measured with three replications. In the rice sample, husks were removed, and then, brown rice grains were photographed by a fixed imaging system. These seed images were analyzed and out-put the data file by SmartGRAIN software (Tanabata et al. 2012) (Figure 1). The grain size classification was referenced according to Standard Evaluation System (SES) for rice (IRRI, 2014), including the long grain (GL > 6.60 mm and GL/GW > 3.0), the medium grain (GL = 5.51-6.60 mm and GL/GW = 2.1-3.0), and the short grain (GL < 5.51 mm and GL/GW < 2.1).

2.3. DNA Extraction and genotyping

Genomic DNA was extracted using the modified cetyltrimethylammonium bromide (CTAB) method based on the protocol of Murray and Thompson (1980). DNA products were air dried and resuspended in 50 µL of TE buffer (10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0). Then, they were diluted with distilled water to a concentration of 20 ng/µL.

PCR amplification of markers was carried out in a Mastercycler (Eppendorf, Germany) in a total volume of 20 µL with the following PCR reaction: 2 µL of DNA at 20 ng/µL, 2 µL of 10× buffer containing 25 mM MgCl₂, 1 µL of 2.5 mM dNTPs, 1 unit of Taq DNA Polymerase (Bioline, England), and 1 µL each of forward and reverse primers (10 µM). All amplifications were performed for a

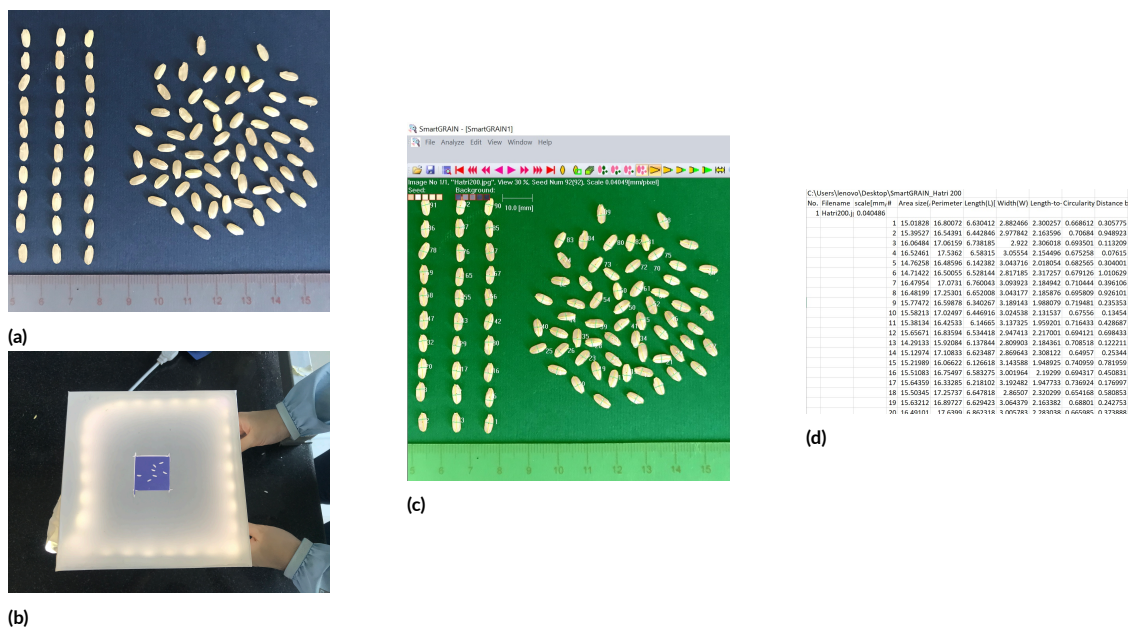


FIGURE 1 Steps to measure rice grain size and collect data based on SmartGrain software. a. Preparing rice grains, b. Image taking of grains by photography system, c. Recognition of grains by SmartGrain, d. Collecting data of grain size through SmartGrain output.

total of 35 cycles of 1 min at 95°C, 30 s at 55°C, and 1 min at 72°C.

PCR products are electrophoresed on 2.5% agarose gel in 1X TBE buffer and containing the 1X GelRed fluorescent nucleic acid stain (Merck, Germany). Sample electrophoresis was carried out at 100V for about 120 min. After that, the gel was photographed using a Quantum-ST4 UV gel camera (France).

2.4. Statistical methods

Data were collected and stored, and standard deviation (SD) was calculated using the Microsoft Office Excel 2013 program. A map of distribution by geographical region was designed by R-studio 3.4.1 software (<https://rstudio.com/products/rstudio/download>) with the 'ggplot2' package.

3. Results and Discussion

3.1. Results

According to the grain size of brown rice, the RDP consisted of long-grain, medium-grain, and short-grain rice varieties. Long-, medium-, and short-grain rice accounted for 122, 114, and 106 of the 342 RDP rice varieties, respectively. Thus, medium-grain rice comprised nearly 33 percent of the total varieties.

The RDP medium-grain rice varieties were widely distributed in Vietnam, China, Bangladesh, the United States, India, and Taiwan (Figure 2 and Table 1). The group of medium-grain rice contained numerous subgroups; the indica subgroup (IND) accounted for 36.0%, the aus subgroup (AUS) accounted for 22.8%, the tropical japonica subgroup (TRJ) accounted for 14.8%, and the remainder were other subgroups (Table 1).

The medium-grain rice is a variety with a brown grain length ranging from 5.51 to 6.60 mm and a length-to-width

ratio ranging from 2.1 to 3.0. A total of 114 medium varieties were identified by SmartGrain software in Table 1. These medium rice were then amplified the *GS3* gene with the DRR-GL marker and the result of PCR products was shown in Figure 3. Three alleles of 150 bp, 260 bp, and 360 bp were detected in the medium-grain rice population. Among them, the 360-bp bands appeared at all of variety samples and called the universal bands. The 99 varieties had the PCR products with a size of 150 bp and the remaining 15 varieties had the 260-bp bands. Thus, if the 150-bp bands represent for medium-grain rice through amplifying the *GS3* gene, the suitable between phenotype and genotype of medium rice was 86.8% (Table 2).

3.2. Discussion

In general, seed shape can be scored in two ways, but using calipers to measure GL and GW is a simple way. Disadvantage of these conventional methods is limiting the number of data, quality of measurements, and diversity of shape data. On the other hand, computational methods using digital imaging technology could permit automatically measuring a variety of shape parameters at very small sizes in high-resolution images (French et al. 2009; Tanabata et al. 2012). SmartGrain automatically recognizes all grains within a digital image, detects outlines, and then calculates GL, GW, seed area, perimeter length, and other parameters. It could accurately recognize seeds not only of rice but also of several other species, including *Arabidopsis* (*Arabidopsis thaliana*) (Tanabata et al. 2012). SmartGrain software was applied on previous studies about genetics in rice (Megersa et al. 2016; Sun et al. 2022).

Populations of long-, medium-, and short-grain rice varieties worldwide are approximately equal in quantity. This inferred that it is important to be concerned with medium-grain breeding and development as other size grain rice.

The medium-grain rice was mainly distributed in

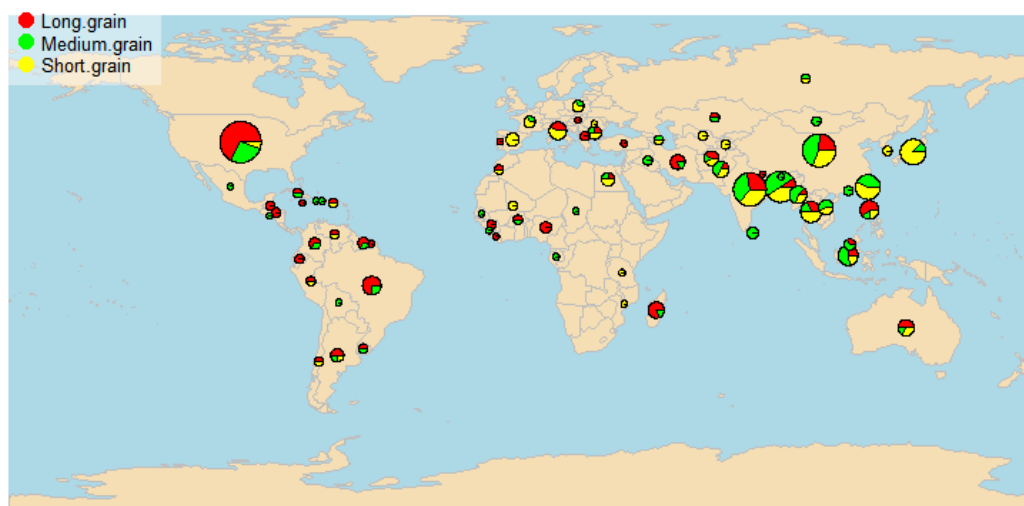


FIGURE 2 Geographical distribution map of rice varieties with varying kinds of grain size designed by R-studio 3.4.1 software. The red, green, and yellow colors indicated long-grain, medium-grain, and short-grain rice, respectively. The size of the circles indicated the quantity of rice varieties.

TABLE 1 Grain size and classification of grain in RDP.

No.	Variety Name	Origin	Sub-group	GL (mm)	SD (1)	GW (mm)	SD (2)	GL/GW	Classification of grain
1	ST24	Vietnam	IND	7.63	0.024	1.79	0.020	4.3	Long
2	OM5451	Vietnam	IND	7.08	0.089	2.09	0.072	3.4	Long
3	IR50404	Philippines	IND	6.87	0.081	2.27	0.069	3.0	Long
4	OM18	Vietnam	IND	7.03	0.018	2.21	0.060	3.2	Long
5	Đài Thơm 8	Vietnam	IND	7.06	0.073	2.17	0.025	3.3	Long
6	Jasmine 85	Vietnam	IND	7.20	0.051	2.18	0.033	3.3	Long
7	Hananomai	Japan	TEJ	5.13	0.093	2.88	0.034	1.8	Short
8	ĐS1	Vietnam	TEJ	5.20	0.063	3.18	0.041	1.6	Short
9	Nipponbare	Philippines	TEJ	5.26	0.053	2.87	0.069	1.8	Short
10	Koshihikari	Japan	TEJ	5.25	0.090	2.93	0.034	1.8	Short
11	27	Dominican Republic	TRJ	6.57	0.075	2.68	0.015	2.5	Medium
12	519	Uruguay	IND	5.78	0.064	2.72	0.021	2.1	Medium
13	9524	India	AUS	5.60	0.083	2.57	0.009	2.2	Medium
14	68-2	France	TEJ	5.92	0.045	2.71	0.013	2.2	Medium
15	Ai-Chiao-Hong	China	IND	5.70	0.030	2.57	0.038	2.2	Medium
16	Aijiaonante	China	IND	5.89	0.053	2.63	0.060	2.2	Medium
17	Arias	Indonesia	TRJ	5.59	0.060	2.19	0.059	2.5	Medium
18	Aswina 330	Bangladesh	AUS	6.29	0.083	2.46	0.072	2.6	Medium
19	BJ 1	India	AUS	5.73	0.056	2.49	0.036	2.3	Medium
20	Black Gora	India	AUS	5.89	0.037	2.62	0.037	2.2	Medium
21	Blue Rose	Louisiana	ADMIX	6.11	0.083	2.82	0.044	2.2	Medium
22	Boa Vista	El Salvador	TRJ	6.60	0.096	2.65	0.005	2.5	Medium
23	C101A51	Colombia	IND	5.55	0.118	2.59	0.046	2.1	Medium
24	C1-6-5-3	Mexico	ADMIX	5.62	0.081	2.73	0.033	2.1	Medium
25	CA 902/B/2/1	Chad	AUS	6.09	0.051	2.56	0.015	2.4	Medium
26	Caawa/Fortuna 6-103-15	Taiwan	TRJ	6.38	0.110	2.89	0.046	2.2	Medium
27	Canella De Ferro	Brazil	TRJ	6.39	0.138	2.77	0.020	2.3	Medium
28	Carolina Gold	United States	TRJ	6.59	0.062	2.65	0.029	2.5	Medium
29	Caucasica	Former Soviet Union	TEJ	6.37	0.089	3.00	0.041	2.1	Medium
30	Cenit	Argentina	TRJ	5.96	0.064	2.48	0.035	2.4	Medium
31	Chau	Vietnam	IND	5.79	0.067	2.48	0.046	2.3	Medium
32	Chiem Chanh	Vietnam	IND	6.39	0.091	2.21	0.015	2.9	Medium
33	China 1039	China	IND	5.86	0.096	2.65	0.032	2.2	Medium
34	CI 11011	United States	ADMIX	5.77	0.134	2.67	0.025	2.2	Medium
35	Coarse	Pakistan	AUS	5.70	0.167	2.45	0.028	2.3	Medium
36	Coppocina	Bulgaria	TEJ	5.66	0.075	2.68	0.023	2.1	Medium
37	CS-M3	United States-CA	TEJ	5.92	0.076	2.77	0.007	2.1	Medium
38	CTG 1516	Bangladesh	AUS	6.13	0.096	2.36	0.041	2.6	Medium
39	Dee Geo Woo Gen	Taiwan	IND	5.69	0.105	2.72	0.072	2.1	Medium
40	Delrex	United States	TRJ	6.09	0.021	2.20	0.056	2.8	Medium
41	Dhala Shaitta	Bangladesh	AUS	6.32	0.067	2.24	0.024	2.8	Medium
42	DK 12	Bangladesh	AUS	5.77	0.062	2.74	0.033	2.1	Medium
43	DM 43	Bangladesh	AUS	5.86	0.011	2.47	0.033	2.4	Medium
44	DM 59	Bangladesh	AUS	6.20	0.118	2.36	0.041	2.6	Medium
45	DZ 193	Bangladesh	AUS	6.00	0.061	2.99	0.029	2.0	Medium
46	ECIA76-S89-1	Cuba	IND	5.79	0.116	2.33	0.011	2.5	Medium
47	EMATA A 16-34	Myanmar	IND	5.97	0.092	2.35	0.017	2.5	Medium
48	Ghati Kamma Nangarhar	Afghanistan	AUS	5.80	0.051	2.79	0.043	2.1	Medium

TABLE 1 (continued).

No.	Variety Name	Origin	Sub-group	GL (mm)	SD (1)	GW (mm)	SD (2)	GL/GW	Classification of grain
49	Ghorbhai	Bangladesh	AUS	5.68	0.066	2.37	0.034	2.4	Medium
50	Guan-Yin-Tsan	China	IND	5.76	0.077	2.57	0.029	2.2	Medium
51	Halwa Gose Red	Iraq	AUS	5.90	0.082	2.66	0.048	2.2	Medium
52	Hon Chim	Hong Kong	IND	6.40	0.122	2.57	0.030	2.5	Medium
53	Hunan Early Dwarf No. 3	China	IND	5.69	0.084	2.58	0.016	2.2	Medium
54	I-Geo-Tze	Taiwan	ADMIX	5.60	0.073	2.45	0.014	2.3	Medium
55	Iguape Cateto	Haiti	TRJ	6.44	0.092	2.73	0.046	2.4	Medium
56	Italica Carolina	Poland	TEJ	6.57	0.050	2.78	0.028	2.4	Medium
57	Jambu	Indonesia	TRJ	5.80	0.069	2.68	0.018	2.2	Medium
58	Jamir	Bangladesh	AUS	5.57	0.041	2.32	0.038	2.4	Medium
59	Jaya	India	IND	6.54	0.060	2.64	0.048	2.5	Medium
60	JC 117	India	IND	5.79	0.040	2.51	0.081	2.3	Medium
61	Jouiku 393G	Japan	TEJ	6.20	0.065	2.76	0.036	2.2	Medium
62	Kachilon	Bangladesh	AUS	5.79	0.085	2.53	0.028	2.3	Medium
63	Kalubala Vee	Sri Lanka	AUS	6.00	0.068	2.47	0.036	2.4	Medium
64	Kon Suito	Mongolia	TEJ	6.22	0.047	2.86	0.064	2.2	Medium
65	KU115	Thailand	ADMIX	6.25	0.098	2.77	0.028	2.3	Medium
66	Lacrosse	United States	ADMIX	6.04	0.105	2.90	0.042	2.1	Medium
67	LD 24	Sri Lanka	TEJ	5.55	0.083	2.41	0.010	2.3	Medium
68	M-202	United States-CA	ADMIX	6.04	0.058	2.78	0.073	2.2	Medium
69	Mehr	Iran	AUS	5.80	0.149	2.40	0.032	2.4	Medium
70	Mudgo	India	IND	6.46	0.077	2.69	0.024	2.4	Medium
71	Nira	United States	IND	6.30	0.035	2.41	0.033	2.6	Medium
72	Nova	United States	ADMIX	6.09	0.074	2.80	0.030	2.2	Medium
73	Okshitmayin	Myanmar	ADMIX	6.07	0.016	2.75	0.022	2.2	Medium
74	Padi Kasalle	Indonesia	TRJ	5.91	0.029	2.71	0.041	2.2	Medium
75	Pai Hok Glutinous	Hong Kong	IND	5.65	0.054	2.68	0.062	2.1	Medium
76	Palmyra	United States	ADMIX	6.21	0.091	2.54	0.025	2.4	Medium
77	Pao-Tou-Hung	China	IND	5.95	0.037	2.59	0.040	2.3	Medium
78	Paraiba Chines Nova	Brazil	IND	5.89	0.007	2.52	0.041	2.3	Medium
79	Pato De Gallinazo	Australia	ADMIX	5.84	0.087	2.80	0.038	2.1	Medium
80	Paung Malaung	Myanmar	AUS	5.94	0.050	2.78	0.022	2.1	Medium
81	Peh-Kuh	Taiwan	IND	5.58	0.054	2.65	0.049	2.1	Medium
82	Peh-Kuh-Tsao-Tu	Taiwan	IND	5.51	0.067	2.66	0.016	2.1	Medium
83	Phudugey	Bhutan	AUS	6.03	0.078	2.30	0.072	2.6	Medium
84	Radin Ebos 33	Malaysia	IND	5.88	0.084	2.38	0.047	2.5	Medium
85	Rathuwee	Sri Lanka	IND	6.07	0.072	2.30	0.041	2.6	Medium
86	Riz Local	Burkina Faso	ADMIX	6.07	0.126	2.88	0.016	2.1	Medium
87	RTS14	Vietnam	IND	6.01	0.074	2.54	0.022	2.4	Medium
88	Sabharaj	Bangladesh	IND	5.79	0.061	2.69	0.079	2.2	Medium
89	Sadri Belyi	Azerbaijan	ARO	6.13	0.045	2.42	0.012	2.5	Medium
90	Saku	Mongolia	ADMIX	6.34	0.111	2.73	0.038	2.3	Medium
91	Santhi Sufaid	Pakistan	AUS	6.41	0.087	2.32	0.022	2.8	Medium
92	Saraya	Fiji	AUS	5.86	0.031	2.59	0.005	2.3	Medium
93	Saturn	United States	ADMIX	6.07	0.037	2.62	0.012	2.3	Medium
94	Shai-Kuh	China	IND	5.92	0.047	2.41	0.036	2.5	Medium
95	Shim Balte	Iraq	AUS	5.92	0.061	2.39	0.043	2.5	Medium
96	Shirogane	Japan	AUS	5.80	0.093	2.69	0.040	2.2	Medium

TABLE 1 (continued).

No.	Variety Name	Origin	Sub-group	GL (mm)	SD (1)	GW (mm)	SD (2)	GL/GW	Classification of grain
97	Short Grain	Thailand	IND	6.32	0.085	2.29	0.033	2.8	Medium
98	Sigadis	Indonesia	IND	6.51	0.060	2.54	0.003	2.6	Medium
99	SL 22-613	Sierra Leone	ADMIX	6.23	0.073	2.24	0.040	2.8	Medium
100	SLO 17	India	IND	6.07	0.072	2.37	0.070	2.6	Medium
101	SML 242	Suriname	IND	5.96	0.063	2.66	0.028	2.2	Medium
102	Sri Malaysia Dua	Malaysia	TEJ	6.27	0.027	2.76	0.030	2.3	Medium
103	Sufaid	Pakistan	AUS	5.88	0.080	2.22	0.026	2.6	Medium
104	Sultani	Egypt	TRJ	6.22	0.072	2.67	0.061	2.3	Medium
105	Surjamkuhi	India	AUS	5.98	0.054	2.19	0.042	2.7	Medium
106	Sze Guen Zim	China	IND	5.94	0.154	2.57	0.024	2.3	Medium
107	Taducan	Philippines	IND	5.79	0.087	2.23	0.013	2.6	Medium
108	Taichung Native 1	Taiwan	IND	5.52	0.098	2.68	0.042	2.1	Medium
109	Tchibanga	Gabon	IND	5.56	0.091	2.69	0.041	2.1	Medium
110	TeQing	China	IND	5.66	0.023	2.65	0.052	2.1	Medium
111	TKM6	India	IND	5.87	0.060	2.12	0.019	2.8	Medium
112	TOg 7178	Senegal	ADMIX	6.14	0.046	2.93	0.051	2.1	Medium
113	Trembese	Indonesia	TRJ	5.65	0.082	2.73	0.035	2.1	Medium
114	Vary Vato 462	Madagascar	ADMIX	6.49	0.063	2.65	0.034	2.5	Medium
115	Vavilovi	Kazakhstan	TEJ	6.54	0.075	2.84	0.043	2.3	Medium
116	WAB 501-11-5-1	Cote D'Ivoire	TRJ	6.60	0.062	2.98	0.012	2.2	Medium
117	WAB 502-13-4-1	Cote D'Ivoire	TRJ	6.60	0.064	2.94	0.041	2.2	Medium
118	WC 4443	Bolivia	TRJ	6.55	0.100	2.92	0.028	2.2	Medium
119	Yodanya	Myanmar	IND	6.00	0.077	2.71	0.038	2.2	Medium
120	ZHE 733	China	IND	6.49	0.051	2.53	0.047	2.6	Medium
121	Zhenshan 2	China	IND	5.74	0.071	2.75	0.040	2.1	Medium
122	Siêu Hàm Châu	Vietnam	ADMIX	6.20	0.087	2.28	0.039	2.7	Medium
123	PY 2	Vietnam	ADMIX	5.65	0.048	2.21	0.040	2.6	Medium
124	ML202	Vietnam	ADMIX	5.55	0.094	2.22	0.067	2.5	Medium

Notes: admixed (ADMIX), aromatic (ARO), aus (AUS), indica (IND), temperate japonica (TEJ) and tropical japonica (TRJ), GL: Grain Length; GW: Grain Width; SD (1): Standard Deviation of grain length; SD (2): Standard Deviation of grain width; GL/GW: The ratio of grain length and grain width

South and East Asia, and North America where the rice plant was grown the largest. The distribution of medium-grain rice varieties in geographically dispersed regions increased the likelihood of variation in their genomes, thereby providing a vast source of genetic diversity and facilitating hybridization to achieve maximum genetic efficiency. Singh et al. (2021) also suggested that information about the relationships between cultivated plants and their relatives is helpful for breeders and geneticists who wish to create hybrids between closely related species. Genetic diversity has played a crucial role in crop enhancement and is a fundamental principle for the comprehension and collection of genetic resources.

To examine the *GS3* gene in the rice populations, the existence of 360 bp band was shown in all forms. The 150 bp and 260 bp bands represented polymorphisms between species (Figure 3, Table 2). In addition to the universal band, almost all PCR products in long-grain rice varieties tended to cluster at the 260 bp position on the agarose

gel electrophoresis. Nonetheless, Jasmine 85 yielded a PCR band of 150 bp. Therefore, assuming the PCR band for long-grain rice to be 260 bp, the conformity between genotype and phenotype in this study was approximately 83.3%, which is comparable to the conclusion of previous studies that the *GS3* gene was responsible for 80–90% of the variation in grain length (Ramkumar et al. 2010). Our results showed similarly with primary reported that PCR products were detected in the 150 bp band besides of the universal band (360 bp) in short-grain rice varieties. Thus, assuming the PCR band for short-grain rice to be 150 bp in size, the conformity between genotype and phenotype in this study was 100%. In this study, in addition to the 360 bp universal band, the PCR band size of the medium-grain varieties was nearly 150 bp. Therefore, it was concluded that the 150 bp band indicated the presence of medium-grain rice. In PCR product result, 99 medium-grain varieties appeared in the 150-bp band, and 86.8% accuracy was predicted between genotype and phenotype. How-

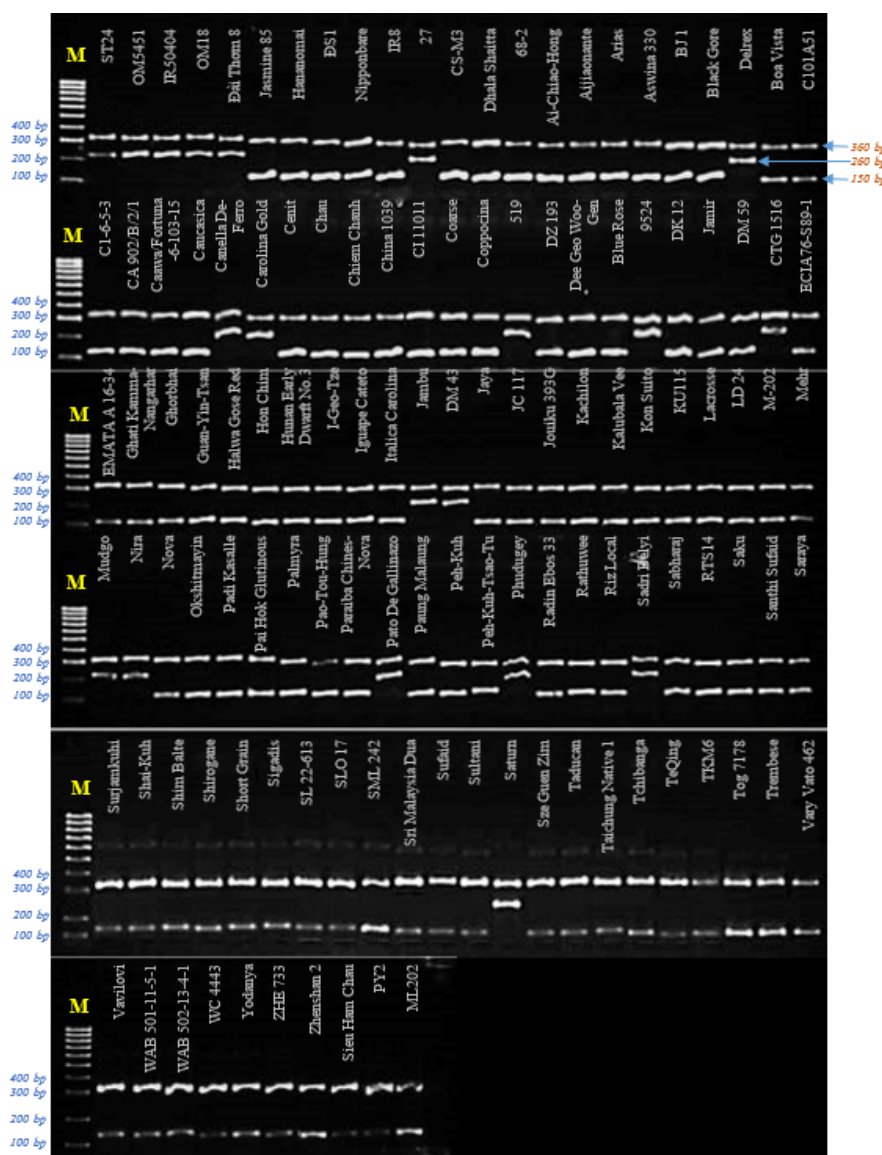


FIGURE 3 PCR products with the DRR-GL marker in rice varieties with diversified sizes in the 2.5% agarose gel. Notes: M: ladder 100 - 1000 b

ever, other 15 medium-grain varieties appeared in bands with 260 bp. This could explain that the trait of grain size in rice is a complex quantitative trait controlled by multiple genes. Although GS3 is one of the major QTLs, the appearance of this gene in rice varieties can only explain about 80–90% of the diversity in kernel length (Ramkumar et al. 2010; Xie et al. 2022). In summary, the PCR products with the DRR-GL molecular marker, amplifying

the GS3 gene related to the grain size trait, were predicted that the long, medium, and short-grain rice appeared bands with the size of 260, 150, and 150 bp, respectively.

Grain size is affected by the light, temperature, and humidity factors (Li et al. 2018). Although influenced by the environment, grain size is predominantly and tightly controlled by genetics (Zuo and Li 2014). Moreover, Jennings et al. (1979) asserted that grain size is quantitatively inher-

TABLE 2 PCR products of GS3 gene in rice varieties.

Grain shape	Number of varieties	PCR products of GS3 gene			Prediction of conformity between genotype and phenotype
		150 bp	260 bp	360 bp	
Long grain	6	1	5	6	83.3%
Short grain	4	4	0	4	100.0%
Medium grain	114	99	15	114	86.8%

ited and fixed exceptionally early in the segregating generations. More than 500 QTL genes related to rice grain size have been identified. Among them, 19 QTL genes have been cloned in rice. Of these cloned QTL genes, the *GS3* major QTL has negative effects on controlling grain length (Yuyu et al. 2020; Huang et al. 2022). Plants with the *GS3* wild-type allele produce medium-sized grains, and the other forms, short or long grains, result from mutations in different functional domains (Huang et al. 2022; Gasparis and Miłoszewski 2023). Liu et al. (2018) also claimed that the *GS3* alleles combined with other components could improve both yield and quality of rice. The *GS3* gene was applied in previous studies in rice grain size (Ramkumar et al. 2010; Zhou et al. 2017; Huang et al. 2022). These inferred that the *GS3* gene is significant in identifying medium grains and supporting rice breeding related to grain size in further studies.

4. Conclusions

The DRR-GL molecular marker linked to the *GS3* gene on chromosome 3 could distinguish medium-grain rice from other grain-size rice. The PCR band size of 150 bp could recognize the presence of medium rice varieties. In electrophoresis gel, the band size of medium rice was different from long rice; however, it was the same as short rice. The PCR products with the DRR-GL marker reflected 86.8% of the phenotypic variation of grain size in medium-grain rice. To exactly determine medium-grain rice, a combination of quantitative measurement and molecular determination is required.

Acknowledgments

The author is highly thankful to the Institute of Food and Biotechnology and College of Agriculture, Can Tho University for technical support in laboratory work, Dr. Pham Thi Be Tu (College of Agriculture, Can Tho University) and Dr. Nguyen Thi Pha (Institute of Food and Biotechnology, Can Tho University) for their critical comments to improve the manuscript.

Authors' contributions

PTBT and NTP conceived of the study and participated in its design and coordination. BPT carried out the laboratory work. BPT analyzed the data. BPT wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

All authors declare that there are not any conflicts of interest.

References

- Badi O. 2013. In : Rice post-harvest technology training program. Japan International Cooperation Agency. [accessed April 01, 2021]. URL https://www.jica.go.jp/project/english/sudan/001/materials/c8h0vm00007vrgs5-att/rice_quality_en.pdf.
- French A, Ubeda-Tomás S, Holman TJ, Bennett MJ, Pridmore T. 2009. High-throughput quantification of root growth using a novel image-analysis tool. *Plant Physiol.* 150(4):1784–1795. doi:10.1104/pp.109.140558.
- Gasparis S, Miłoszewski MM. 2023. Genetic basis of grain size and weight in rice, wheat, and barley. *Int. J. Mol. Sci.* 24(23):16921. doi:10.3390/ijms242316921.
- Hori K, Sun J. 2022. Rice grain size and quality. *Rice* 15(1):33. doi:10.1186/s12284-022-00579-z.
- Hu J, Wang Y, Fang Y, Zeng L, Xu J, Yu H, Shi Z, Pan J, Zhang D, Kang S, Zhu L, Dong G, Guo L, Zeng D, Zhang G, Xie L, Xiong G, Li J, Qian Q. 2015. A rare allele of *GS2* enhances grain size and grain yield in rice. *Mol. Plant* 8(10):1455–1465. doi:10.1016/j.molp.2015.07.002.
- Huang J, Gao L, Luo S, Liu K, Qing D, Pan Y, Dai G, Deng G, Zhu C. 2022. The genetic editing of *GS3* via CRISPR/Cas9 accelerates the breeding of three-line hybrid rice with superior yield and grain quality. *Mol. Breed.* 42(4):22. doi:10.1007/s11032-022-01290-z.
- Huang R, Jiang L, Zheng J, Wang T, Wang H, Huang Y, Hong Z. 2013. Genetic bases of rice grain shape: So many genes, so little known. *Trends Plant Sci.* 18(4):218–226. doi:10.1016/j.tplants.2012.11.001.
- International Rice Research Institute. 2014. Standard Evaluation System for Rice (SES). 5th ed. Technical report, International Rice Research Institute, Los Banos, Philippines.
- Ishimaru K, Hirotsu N, Madoka Y, Murakami N, Hara N, Onodera H, Kashiwagi T, Ujiie K, Shimizu BI, Onishi A, Miyagawa H, Katoh E. 2013. Loss of function of the IAA-glucose hydrolase gene *TGW6* enhances rice grain weight and increases yield. *Nat. Genet.* 45:707–711. doi:10.1038/ng.2612.
- Jennings PR, Coman WR, Kauman HE. 1979. Rice improvement. International Rice Research Institute, Los Banos, Philippines.
- Jiang H, Zhang A, Liu X, Chen J. 2022. Grain size associated genes and the molecular regulatory mechanism in rice. *Int. J. Mol. Sci.* 23(6):3169. doi:10.3390/ijms23063169.
- Kim B, Kim DG, Lee G, Seo J, Choi IY, Choi BS, Yang TJ, Kim KS, Lee J, Chin JH, Koh HJ. 2014. Defining the genome structure of ‘Tongil’ rice, an important cultivar in the Korean ‘Green Revolution’. *Rice* 7(1):22. doi:10.1186/s12284-014-0022-5.
- Li X, Wu L, Geng X, Xia X, Wang X, Xu Z, Xu Q. 2018. Deciphering the environmental impacts on rice quality for different rice cultivated areas. *Rice* 11:7. doi:10.1186/s12284-018-0198-1.
- Liu Q, Han R, Wu K, Zhang J, Ye Y, Wang S, Chen J, Pan

- Y, Li Q, Xu X, Zhou J, Tao D, Wu Y, Fu X. 2018. G-protein $\beta\gamma$ subunits determine grain size through interaction with MADS-domain transcription factors in rice. *Nat. Commun.* 9:852. doi:10.1038/s41467-018-03047-9.
- Megersa A, Seo J, Chin JH, Kim B, Koh HJ. 2016. Characterization of selected rice varieties adapted in Africa. *Plant Breed. Biotechnol.* 4(3):297–305. doi:10.9787/pbb.2016.4.3.297.
- Murray MG, Thompson WF. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* 8(19):4321–4325. doi:10.1093/nar/8.19.4321.
- Ngangkham U, Samantaray S, Yadav MK, Kumar A, Chidambaramanathan P, Katara JL. 2018. Effect of multiple allelic combinations of genes on regulating grain size in rice. *PLoS One* 13(1):e0190684. doi:10.1371/journal.pone.0190684.
- Ramkumar G, Sivaranjani AK, Pandey MK, Sakthivel K, Shobha Rani N, Sudarshan I, Prasad GS, Neeraja CN, Sundaram RM, Viraktamath BC, Madhav MS. 2010. Development of a PCR-based SNP marker system for effective selection of kernel length and kernel elongation in rice. *Mol. Breed.* 26(4):735–740. doi:10.1007/s11032-010-9492-3.
- Singh DP, Singh AK, Singh A. 2021. Chapter 5 - Plant genetic resources. USA: Academic Press.
- Sun K, Li D, Xia A, Zhao H, Wen Q, Jia S, Wang J, Yang G, Zhou D, Huang C, Wang H, Chen Z, Guo T. 2022. Targeted identification of rice grain-associated gene allelic variation through mutation induction, targeted sequencing, and whole genome sequencing combined with a mixed-samples strategy. *Rice* 15(1):57. doi:10.1186/s12284-022-00603-2.
- Tanabata T, Shibaya T, Hori K, Ebana K, Yano M. 2012. SmartGrain: High-throughput phenotyping software for measuring seed shape through image analysis. *Plant Physiol.* 160(4):1871–1880. doi:10.1104/pp.112.205120.
- Wailles E, Chavez E. 2015. International Rice Outlook, Baseline Projections 2014-2024 (Staff papers 199846). Technical report, University of Arkansas, USA.
- Wang S, Li S, Liu Q, Wu K, Zhang J, Wang S, Wang Y, Chen X, Zhang Y, Gao C, Wang F, Huang H, Fu X. 2015a. The *OsSPL16-GW7* regulatory module determines grain shape and simultaneously improves rice yield and grain quality. *Nat. Genet.* 47(8):949–954. doi:10.1038/ng.3352.
- Wang Y, Xiong G, Hu J, Jiang L, Yu H, Xu J, Fang Y, Zeng L, Xu E, Xu J, Ye W, Meng X, Liu R, Chen H, Jing Y, Wang Y, Zhu X, Li J, Qian Q. 2015b. Copy number variation at the *GL7* locus contributes to grain size diversity in rice. *Nat. Genet.* 47(8):944–948. doi:10.1038/ng.3346.
- Xie X, Li S, Liu H, Xu Q, Tang H, Mu Y, Deng M, Jiang Q, Chen G, Qi P, Li W, Pu Z, Ahsan Habib, Wei Y, Zheng Y, Lan X, Ma J. 2022. Identification and validation of a major QTL for kernel length in bread wheat based on two F3 biparental populations. *BMC Genomics* 23:386. doi:10.1186/s12864-022-08608-3.
- Xing Y, Zhang Q. 2010. Genetic and molecular bases of rice yield. *Annu. Rev. Plant Biol.* 61:421–442. doi:10.1146/annurev-arplant-042809-112209.
- Xue D, Qian Q, Teng S. 2014. Identification and utilization of elite genes from elite germplasms for yield improvement. *InTechOpen.* doi:10.5772/56390.
- Yuyu C, Aike Z, Pao X, Xiaoxia W, Yongrun C, Beifang W, Yue Z, Liaqat S, Shihua C, Liyong C, Yingxin Z. 2020. Effects of *GS3* and *GL3.1* for grain size editing by CRISPR/Cas9 in rice. *Rice Sci.* 27(5):405–413. doi:10.1016/j.rsci.2019.12.010.
- Zhao DS, Li QF, Zhang CQ, Zhang C, Yang QQ, Pan LX, Ren XY, Lu J, Gu MH, Liu QQ. 2018. *GS9* acts as a transcriptional activator to regulate rice grain shape and appearance quality. *Nat. Commun.* 9:1240. doi:10.1038/s41467-018-03616-y.
- Zhou H, Li P, Xie W, Hussain S, Li Y, Xia D, Zhao H, Sun S, Chen J, Ye H, Hou J, Zhao D, Gao G, Zhang Q, Wang G, Lian X, Xiao J, Yu S, Li X, He Y. 2017. Genome-wide association analyses reveal the genetic basis of stigma exertion in rice. *Mol. Plant* 10(4):634–644. doi:10.1016/j.molp.2017.01.001.
- Zuo J, Li J. 2014. Molecular genetic dissection of quantitative trait loci regulating rice grain size. *Annu. Rev. Genet.* 48:99–118. doi:10.1146/annurev-genet-120213-092138.