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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 vield, 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.

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 (Wailes 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.

Rice grain size and shape are the appearance of the

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.

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### 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'-aggctaaacacatgcccatcc-3', ERP: 5'-cccaacgttcagaaattaaatgtgctg-3', IRSP: 5'-aacagcaggctggcttactctctg-3', IFLP: 5'acgctgcctccagatgctga-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  $\mu$ L 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/ $\mu$ L.

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



(b)

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 throught amplificating 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). Smart-Grain 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.

1ST24VietnamIND7.630.6241.790.0204.31Long2OM541VietnamIND7.080.692.270.6073.4Long4OM180VietnamIND7.080.712.210.603.2Long5Dial'norm 85VietnamIND7.080.702.180.7033.18Long7HananomaiVietnamIND7.000.712.180.7033.18Short8Ontomine 85VietnamTEU5.130.792.880.0441.8<Short10KoshinikariJapanTEU5.250.702.880.0341.8<Short1127Oringian RepublicTRU5.70.752.870.0092.27Medium13S24IndiaAUS5.00.832.570.0092.24Medium14462IndiaAUS5.00.832.570.0092.24Medium15Aichan-HongChinaIND5.890.602.730.032.24Medium16AijananteIndiaAUS5.90.602.730.032.24Medium16AijananteIndiaAUS5.90.602.740.032.24Medium17AisaIndiaAUS5.90.602.740.632.044.0418AisaIndia<	No.	Variety Name	Origin	Sub-group	GL (mm)	SD (1)	GW (mm)	SD (2)	GL/GW	Classification of grain
2         OMS451         Vietnam         IND         7.08         7.08         2.09         7.07         3.4         Long           3         IR5004         Philipnes         IND         6.70         2.21         0.059         3.21         Longer           5         JaiThorn 8         Vietnam         IND         7.00         0.701         2.18         0.033         3.21         Longer           6         Jasnine 85         Vietnam         IND         7.02         0.603         3.18         0.033         4.01           7         Haanomai         Japan         TEU         5.20         0.633         2.81         0.044         1.8         Short           10         Koshihkir         Japan         TEU         5.20         0.63         2.87         0.69         1.8         Orter           11         Strot         Japan         TEU         5.20         0.63         2.60         0.51         1.60         Orter         Medium           12         Strot         India         NUD         5.78         0.61         2.01         2.21         Medium           13         Acchiao-Hong         India         AUS         5.87         0.63 <td>1</td> <td>ST24</td> <td>Vietnam</td> <td>IND</td> <td>7.63</td> <td>0.024</td> <td>1.79</td> <td>0.020</td> <td>4.3</td> <td>Long</td>	1	ST24	Vietnam	IND	7.63	0.024	1.79	0.020	4.3	Long
3 ISOMA4 Philippines IND 6.87 0.01 2.27 0.069 3.00 Ing   4 OMBA Victuam IND 7.03 0.01 2.10 0.060 3.10 Ing   6 Jamine S5 Victuam IND 7.01 0.02 2.10 0.03 3.10 Ing   7 Hananomai Japan TEI 5.20 0.03 3.10 0.034 1.8 Short   9 Niponbare Pilippines TEI 5.20 0.05 2.80 0.04 1.8 Short   10 Koshihikari Japan PIL 5.20 0.05 2.80 0.05 2.61 0.05 1.61   11 270 OminicanRepuble TEU 5.20 0.05 2.62 0.02 1.61 Medium   12 Stahihikari Japan Pilippines 1.81 5.70 0.63 2.71 0.01 2.40 Medium   13 Stahihikari Japan Pilippines 1.81 5.70 0.63 2.71 0.01 2.40 Medium   14 640-4 Pinace Pinace 1.81 5.70 0.63 2.71 0.02 2.61 Medium   15 Stahihikari Pinace Pinace Pinace 1.81 5.70 0.63 2.71 0.63 2.61 Medium   16 Aligonarte China Aligonarte Pinace Pinace Pinace Pinace Pinace Pinace   16 Aligonarte Rida <td>2</td> <td>OM5451</td> <td>Vietnam</td> <td>IND</td> <td>7.08</td> <td>0.089</td> <td>2.09</td> <td>0.072</td> <td>3.4</td> <td>Long</td>	2	OM5451	Vietnam	IND	7.08	0.089	2.09	0.072	3.4	Long
4OM18VietnamIND7.030.0132.110.0603.2Long5Jairhorn BVietnamIND7.000.0732.170.0233.3Long7HananomiaJapanTEU5.130.0732.180.0341.8Short8DS1VietnamTEU5.200.0633.180.0411.6Short10KoshihkariJapanTEU5.200.0502.880.0341.8Short1127Dominkan RepubliTRU5.700.0752.680.0122.1Medium13S24UrguayTEU5.200.0402.710.0132.2Medium14AlS-FanceTEU5.200.0402.100.0122.1Medium15AiChai-HongChinaNID5.700.0512.10Medium16AljaonateChinaNID5.700.0522.54Medium17AriasMonsiaAUS5.790.0522.54Medium18Asina 330BagladshAUS5.790.0522.640.0722.54Medium19Balc GoraIndiaAUS5.790.0522.640.6144.64Medium10Balc GoraIndiaAUS5.790.0522.64MediumMedium10Balc MarcaBalgadshAUS5.790.0522.64Medium	3	IR50404	Philippines	IND	6.87	0.081	2.27	0.069	3.0	Long
5Dia ThomâVietnamIND7.640.720.710.7250.730.731.810.733Long6Jasmine 85VietnamIND7.200.0512.180.0341.8Short8DS1VietnamTEJ5.200.0633.180.0411.8Short9NipponbarePhilippinesTEJ5.250.0752.670.0711.8Short10KoshihikariJapanTEJ5.270.0722.680.0152.5Medium1127Dominican RepublicTRU6.770.0752.680.0152.2Medium12S19Ominican RepublicTRU5.700.0752.680.0152.2Medium139524IndiaAUS5.700.0352.570.0382.2Medium14682-FranceTIND5.700.0352.570.0382.2Medium15Aifchiac-HongIndonesiaIND5.700.0352.630.0612.3Medium16AijaonarteIndonesiaAUS5.700.0352.640.0372.2Medium18Aswina 330BagladeshAUS5.870.0572.630.0512.5Medium19Bulk GoraIndiaAUS5.870.0552.490.302.4Medium10Busk GoraIndiaAUS5.800.057	4	OM18	Vietnam	IND	7.03	0.018	2.21	0.060	3.2	Long
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7HananomaiJapanTEJ5.130.0932.880.0341.8Short8OS1VeltamTEJ5.200.0633.180.0411.6Short10KoshihkariJapanTEJ5.200.0502.830.0512.50Note1127Dominca RepublicTRU6.570.0002.730.0202.20Medium12519UruguayIND5.700.0002.570.0022.20Medium1468-2IndiaAUS5.700.0302.570.0302.20Medium15Al-Chiao-HongChinaIND5.700.0302.630.0602.20Medium16AjiaonateChinaIND5.700.0302.640.0502.64Medium17AriasIndonesiaIND5.700.0302.640.0502.64Medium18Aswina 330BangladeshAUS5.700.0502.64Medium19BlaIndiaAUS5.700.0512.64Medium10Bas VistaMaxinAUS5.700.0532.640.0512.4410Bas VistaMaxinAUS5.710.0532.64Medium11BlaBas VistaMaxinAUS5.710.0532.64Medium12Bas VistaMaxinAUS5.710.0532.64Medium <td>6</td> <td>Jasmine 85</td> <td>Vietnam</td> <td>IND</td> <td>7.20</td> <td>0.051</td> <td>2.18</td> <td>0.033</td> <td>3.3</td> <td>Long</td>	6	Jasmine 85	Vietnam	IND	7.20	0.051	2.18	0.033	3.3	Long
8     DS1     Vietnam     TEJ     5.20     0.063     3.18     0.041     1.80     Short       9     Nipponbare     Pilippines     TEJ     5.26     0.000     2.03     0.034     1.80     Short       10     Koshihikari     Japan     TEJ     5.25     0.000     2.03     0.034     1.80     Short       12     Sth     Dominan Republi     TR     5.25     0.005     2.72     0.012     2.10     Medium       13     9524     France     France     NID     5.80     0.053     2.63     0.030     2.20     Medium       15     Aichiao-Hong     China     NID     5.80     0.053     2.63     0.040     2.20     Medium       16     Aijaonante     China     NID     5.89     0.035     2.64     0.020     2.64     Medium       17     Arias     Banglach     AUS     5.89     0.053     2.64     0.020     2.64     Medium       18     Javina 302     Balyador     TR     6.80     0.054     2.65     0.054     2.65     Medium       19     Black Gora     Louisan     ADM     5.89     0.015     2.65     0.015     2.64     Medium <td>7</td> <td>Hananomai</td> <td>Japan</td> <td>TEJ</td> <td>5.13</td> <td>0.093</td> <td>2.88</td> <td>0.034</td> <td>1.8</td> <td>Short</td>	7	Hananomai	Japan	TEJ	5.13	0.093	2.88	0.034	1.8	Short
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1127Dominican RepublicTRJ6.770.780.800.71 </td <td>10</td> <td>Koshihikari</td> <td>Japan</td> <td>TEJ</td> <td>5.25</td> <td>0.090</td> <td>2.93</td> <td>0.034</td> <td>1.8</td> <td>Short</td>	10	Koshihikari	Japan	TEJ	5.25	0.090	2.93	0.034	1.8	Short
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139524IndiaAUS5.600.0832.710.0092.2Medium1468-2FranceTEJ5.720.4542.710.0132.2Medium15Ai-Chiao-HongChinaIND5.700.3032.310.0302.2Medium16AijananteChinaIND5.790.6032.430.0302.2Medium17ArasIndonesiaTR5.590.602.190.592.6Medium18Asvina 330BangladeshAUS5.730.652.490.3032.2Medium19Blak GoraIndiaAUS5.890.3072.400.3042.2Medium20Blak GoraIndiaADMX5.890.3072.500.0552.4Medium21Blak GoraIndiaADMX5.890.3072.500.0552.4Medium22Boa VistaCalombiaTR6.600.962.550.0552.4Medium23C10451GaomáMexicoADMX5.620.812.750.3032.1Medium24CalosiraMexicoADMX5.620.812.750.812.4Medium25CAO2/P//1GaomáGaomáGao0.612.650.052.4Medium26CalosiraArgentiaTR6.390.162.850.042.3Medium	12	519	Uruguay	IND	5.78	0.064	2.72	0.021	2.1	Medium
1468-2FranceTEJ5.720.0452.710.0132.2Medium15Ai-Chiao-HongChinaIND5.700.0302.570.0382.2Medium16AijaonanteChinaIND5.890.0532.500.0602.190.0552.5Medium17AriasIndonesiaIRU5.790.0602.190.0522.6Medium18Asvina 330BagladeshAUS5.730.0562.490.0322.6Medium19Bl 1IndiaAUS5.890.0372.400.0322.6Medium10Back GoraIndiaAUS5.890.0372.600.0322.5Medium12Back GoraIndiaAUS5.890.0372.650.0442.2Medium13Bloe SocISlavadorTR6.600.0962.650.0442.1Medium14C14-5-3MexicoADMIX5.620.182.730.0332.1Medium15CA902/B2/1/1ChadAUS5.690.182.790.0462.2Medium16CausirFarineATM6.690.812.790.0462.2Medium17Canale DeferroBraivaTR6.390.182.790.0202.5Medium18Carolina GoldMited StatesTR6.370.6622.480.62 <td>13</td> <td>9524</td> <td>India</td> <td>AUS</td> <td>5.60</td> <td>0.083</td> <td>2.57</td> <td>0.009</td> <td>2.2</td> <td>Medium</td>	13	9524	India	AUS	5.60	0.083	2.57	0.009	2.2	Medium
15Ai-Chiao-HongChinaINDINDS.700.3032.570.0382.2Medium16AijaonanteChinaINDS.890.0532.630.0602.2Medium17AriasIndonesiaTRJS.590.0602.190.0592.50Medium18Aswina 330BangladeshUSS.270.0652.490.0722.6Medium10B1 1IndiaAUSS.890.0372.620.0372.2Medium20Back GoraIndiaAUSS.890.0372.620.0442.2Medium21Blue RoseLouisianaADMIX6.110.0832.820.0442.2Medium23C10451ColombiaITN6.550.1182.570.0332.1Medium24C1-6-53MedicoADMIX5.610.1812.750.0332.1Medium25CA902/B/2/1ChadALGNT6.390.1182.570.0232.4Medium26Cawa/Fortuna 6-103-15TaiwanTR6.370.0812.750.0242.5Medium27Cacela ColoMarceTR6.370.0842.480.0352.44Medium28Carolina GoldUnited StatesTR6.370.0672.480.0462.3Medium29Cacela ColoMitenTR5.760.047	14	68-2	France	TEJ	5.92	0.045	2.71	0.013	2.2	Medium
16AijaonanteChinaIND5.890.0532.630.0602.2Medium17AriasIndonesiaTR5.590.0602.190.0592.5Medium18Aswina 330BangladeshAUS5.730.0602.490.0362.40Medium19BJ 1IndiaAUS5.730.0572.62Medium10Black GoraIndiaAUS5.730.0572.620.0372.20Medium21Black GoraIndiaADMIX6.110.0832.820.0442.20Medium22Boa VistaEl SalvadorTR6.600.0612.550.0532.10Medium23C10451ColombiaIND5.550.1182.590.0462.10Medium24C1-6-53MexicoADMIX5.620.1812.560.0132.41Medium25CA902/B/21ChadTR6.390.0512.560.0522.40Medium26Caawa/Fortuna 6-103-15TaiwanTR6.370.0812.770.0202.31Medium26CaavalaCateiraMeritoraTR6.370.0812.480.0412.1<	15	Ai-Chiao-Hong	China	IND	5.70	0.030	2.57	0.038	2.2	Medium
11AriasIndonesiaTRJ5.590.0602.190.0592.5Medium18Aswina 330BangladeshAUS6.290.0832.460.0722.64Medium19B.1IndiaAUS5.730.0562.490.0332.2Medium20Black GoraIndiaAUS5.730.0582.820.0442.2Medium21Blue RoseLouisianaADMIX6.110.0832.820.0442.1Medium23C101A51ColombiaTRJ6.600.0962.650.0332.1Medium24C1-6-5-3MexicoADMIX5.520.1182.920.0462.1Medium25CA 902/B/2/1ChadADA7.820.0312.14Medium26Caawa/Fortuna 6-103-15TaiwanTRJ6.390.1012.890.0462.2Medium27Cacella De FerroBrailTRJ6.390.1022.650.0292.5Medium28Carolina GoldUnited StatesTRJ6.390.0672.480.0352.4Medium29CaucaicaFormer Soviet UnionTL6.370.0812.480.0352.4Medium29CaucaicaFormer Soviet UnionTL6.370.0672.550.0242.3Medium30CenitArgentinaNDL5.700.0672.540.025<	16	Aijiaonante	China	IND	5.89	0.053	2.63	0.060	2.2	Medium
18Aswina 330BangladeshAUS6.290.0832.460.0722.6Medium19BJ 1IndiaAUS5.730.0562.490.0362.3Medium20Black GoraIndiaAUS5.890.0372.620.0372.2Medium21Blue RoseLouisianaADMIX6.110.0832.820.0442.2Medium22Boa VistaEl SalvadorTRU6.000.0962.550.0452.5Medium23C101A51ColombiaIND5.550.1812.590.0462.2Medium24C1-6-5-3MexicoADMIX6.090.0512.560.0152.4Medium25CA 902/B/2/1ChadALGALG6.390.1012.890.0462.2Medium26Casawa/Fortuna 6-103-15TaiwanTRJ6.380.1102.890.0412.2Medium27Canella D FerroBrazilTRJ6.390.0422.650.0222.5Medium28Carolina GoldUnited StatesTRJ5.590.0642.480.0352.4Medium29CausaicaFormer SovietUnionTL5.590.0642.480.0352.4Medium30China ChanhVietnamIND5.790.0672.480.0352.4Medium31ChanNatioS.600.021	17	Arias	Indonesia	TRJ	5.59	0.060	2.19	0.059	2.5	Medium
19B11IndiaAUS5.730.0562.490.0362.3Medium20Black GoraIndiaAUS5.890.0372.620.0372.2Medium21Blue RoseLouisianaADMIX6.110.0832.820.0442.2Medium22Boa VistaEl SalvadorTRJ6.600.0962.650.0052.5Medium23C101A51ColombiaIND5.500.182.730.0332.1Medium24C1-6-5-3MexicoADMIX5.600.812.730.332.1Medium25CA902/B/2/1ChadAUS6.090.512.560.0152.4Medium26Cawa/Fortuna 6-103-15TaiwanTRJ6.390.182.770.0202.3Medium27Canella De FerroBrazilTRJ6.390.182.770.0202.3Medium28Carolina GoldUnited StatesTRJ5.900.0622.650.0212.6Medium29CausaicaFormer Soviet UnitTL5.700.0622.650.0222.5Medium31ChauArgentinaTRJ5.700.0622.480.0512.4Medium33China ObayVietnamIND5.700.0622.480.0522.2Medium34China 1039China 400Not5.700.1632.45 <td>18</td> <td>Aswina 330</td> <td>Bangladesh</td> <td>AUS</td> <td>6.29</td> <td>0.083</td> <td>2.46</td> <td>0.072</td> <td>2.6</td> <td>Medium</td>	18	Aswina 330	Bangladesh	AUS	6.29	0.083	2.46	0.072	2.6	Medium
20Black GoraIndiaAUS5.890.0372.62Nodium21Blue RoseLouisianaADMIX6.110.0832.820.0442.2Medium22Boa VistaEl SalvadorTRU6.600.0962.650.0052.5Medium23C101A51ColombiaIND5.550.1182.730.0332.1Medium24C1-6-53MexicoADMIX5.620.812.730.0332.1Medium25CA 902/B/2/1ChadAUS6.090.512.560.0292.5Medium26Caswa/Fortuna 6-103-15TaiwanTR6.380.1382.770.0202.3Medium26Caswa/Fortuna 6-103-15TaiwanTR6.390.1382.700.0202.5Medium27Canella De FerroBrazilTR6.370.622.650.0292.5Medium28Caoraira GoldUnited StatesTR6.370.660.422.4Medium29CacutaicaArgentinaTR5.960.0642.480.0352.4Medium30CenitArgentinaND5.790.6672.480.0452.9Medium31ChanChanND5.790.672.480.0452.9Medium32Chien ChanhVietnamND5.700.1672.450.0282.1Medium <td>19</td> <td>BJ 1</td> <td>India</td> <td>AUS</td> <td>5.73</td> <td>0.056</td> <td>2.49</td> <td>0.036</td> <td>2.3</td> <td>Medium</td>	19	BJ 1	India	AUS	5.73	0.056	2.49	0.036	2.3	Medium
11Blue RoseLouisianaADMIX6.110.0832.820.0442.2Medium22Boa VistaEl SalvadorTRJ6.600.0962.650.0052.5Medium23C101A51ColombiaIND5.550.1182.590.0462.1Medium24C1-6-5-3MexicoADMIX5.620.0812.730.0332.1Medium25CA 902/B/2/1ChadAUS6.090.0512.560.0152.4Medium26Caaw/Fortuna 6-103-15TaiwanTRJ6.380.1102.890.0462.2Medium27Canella De FerroBrazilTRJ6.390.1382.770.0202.5Medium28Carolina GoldUnited StatesTRJ6.370.0622.650.0292.5Medium29CacuasicaFormer Soviet UnioTL6.370.0612.480.0552.4Medium30CenitArgentinaTRD5.790.0672.480.0552.4Medium31ChauVietnamIND5.790.0672.480.0522.2Medium32Chien ChanhVietnamIND5.790.1672.450.0282.3Medium33Chien ChanhUnited StatesADMIX5.770.1472.450.0282.2Medium34Cl 11011United States-CATEJ5.66<	20	Black Gora	India	AUS	5.89	0.037	2.62	0.037	2.2	Medium
22Boa VistaEl SalvadorTRJ6.600.0962.650.0052.5Medium23C101A51ColombiaIND5.550.1182.590.0442.1Medium24C1-6-5-3MexicoADMIX5.620.0812.730.0332.1Medium25CA 902/B/2/1ChadAUS6.090.0512.640.0152.4Medium26Caawa/Fortuna 6-103-15TaiwanTRJ6.380.1102.890.0462.2Medium27Caclela De FerroBrazilTRJ6.390.1382.770.0202.3Medium28Carolina GoldUnited StatesTRJ6.590.6620.642.4Medium29CacasicaFormer Soviet UnionTEJ6.370.0672.480.0462.3Medium30CenitArgentinaIND5.960.6642.480.0452.4Medium31ChauVietnamIND5.970.6672.480.0452.3Medium32Chien ChanhVietnamIND5.860.0952.650.0222.2Medium33China 1039ChinaMaitsantALMS5.700.1672.480.0232.44Medium34China 1039ChinaStatesALMS5.700.1672.480.0232.4Medium35CoarsePakistanALMS5.70 <td>21</td> <td>Blue Rose</td> <td>Louisiana</td> <td>ADMIX</td> <td>6.11</td> <td>0.083</td> <td>2.82</td> <td>0.044</td> <td>2.2</td> <td>Medium</td>	21	Blue Rose	Louisiana	ADMIX	6.11	0.083	2.82	0.044	2.2	Medium
23C101A51ColombiaIND5.550.1182.590.0462.1Medium24C1-6-5-3MexicoADMIX5.620.0812.730.0332.1Medium25CA 902/B/2/1ChadAUS6.090.512.560.0152.4Medium26Cawa/Fortuna 6-103-15TaiwanTRJ6.380.1102.890.0462.2Medium27Canella De FerroBrazilTRJ6.390.0622.650.0292.5Medium28Carolina GoldUnited StatesTRJ6.370.0893.000.0412.1Medium29CaucasicaFormer Soviet UnionTEJ6.370.0893.000.0412.1Medium30CenitArgentinaTRJ5.960.0642.480.0352.4Medium31ChauVietnamIND5.790.0672.480.0462.3Medium32Chin ChanhVietnamIND5.790.0672.480.0232.2Medium33China 1039Ointe StatesADMIX5.770.1342.650.0232.2Medium34Cl 11011United StatesADMIX5.770.1342.640.0232.1Medium35CoarsePakistanAUS5.700.1542.480.0232.1Medium36CogocionaBulgariaTEJ5.640.07	22	Boa Vista	El Salvador	TRJ	6.60	0.096	2.65	0.005	2.5	Medium
24C1-6-5-3MexicoADMIX5.620.0812.730.0332.1Medium25CA 902/B/2/1ChadAUS6.090.0512.560.0152.4Medium26Cawa/Fortuna 6-103-15TaiwanTRJ6.380.1102.890.0462.2Medium27Canella De FerroBrazilTRJ6.390.0622.650.0292.5Medium28Carolina GoldUnited StatesTRJ6.570.0622.650.0292.5Medium29CaucasicaFormer Soviet UnionTLJ6.370.0642.480.0352.44Medium30CenitArgentinaTRJ5.960.0642.480.0452.3Medium31ChauVietnamIND5.790.0672.480.0452.3Medium32Chine ChanhVietnamIND5.790.0572.480.0452.3Medium33China 1039ChinaNID5.860.0962.650.0222.2Medium34C11011United StatesADMIX5.770.1342.670.0232.1Medium35CoarsePalgashTLJ5.660.0752.680.0232.1Medium36CopocinaBulgariaTLJ5.690.0762.720.0722.14Medium37CS-M3United States-CATL5.690.075 <td>23</td> <td>C101A51</td> <td>Colombia</td> <td>IND</td> <td>5.55</td> <td>0.118</td> <td>2.59</td> <td>0.046</td> <td>2.1</td> <td>Medium</td>	23	C101A51	Colombia	IND	5.55	0.118	2.59	0.046	2.1	Medium
25CA 902/B/2/1ChadAUS6.090.0512.560.0152.4Medium26Caawa/Fortuna 6-103-15TaiwanTRJ6.380.1102.890.0462.2Medium27Canella De FerroBrazilTRJ6.390.1382.770.0202.3Medium28Carolina GoldUnited StatesTRJ6.590.0622.650.0292.5Medium29CaucasicaFormer Soviet UnionTEJ6.370.0893.000.0412.1Medium30CenitArgentinaTRJ5.960.0622.480.0352.4Medium31ChauVietnamIND5.790.0672.480.0462.3Medium32Chien ChanhVietnamIND5.860.0912.110.0152.9Medium33China 1039ChinaInited StatesADMIX5.770.1342.670.0252.2Medium34C11011United States-CATEJ5.660.0752.680.0232.1Medium35CoarseBulgariaTEJ5.690.0152.720.0722.1Medium36C15156BangladeshAUS5.790.0562.88Medium37DefeaxUnited StatesTRJ6.990.0152.740.0242.8Medium38CTG 1516BangladeshAUS6.320.677 <td>24</td> <td>C1-6-5-3</td> <td>Mexico</td> <td>ADMIX</td> <td>5.62</td> <td>0.081</td> <td>2.73</td> <td>0.033</td> <td>2.1</td> <td>Medium</td>	24	C1-6-5-3	Mexico	ADMIX	5.62	0.081	2.73	0.033	2.1	Medium
26Caawa/Fortuna 6-103-15TaiwanTRJ6.380.1102.890.0462.2Medium27Canella De FerroBrazilTRJ6.390.1382.770.0202.3Medium28Carolina GoldUnited StatesTRJ6.590.0622.650.0292.5Medium29CaucasicaFormer Soviet UnionTEJ6.370.0893.000.0412.1Medium30CenitArgentinaTRJ5.960.0642.480.0352.4Medium31ChauVietnamIND5.790.0672.480.0452.9Medium33China ChanhVietnamIND5.860.0962.650.0322.2Medium34C11011United StatesADMIX5.770.1342.670.0252.2Medium35CoarsePakistanAUS5.700.1672.480.0242.1Medium36CopocinaBulgariaTEJ5.660.0752.680.0232.1Medium36CopocinaBungladeshAUS5.790.0762.770.0072.1Medium37CS-M3United States-CATEJ5.690.0152.720.0722.1Medium37De Geo Woo GenTaiwanIND5.690.0152.740.0242.8Medium40DelrexUnited StatesTRJ6.09<	25	CA 902/B/2/1	Chad	AUS	6.09	0.051	2.56	0.015	2.4	Medium
27Canella De FerroBrazilTRJ6.390.1382.770.0202.3Medium28Carolina GoldUnited StatesTRJ6.590.0622.650.0292.5Medium29CaucasicaFormer Soviet UnionTEJ6.370.0893.000.0412.1Medium30CenitArgentinaTRJ5.960.0672.480.0352.4Medium31ChauVietnamIND5.790.0672.480.0462.3Medium32Chien ChanhVietnamIND5.860.0962.650.0322.2Medium33China 1039ChinaUnited StatesADMIX5.770.1342.670.0252.2Medium34Cl 11011United StatesADMIX5.770.1472.450.0282.3Medium35CoarsePakistanAUS5.700.1672.480.0282.3Medium36CoppocinaBulgariaTEJ5.660.0752.680.0282.1Medium37CS-M3United States-CATEJ5.920.0762.770.0702.1Medium38CTG 1516BangladeshAUS5.490.0242.8Medium39Deceo Woo GenTaiwanIND5.690.0512.740.0342.4Medium40DelrexUnited StatesTRJ6.990.051<	26	Caawa/Fortuna 6-103-15	Taiwan	TRJ	6.38	0.110	2.89	0.046	2.2	Medium
28Carolina GoldUnited StatesTRJ6.590.0622.650.0292.5Medium29CaucasicaFormer Soviet UnionTEJ6.370.0893.000.0412.1Medium30CenitArgentinaTRJ5.960.0642.480.0352.4Medium31ChauVietnamIND5.790.0672.480.0452.3Medium32Chien ChanhVietnamIND6.390.0912.210.0152.9Medium33China 1039ChinaIND5.860.0962.650.0322.2Medium34Cl 11011United StatesADMIX5.770.1342.670.0282.3Medium35CoarsePakistanAUS5.700.1672.480.0232.1Medium36CoppocinaBulgariaTEJ5.660.0752.680.0232.1Medium37CS-M3United States-CATEJ5.920.0762.770.0722.1Medium38CTG 1516BangladeshAUS5.690.0152.720.0722.1Medium40DelrexUnited StatesTRJ6.090.0212.200.0562.8Medium41Dhala ShaittaBangladeshAUS5.770.0622.740.0332.1Medium42DK 12BangladeshAUS5.770.062<	27	Canella De Ferro	Brazil	TRJ	6.39	0.138	2.77	0.020	2.3	Medium
29CaucasicaFormer Soviet UnionTEJ6.370.0893.000.0412.1Medium30CenitArgentinaTRJ5.960.0642.480.0352.4Medium31ChauVietnamIND5.790.0672.480.0462.3Medium32Chiem ChanhVietnamIND6.390.0912.210.0152.9Medium33China 1039ChinaIND5.860.0962.650.0322.2Medium34Cl 11011United StatesADMIX5.770.1342.670.0252.2Medium35CoarsePakisanAUS5.700.1672.480.0232.1Medium36CopocinaBulgariaTEJ5.660.0752.680.0232.1Medium37CS-M3United States-CATEJ5.690.0752.680.0212.1Medium39Dec Geo Woo GenIniwanIND5.690.0752.740.0722.1Medium40DelrexUnited StatesTRJ6.090.0212.200.0562.8Medium41Dhala ShaittaBangladeshAUS5.770.0622.740.0332.1Medium42DK 12BangladeshAUS5.770.0622.740.0332.1Medium43DM 43BangladeshAUS5.770.0622.74 <td>28</td> <td>Carolina Gold</td> <td>United States</td> <td>TRJ</td> <td>6.59</td> <td>0.062</td> <td>2.65</td> <td>0.029</td> <td>2.5</td> <td>Medium</td>	28	Carolina Gold	United States	TRJ	6.59	0.062	2.65	0.029	2.5	Medium
30CenitArgentinaTRJ5.960.0642.480.0352.4Medium31ChauVietnamIND5.790.0672.480.0462.3Medium32Chiem ChanhVietnamIND6.390.0912.210.0152.9Medium33China 1039ChinaIND5.860.0962.650.0322.2Medium34C1 11011United StatesADMIX5.770.1342.670.0252.2Medium35CoarsePakistanAUS5.700.1672.480.0232.1Medium36CoppocinaBulgariaTEJ5.660.0752.680.0232.1Medium37CS-M3United States-CATEJ5.920.0762.770.0072.1Medium38CTG 1516BangladeshAUS5.690.0152.720.0722.1Medium39DefexUnited StatesTRJ6.090.0212.200.0562.8Medium40DelrexUnited StatesAUS6.320.0672.440.0242.8Medium41Dhala ShaittaBangladeshAUS6.320.0672.440.0242.8Medium42DK 12BangladeshAUS5.770.0622.740.0332.1Medium43DM 43BangladeshAUS5.770.0622.740.033	29	Caucasica	Former Soviet Union	TEJ	6.37	0.089	3.00	0.041	2.1	Medium
31ChauVietnamIND5.790.0672.480.0462.3Medium32Chiem ChanhVietnamIND6.390.0912.210.0152.9Medium33China 1039ChinaIND5.860.0962.650.0322.2Medium34Cl 11011United StatesADMIX5.770.1342.670.0252.2Medium35CoarsePakistanAUS5.700.1672.450.0282.3Medium36CoppocinaBulgariaTEJ5.660.0752.680.0232.1Medium38CTG 1516BangladeshAUS5.920.0762.770.0072.1Medium39Dee Geo Woo GenTaiwanIND5.690.1052.720.0722.1Medium40DelrexUnited StatesTRJ6.090.0212.200.0562.8Medium41Dhala ShaittaBangladeshAUS5.770.0622.740.0332.1Medium42DK 12BangladeshAUS5.770.0622.740.0332.1Medium43DM 43BangladeshAUS5.860.0112.470.0332.4Medium44DM 59BangladeshAUS5.860.0112.470.0332.4Medium	30	Cenit	Argentina	TRJ	5.96	0.064	2.48	0.035	2.4	Medium
32Chiem ChanhVietnamIND6.390.0912.210.0152.9Medium33China 1039ChinaIND5.860.0962.650.0322.2Medium34Cl 11011United StatesADMIX5.770.1342.670.0252.2Medium35CoarsePakistanAUS5.700.1672.450.0282.3Medium36CoppocinaBulgariaTEJ5.660.0752.680.0232.1Medium37CS-M3United States-CATEJ5.920.0762.770.0072.1Medium38CTG 1516BangladeshAUS6.130.0962.360.0412.6Medium39Dee Geo Woo GenTaiwanIND5.690.1052.720.0722.1Medium40DelrexUnited StatesTRJ6.090.0212.200.0562.8Medium41Dhala ShaittaBangladeshAUS6.320.0672.740.0332.1Medium42DK 12BangladeshAUS5.770.0622.740.0332.1Medium43DM 43BangladeshAUS5.860.0112.470.0332.4Medium44DM 59BangladeshAUS5.860.0112.470.0332.4Medium	31	Chau	Vietnam	IND	5.79	0.067	2.48	0.046	2.3	Medium
33China 1039ChinaIND5.860.0962.650.0322.2Medium34Cl 11011United StatesADMIX5.770.1342.670.0252.2Medium35CoarsePakistanAUS5.700.1672.450.0282.3Medium36CoppocinaBulgariaTEJ5.660.0752.680.0232.1Medium37CS-M3United States-CATEJ5.920.0762.770.0072.1Medium38CTG 1516BangladeshAUS6.130.0962.360.0412.6Medium40DelrexUnited StatesTRJ6.090.1052.720.0722.1Medium41Dhala ShaittaBangladeshAUS5.690.0212.200.0562.8Medium42DK 12BangladeshAUS6.320.0672.440.0332.1Medium43DM 43BangladeshAUS5.770.0622.740.0332.1Medium44DM 59BangladeshAUS5.860.0112.470.0332.4Medium	32	Chiem Chanh	Vietnam	IND	6.39	0.091	2.21	0.015	2.9	Medium
34Cl 11011United StatesADMIX5.770.1342.670.0252.2Medium35CoarsePakistanAUS5.700.1672.450.0282.3Medium36CoppocinaBulgariaTEJ5.660.0752.680.0232.1Medium37CS-M3United States-CATEJ5.920.0762.770.0072.1Medium38CTG 1516BangladeshAUS6.130.0962.360.0412.6Medium39Dee Geo Woo GenTaiwanIND5.690.1052.720.0722.1Medium40DelrexUnited StatesTRJ6.090.0212.200.0562.8Medium41Dhala ShaittaBangladeshAUS6.320.0672.240.0242.8Medium42DK 12BangladeshAUS5.770.0622.740.0332.1Medium43DM 43BangladeshAUS5.860.0112.470.0332.4Medium44DM 59BangladeshAUS5.860.0112.470.0332.4Medium	33	China 1039	China	IND	5.86	0.096	2.65	0.032	2.2	Medium
35CoarsePakistanAUS5.700.1672.450.0282.3Medium36CoppocinaBulgariaTEJ5.660.0752.680.0232.1Medium37CS-M3United States-CATEJ5.920.0762.770.0072.1Medium38CTG 1516BangladeshAUS6.130.0962.360.0412.6Medium39Dee Geo Woo GenTaiwanIND5.690.1052.720.0722.1Medium40DelrexUnited StatesTRJ6.090.0212.200.0562.8Medium41Dhala ShaittaBangladeshAUS6.320.0672.440.0232.1Medium42DK 12BangladeshAUS5.770.0622.740.0332.1Medium43DM 43BangladeshAUS5.860.0112.470.0332.4Medium44DM 59BangladeshAUS6.200.1182.360.0412.6Medium	34	CI 11011	United States	ADMIX	5.77	0.134	2.67	0.025	2.2	Medium
36CoppocinaBulgariaTEJ5.660.0752.680.0232.1Medium37CS-M3United States-CATEJ5.920.0762.770.0072.1Medium38CTG 1516BangladeshAUS6.130.0962.360.0412.6Medium39Dee Geo Woo GenTaiwanIND5.690.1052.720.0722.1Medium40DelrexUnited StatesTRJ6.090.0212.200.0562.8Medium41Dhala ShaittaBangladeshAUS6.320.0672.240.0242.8Medium42DK 12BangladeshAUS5.770.0622.740.0332.1Medium43DM 43BangladeshAUS5.860.0112.470.0332.4Medium44DM 59BangladeshAUS6.200.1182.360.0412.6Medium	35	Coarse	Pakistan	AUS	5.70	0.167	2.45	0.028	2.3	Medium
37CS-M3United States-CATEJ5.920.0762.770.0072.1Medium38CTG 1516BangladeshAUS6.130.0962.360.0412.6Medium39Dee Geo Woo GenTaiwanIND5.690.1052.720.0722.1Medium40DelrexUnited StatesTRJ6.090.0212.200.0562.8Medium41Dhala ShaittaBangladeshAUS6.320.0672.240.0242.8Medium42DK 12BangladeshAUS5.770.0622.740.0332.1Medium43DM 43BangladeshAUS5.860.0112.470.0332.4Medium44DM 59BangladeshAUS6.200.1182.360.0412.6Medium	36	Coppocina	Bulgaria	TEJ	5.66	0.075	2.68	0.023	2.1	Medium
38CTG 1516BangladeshAUS6.130.0962.360.0412.6Medium39Dee Geo Woo GenTaiwanIND5.690.1052.720.0722.1Medium40DelrexUnited StatesTRJ6.090.0212.200.0562.8Medium41Dhala ShaittaBangladeshAUS6.320.0672.240.0242.8Medium42DK 12BangladeshAUS5.770.0622.740.0332.1Medium43DM 43BangladeshAUS5.860.0112.470.0332.4Medium44DM 59BangladeshAUS6.200.1182.360.0412.6Medium	37	CS-M3	United States-CA	TEJ	5.92	0.076	2.77	0.007	2.1	Medium
39Dee Geo Woo GenTaiwanIND5.690.1052.720.0722.1Medium40DelrexUnited StatesTRJ6.090.0212.200.0562.8Medium41Dhala ShaittaBangladeshAUS6.320.0672.240.0242.8Medium42DK 12BangladeshAUS5.770.0622.740.0332.1Medium43DM 43BangladeshAUS5.860.0112.470.0332.4Medium44DM 59BangladeshAUS6.200.1182.360.0412.6Medium	38	CTG 1516	Bangladesh	AUS	6.13	0.096	2.36	0.041	2.6	Medium
40DelrexUnited StatesTRJ6.090.0212.200.0562.8Medium41Dhala ShaittaBangladeshAUS6.320.0672.240.0242.8Medium42DK 12BangladeshAUS5.770.0622.740.0332.1Medium43DM 43BangladeshAUS5.860.0112.470.0332.4Medium44DM 59BangladeshAUS6.200.1182.360.0412.6Medium	39	Dee Geo Woo Gen	Taiwan	IND	5.69	0.105	2.72	0.072	2.1	Medium
41Dhala ShaittaBangladeshAUS6.320.0672.240.0242.8Medium42DK 12BangladeshAUS5.770.0622.740.0332.1Medium43DM 43BangladeshAUS5.860.0112.470.0332.4Medium44DM 59BangladeshAUS6.200.1182.360.0412.6Medium	40	Delrex	United States	TRJ	6.09	0.021	2.20	0.056	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	41	Dhala Shaitta	Bangladesh	AUS	6.32	0.067	2.24	0.024	2.8	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	42	DK 12	Bangladesh	AUS	5.77	0.062	2.74	0.033	2.1	Medium
44 DM 59 Bangladesh AUS 6.20 0.118 2.36 0.041 2.6 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	45	DZ 193	Bangladesh	AUS	6.00	0.061	2.99	0.029	2.0	Medium
46 FCIA76-S89-1 Cuba IND 5.79 0.116 2.33 0.011 2.5 Medium	46	ECIA76-589-1	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	47	EMATA A 16-34	Mvanmar	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	48	Ghati Kamma Nangarhar	Afghanistan	AUS	5.80	0.051	2.79	0.043	2.1	Medium

#### **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
19	Charbhai	Pangladash	ALIC	5 (0 00()	0.044	0.07	0.024	0.4	Madium
47 50	Guordia Guor-Vin-Teon	Chipa		5.76	0.000	2.37	0.034	2.4	Medium
51	Halwa Gose Red	Iraq		5.90	0.077	2.57	0.027	2.2	Medium
52	Hon Chim	Hong Kong		6.40	0.002	2.00	0.040	2.2	Medium
53	Hunan Farly Dwarf No. 3	China		5.49	0.122	2.57	0.000	2.5	Medium
54	I-Geo-Tze	Taiwan		5.60	0.004	2.30	0.010	2.2	Medium
55	Iguane Cateto	Haiti	TRI	6.44	0.070	2.43	0.014	2.5	Medium
56	Italica Carolina	Poland	TEI	6 57	0.072	2.73	0.040	2. <del>4</del> 2.4	Medium
57	Jambu	Indonesia	TDI	5.80	0.050	2.70	0.020	2. <del>1</del> 2.2	Medium
58	Jamir	Bangladech		5.57	0.007	2.00	0.010	2.2	Medium
50		India		6.54	0.041	2.52	0.030	2.4	Medium
57 60	Jaya	India		5 70	0.000	2.04	0.040	2.5	Medium
21		lanan		5.77	0.040	2.31	0.001	2.3	Medium
40	Jourku 373G	Japan		6.20 5.70	0.005	2.70	0.030	2.2	
02 (2	Kachilon Kabubaba Mala	Bangladesh	AUS	5.79	0.065	2.53	0.028	2.3	Medium
63		Sri Lanka	AUS	6.00	0.068	2.47	0.036	2.4	Medium
64	Kon Suito	Mongolia	IEJ	6.22	0.047	2.86	0.064	2.2	Medium
65	KU115	Ihailand		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	Sabharai	Bangladesh	IND	5.79	0.061	2.69	0.079	2.2	Medium
89	Sadri Belvi	Azerbaijan	ARO	6.13	0.045	2.42	0.012	2.5	Medium
90	Saku	Mongolia		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	Sarava	Fiii		5.86	0.031	2.52	0.005	23	Medium
93	Saturn	United States		6.07	0.037	2.62	0.012	2.3	Medium
94	Shai-Kuh	China		5.92	0.047	2.02	0.036	2.5	Medium
95	Shim Balte	Iroa		5.02	0.041	2.71 2.20	0.042	2.5	Medium
7J 06	Shirogana	lanan		5.90	0.001	2.07	0.040	2.J 2.2	Modium
70	Shirogane	Jahan	A03	J.00	0.073	2.07	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

#### TABLE 1 (continued).

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 mediumgrain 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 vielded 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 mediumgrain 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 p	oroducts	of GS3	gene in	rice	varieties
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Grain shano	Number of variaties	PCR	products of GS3	3 gene	Prediction of conformity		
Grain shape	Number of varieties	150 bp	260 bp	360 bp	between genotype and phenotype		
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 identifed. Among them, 19 QTL genes have been cloned in rice. Of these cloned QTL genes, the GS3 major QTL has negative efects on controlling grain length (Yuvu 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.

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# 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.

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