Original Research Article

Identification of SSR markers linked to mung bean yellow mosaic virus resistance in blackgram (*Vigna mungo* (L.) Hepper) using bulked segregant analysis

ABSTRACT

Aims: To use bulked segregant analysis and simple sequence repeat (SSR) marker validation tests to find the SSR markers associated with mung bean yellow mosaic virus (MYMV) resistance in the blackgram segregating population ($F_{2:3}$) of ADT 3 x IC343856.

Study Design: $F_{2:3}$ generated 60 single plants of ADT 3 x IC343856 were raised with regular rows of MYMV susceptible check variety CO 5 to draw white flies and thus to improve MYMV infection under field screening. To identify the SSR markers associated to MYMV resistance, bulk segregant analysis and marker validation tests were conducted.

Place and Duration of Study: The experiment was carried out at Department of Genetics and Plant Breeding, VOC Agricultural College and Research Institute, Killikulam, Tamil Nadu Agricultural University, India from 2020 to 2022.

Methodology: The $F_{2:3}$ mapping population of the cross, ADT 3 x IC 343856 was used for bulked segregant analysis (BSA) using 50 simple sequence repeat (SSR) markers for mung bean yellow mosaic virus (MYMV) resistance studies in blackgram (*Vigna mungo (L.)* Hepper).

Results: Four markers *viz.,* CEDG008, CEDG271, VM6 and CEDG264 exhibited polymorphism between the parents. The $F_{2:3}$ segregants were raised along with their parents and check variety CO 5 was raised as infector rows to ensure the disease incidence in the population. An equal number of two extreme genotypes (10 resistant and 10 susceptible respectively) were pooled to form the bulks. Among the four polymorphic markers studied, CEDG 008 was able to differentiate resistant and susceptible bulks and their corresponding individuals (120 bp and 110 bp respectively). From this study, it was concluded that CEDG 008 can be used for marker assisted selection for MYMV resistance breeding programs in blackgram.

Key words: *Blackgram, Bulked segregant analysis, simple sequence repeat markers, mung bean yellow mosaic virus resistance*

1. INTRODUCTION

The main protein source in the diet is pulses. *Vigna* species is most important among them. Due to its vegetable protein content and ability to supplement a diet focused on cereals, the pulse known as "blackgram" (*Vigna mungo* (L.) Hepper) is an essential part of the Indian diet. About 26% of blackgram is protein, which is about three times as much than cereals, along with other vitamins and minerals (Priya *et al.* 2021). Besides, it is also used as nutritive fodder, especially for milch animals. By fixing the

atmospheric nitrogen in the soil it can improve the soil fertility level (Gomathi *et al.* 2023). Its primary origin is India, and it is mostly grown in Asian nations such as Pakistan, Myanmar, and some regions of Southern Asia. India produces over 70% of the blackgram produced worldwide. India is the biggest producer and user of blackgram worldwide. From 4.6 million hectares of land, India produces roughly 24.5 lakh tons of blackgram annually, with an average productivity of 533 kg per hectare in 2020–21 (agricoop.nic.in). About 19% of India's total pulse acreage, or 23% of the country's entire pulse production, is made up of blackgram.

The productivity is significantly lower than the global average, even though the country has the largest production area. Low variability, a poor harvest index, a lack of suitable ideotypes for various cropping systems, and the crop's vulnerability to biotic and abiotic stresses are the primary obstacles to the development of high-yielding blackgram cultivars. When biotic and abiotic stressors are prevalent, selection becomes more difficult. A major issue with blackgram production is the Yellow Mosaic Virus (YMV) disease, which is brought on by the begomo virus and spread by the White fly (*Bemisia tabaci*). Yield reductions of up to 100% have been documented (Nene, 1972). A cost-effective and long-lasting way to manage viral infections is to cultivate MYMV resistant blackgram cultivars.

MYMIV (Mung bean Yellow Mosaic India Virus) is the primary cause of yellow mosaic illness in Northern and Central India, whereas MYMV (Mung bean Yellow Mosaic Virus) is the primary cause in Southern and Western India (Usharani *et al.* 2004). Blackgram has a genetically regulated resistance to MYMV and MYMIV. Blackgram's MYMV resistance is inherited by a single recessive gene (Singh and Chaudhary 1979); (Thakur *et al.* 1977); (Saleem *et al.* 1998); (Malik *et al.* 1986); (Reddy and Singh 1995) and (Reddy 2009), dominant gene (Gupta *et al.* 2005); (Ammavasai *et al.* 2004) and (Singh and Singh 2006) and complementary recessive genes (Shukla and Pandya 1985). MYMV resistance blackgram genotypes could be created using molecular biology and biotechnology methods like genetic transformation and marker-assisted selection (Xu *et al.* 2000).

Simple sequence repeat (SSR) markers are the most user-friendly, highly polymorphic, and reproducible of all the marker kinds. These markers are tandemly repeated, locus-specific, and short sequence repeats of the genome's mono, di, tri, and tetra nucleotides (Tóth *et al.* 2000). Blackgram contains lack of genomic resources and limited SSR markers (Souframanien and Reddy 2015) and this is the main cause of the dearth of research on mapping with SSR markers. To find the gene of interest, it is crucial to determine the markers associated with a trait. The genetic map was first created using a technique called bulked segregant analysis (BSA) (Michelmore *et al.* 1991). Using molecular markers, BSA is used to target areas of segregation or regions of interest in breeding populations. This technique is particularly helpful for quickly isolating genes that do not segregate in breeding populations. Considering these factors, the current study was conducted in order to identify the SSR markers linked to MYMV resistance in the blackgram segregating population ($F_{2:3}$) of ADT 3 x IC343856 using BSA and SSR marker validation studies.

2. MATERIALS AND METHODS

2.1. Experimental materials

The field experiments were carried out at Department of Genetics and Plant Breeding, VOC Agricultural College and Research Institute, Killikulam, Tamil Nadu Agricultural University, India from the year 2020 to 2022. The genotype ADT 3, a popular rice fallow blackgram variety but susceptible to MYMV and IC 343856, an MYMV resistant genotype and an indigenous collection received from NBPGR, New Delhi were used as female and male parents respectively for the hybridization. $F_{2:3}$ mapping population developed from this cross was used for screening on MYMV disease resistance. The molecular analysis was carried out at Molecular Biology Laboratory of Department of Genetics and Plant Breeding, VOC Agricultural College and Research Institute, Killikulam, Tamil Nadu Agricultural University during 2020–2022.

2.2. Crossing block and hybridization

During *rabi* 2020, the parents were raised in crossing block. The male and female parents were raised in 3 m rows with 20 plants each, spaced 30 × 10 cm apart. The female lines' flower buds were selected for emasculation. For identification, each emasculated flower bud was tagged. The stigma of previously emasculated flowers was sprinkled with anthers from recently opened male flowers. Bagging the pollinated flowers allowed for easy identification and protection.

2.3. Mapping population development

Identified F_1s were allowed to selfing to raise F_2 followed by $F_{2:3}$ derived single plants (60 number) were raised in ridges and furrows in a row spacing of 30cm x10cm with frequent rows of MYMV susceptible check CO 5 to attract white flies and to increase infection of MYMV under field screening during summer 2021. No insecticide was sprayed to maintain the natural whitefly populations. The MYMV disease score is recorded on 60 days after sowing by using a phenotype rating scale from 1 (resistant) to 9 (highly susceptible) as suggested by Alice and Nadarajan (2007) (**Table 1)**. The MYMV disease reaction as follows, resistant (1.0 to 2.0); moderately resistant (2.1 to 4); moderately susceptible (4.1 to 5); susceptible (5.1 to 7); highly susceptible (7.1 to 9).

Table 1. MYMV disease resistant scale in blackgram (Alice and Nadarajan, 2007)

Sl. No.	Symptoms	Scale
1.	No visible symptoms on leaves or very minute yellow specks on leaves.	1
2.	Small yellow specks with restricted spread covering 0.1 to 5% leaf area	2
3.	Yellow mottling of leaves covering 5.1 to 10% leaf area.	3
4.	Yellow mottling of leaves covering 10.1 to 15% leaf area.	4
5.	Yellow mottling and discoloration of 15.1 to 30% leaf area.	5
6.	Yellow discoloration of 30.1 to 50 % leaf area.	6
7.	Pronounced yellow mottling and discoloration of leaves and pods, reduction in leaf size and stunting of plants covering 50.1 to 75% foliage.	7

8.	Severe yellow discoloration of leaves covering 75.1 to 90% of foliage, stunting of plants and reduction in pod size.	8
9.	Severe yellow discoloration of entire leaves covering above 90.1% of foliage, stunting of plants and no pod formation.	9

2.4. Molecular analysis

Genomic DNA of parents was extracted from 2-3 weeks old leaf tissues of $F_{2:3}$ plants using CTAB method (Saghai-Maroof *et al.* 1984). The quality and quantity of DNA was analysed by 0.8% agarose gel electrophoresis. PCR amplification was performed by using 50 MYMV specific SSR primers **(Supplementary Table 1)** from the previous study based on high polymorphism (Sathees, 2019). For every reaction 10µl of reaction mixture was used. The PCR profile was programmed for an initial denaturation of 95˚C for 5 minutes followed by 35 cycles of denaturation for 95˚C for 30 seconds, annealing of 56˚C for 30 seconds, extension of 72˚C for 1 minute and final extension of 72˚C for 7 minutes and ends with the final hold for 4˚C. After the samples withdrawal, the PCR products were resolved in 3% agarose gel electrophoresis and visualized in gel documentation system (Bio Rad).

2.5. Bulked Segregant Analysis (BSA)

Bulked Segregant Analysis (BSA) plays a major role in rapid selection of genotypes in mapping population associated to MYMV resistance. In this method two phenotypes *i.e.,* IC 343856 as resistance genotype and ADT 3 as susceptible genotype were used for developing $F_{2:3}$ mapping population. DNA from ten individual plants of resistant and susceptible genotypes from $F_{2:3}$ population were pooled to form a separate resistant and susceptible bulks. The bulks and individual genotypes were analysed along with the parents using the identified polymorphic markers in 3% agarose gel electrophoresis. The amplified products were scored based on the presence and absence of bands.

2.6. Marker Validation

Since excluding pedigree information from the discovery population study may result in spurious relationships between markers and the characteristic of interest, marker validation is required. Unvalidated markers are useless as instruments for marker-assisted selection. The potentiality of the markers was established by comparing the association of the MYMV related markers found in this investigation with those found in earlier related studies.

3. RESULTS AND DISCUSSION

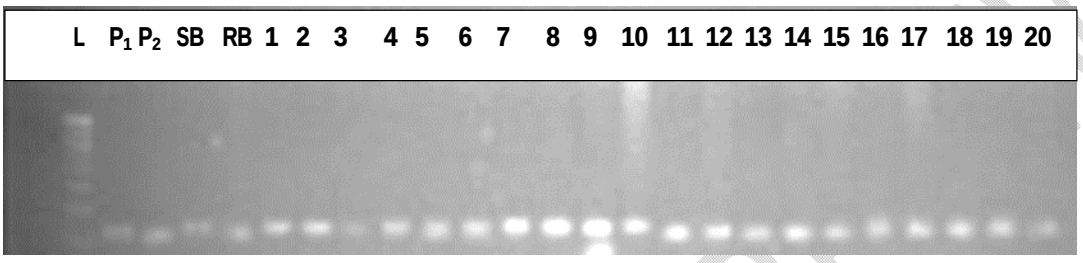
The screening for MYMV resistance was carried out for 60 F_2 derived F_3 plants of the cross ADT 3 x IC 343856. The disease incidence was ensured with the MYMV susceptible check CO 5 for every 2 to 3 rows of plants.

3.1. Parental polymorphism

Among the studied fifty MYMV specific SSR markers, four markers *viz*., CEDG008, CEDG271, CEDG264 and VM6 showed polymorphism between the parents ADT 3 (Susceptible) x and IC343856 (resistant) while other markers were found to be monomorphic. The polymorphic markers were used further in the MYMV resistance studies.

3.2. Bulked Segregant Analysis (BSA)

Bulked Segregant Analysis was done using ten resistant and susceptible genotypes identified under field screening for MYMV resistance. Resistant parent, susceptible parent and their bulks were analysed using the identified four polymorphic markers. Among the studied markers, CEDG008 alone was able to differentiate the resistant and susceptible bulks. CEDG008 showed bands at 120bp for ADT 3 (susceptible) and at 110 bp for IC343856 (resistant). From the study, it was found that CEDG008 associated to MYMV resistance in the $F_{2:3}$ population of blackgram **(Fig 1)**. Similar results were reported by various authors.



P_1 – ADT3 (Susceptible) P_2 – IC343856 (Resistant)
SB – Susceptible Bulk RB – Resistant Bulk
1-10 – Susceptible genotypes 11-20 – Resistant genotypes

Fig 1. Bulked segregant analysis using the SSR marker CEDG008 in $F_{2:3}$ cross ADT 3 x IC343856

Sathees *et al*. (2019) studied the SSR markers linked to MYMV resistance in 162 F_2 plants of IC 435566 X KKB14045 in blackgram through BSA. The SSR marker CEDG141 distinguished resistant and susceptible bulks and found to be associated to MYMV resistance. Naik *et al.* (2017) studied F_2 individuals of T9 (resistant) × LBG-759 (susceptible) cross to screen and identify the yellow mosaic virus (YMV) resistant gene in blackgram using Simple Sequence Repeats (SSR) and Bulk Segregant Analysis (BSA). The study revealed that 12 SSR markers showed polymorphism between the parents among 59 primers. One primer VR9 was able to distinguish the resistant and susceptible bulks and individuals indicated that this marker was tightly linked to yellow mosaic virus resistance gene in blackgram. Rambabu *et al.* (2018) reported SSR marker CEDG185 linked to the YMV resistance in F_2 blackgram population of a cross LGG-759 X T9 using bulked segregant analysis.

3.3. Validation of SSR markers for MYMV resistance

The correlation between MYMV resistance and the SSR marker CEDG 008 was confirmed across a range of susceptible and resistant genotypes (Table 2). For MYMV resistance research in blackgram, CEDG 008 differentiated between susceptible and resistant genotypes. Gomathi (2020) and Narayanan (2021) reported the similar outcome in F_2 and RILs of the cross KKM 1×VBN 6. In the earlier MYMV resistance tests in blackgram, CEDG 008 also shown a favourable polymorphic information content (PIC) value (>0.5) (Sathees *et al.* 2021).

Table 2. Marker validation studies for MYMV resistance in blackgram

Genotypes	Disease	MYMV	CEDG 008	Pedigree	Reference

	reaction to MYMV	Score	120 bp	110bp		
ADT 3	Susceptible	7	Present ($F_{2:3}$ population)	Absent ($F_{2:3}$ population)	Pureline selection from Tirunelveli local	Present study
IC343856	Resistant	1	Absent ($F_{2:3}$ population)	Present ($F_{2:3}$ population)	Land race, NBPGR, New Delhi, India	
IC 436656	Susceptible	7	Present (RILs)	Absent (RILs)	Land race, NBPGR, New Delhi, India	Sathees *et al.* (2022)
KKB 14045	Resistant	1	Absent (RILs)	Present (RILs)	PU0620 ×ADT3	
KKM 1	Susceptible	4	Absent (F_2 and RILs)	Present (F_2 and RILs)	COBG 653 ×VBN 3	Gomathy (2020) and Narayanan (2021)
VBN 6	Resistant	1	Present (F_2 and RILs)	Absent (F_2 and RILs)	VBN 1 × *Vigna mungo* var. *silvestris*	

RILs – Recombinant Inbred Lines

4. CONCLUSION

It is concluded that the present research on MYMV resistance study in the segregating population of the cross ADT 3 x IC343856 would be utilized for MYMV resistance studies in blackgram.

REFERENCES

Alice, D., & Nadarajan, N. (2007). Screening techniques and assessment methods for disease resistance, Department of Pulses, TNAU. All India Coordinated Research Project on MULLaRP-Tamil Nadu Agricultural University.

Ammavasai, S., Phogat, D.S., & Solanki, I.S. (2004). Inheritance of resistance to mungbean yellow mosaic virus (MYMV) in green gram (*Vigna radiata* (L.) Wilczek). Indian Journal of Genetics and Plant Breeding, 64:146–146.

Gomathi, D. (2020). Identification of YMV resistance linked SSR markers in segregating population of black gram (*Vigna mungo* (L.) Hepper). M.Sc., Thesis. Tamil Nadu Agricultural University, Killikulam, Tamil Nadu.

Gomathi, D., Shoba, D., Ramamoorthy, V., & Arumugam Pillai, M. (2020). Studies on variability, heritability, correlation and path analysis in segregating population of blackgram (*Vigna mungo* (L.) Hepper). Legume Research, 46(6): 690-694

Gupta, S., Kumar S., Singh, R.A., & Chandra, S. (2005). Identification of a single dominant gene for resistance to Mungbean Yellow Mosaic Virus in blackgram (*Vigna mungo* (L.) Hepper). SABRAO Journal of Breeding and Genetics, 37:85–89.

Malik, I.A., Sarwar, G., & Ali, Y. (1986). Genetic studies in mungbean (*Vigna radiata* (L) Wilczek). Inheritance of tolerance to mungbean yellow mosaic virus and some morphological characters. Pakistan Journal of Botany, 18:189–198.

Michelmore, R.W., Paran, I., & Kesseli, R.V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proceedings of the National Academy of Sciences, 88:9828–9832.

Naik, B., Jeevula, C.H., Anuradha, P., Ananda Kumar., Sreedhar V., & Sreenivasa Chary. (2017). Identification of simple sequence repeats (SSR) markers linked to yellow mosaic virus (YMV) resistance in blackgram [*Vigna mungo (L). Hepper*]. Agriculture Update, 12:812-819.

Narayanan, M. (2021). Genetics of yellow mosaic virus resistance in black gram (*Vigna mungo* (L.) Hepper). M.Sc., Thesis. Tamil Nadu Agricultural University, Killikulam, Tamil Nadu.

Nene, Y.L. (1972). A survey of viral diseases of pulse crops in Uttar Pradesh, In book: Legumes pp. 87.

Priya, L., Arumugam Pillai, M., & Shoba, D. (2021). Genetic divergence, variability and correlation studies in blackgram (*Vigna mungo* (*L.*) Hepper). Legume Research, 44(1): 36-40

Rambabu, E., Anuradha C.H., Sridhar, V., & Sokka Reddy S. (2018). Identification of molecular markers linked to yellow mosaic virus resistance in blackgram (*Vigna mungo (L.)* Hepper). International Journal of Current Microbiology and Applied Sciences, 7(2): 3810-3817.

Reddy, K.S. (2009). A new mutant for yellow mosaic virus resistance in mungbean (*Vigna radiata* (L.) Wilczek) variety SML-668 by recurrent gamma-ray irradiation. Induced Plant Mutations in the Genomics Era. Food and Agriculture Organization of the United Nation, Rome pp.361–362.

Reddy. K.R., & Singh, D.P. (1995). Inheritance of resistance to mungbean yellow mosaic virus. Madras Agricultural Journal, 88:199–201.

Saghai-Maroof, M.A., Soliman, K.M., Allard Jorgensen, R., & Allard, R.W.L. (1984). Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences, 81 (24):8014-8018.

Saleem, M., Haris, W.A., & Malik, I.A. (1998). Inheritance of yellow mosaic virus in mungbean (*Vigna radiata* L. Wilczek). Pakistan Journal of Phytopathology, 10:30–32.

Sathees, N. (2019). Identification of SSR markers linked to YMV resistance in blackgram [*Vigna mungo* (l.) Hepper]. M.Sc (Ag) Thesis in Genetics and Plant Breeding, Department of Plant Breeding and Genetics, Agricultural College and Research Institute, TNAU, Killikulam.

Sathees, N., Shoba, D., Saravanan, S., Merina Perm Kumari, S., & Arumugam Pillai, M. (2019). Studies on genetic variability, association and path coefficient analysis in blackgram (*Vigna mungo L. Hepper*). International Journal of Current Microbiology and Applied Sciences, 8 (6):1892-1899.

Sathees, N., Shoba, D., Saravanan, S., Merina Perm Kumari, S., & Arumugam Pillai, M. (2021). Assessment of genetic diversity of blackgram (*Vigna mungo L. Hepper*) collections for yellow mosaic virus resistance using simple sequence repeat markers. Legume Research, 44 (7):743-750.

Sathees, N., Shoba, D., Narayanan Mani., Saravanan, S., Merina Prem Kumari, S., & Arumugam Pillai, M. (2022). Tagging of SSR markers associated to yellow mosaic virus resistance in black gram (*Vigna mungo* (L.) Hepper). Euphytica, 218(3): 1-11.

Shukla, G.P., & Pandya, B.P. (1985). Resistance to yellow mosaic in green gram. SABRAO Journal, 17:165–177.

Singh, R.K., & Chaudhary, B.D. (1979). In book: Biometrical methods in quantitative genetic analysis, pp. 82.

Singh, S.K., & Singh, M.N. (2006). Inheritance of resistance to mungbean yellow mosaic virus in mungbean. Indian Journal of Pulses Research, 19:21.

Souframanien, J., & Reddy, K.S. (2015). De novo assembly, characterization of immature seed transcriptome and development of genic-SSR markers in black gram (*Vigna mungo* (L.) Hepper). PLoS ONE, 10: e01287.

Thakur, R.P., Patel, P.N., & Verma, J.P. (1977). Genetical relationships between reactions to bacterial leaf spot, yellow mosaic and *Cercospora* leaf spot diseases in mungbean (*Vigna radiata*). Euphytica, 26:765–774.

Tóth, G., Gaspari, Z., & Jurka, J. (2000). Microsatellites in different eukaryotic genomes: Survey and analysis. Genome Research, 10:967-81.

Usharani, K.S., Surendranath, B., Haq, Q.M., & Malathi, V.G. (2004). Yellow mosaic virus infecting soybean in northern India is distinct from the species infecting soybean in southern and western India. Current Science, 25:845–850.

Xu, R.Q., Tomooka, N., Vaughan, D.A. & Doi K. (2000). The *Vigna angularis* complex: genetic variation and relationships revealed by RAPD analysis and their implications for in-situ conservation and domestication. Genetic Resources and Crop Evolution, 46: 136 -145.

Supplementary Table 1. List of SSR markers used for MYMV resistance studies in blackgram (Sathees, 2019)

Sl No	SSR MARKERS	SEQUENCES
1	CEDG245(F)	CATCTTCCTCACCTGCATTC
	CEDG245(R)	TTTGGTGAAGATGACAGCCC
2	VM12(F)	TTGTCAGCGAAATAAGCAGAG
	VM12(R)	CAACAGCAGACGCCCAACT
3	VM6(F)	GAGGAGCCATATGAAGTGAAAAT
	VM6(R)	TCGGCCAGCAACAGATGC
4	VM9(F)	ACCGCACCCGATTTATTTCAT
	VM9(R)	ATCAGCAGACAGGCAAGACCA
5	VM25(F)	CCACAATCACCGATGTCCAA
	VM25(R)	CAATTCCACTGCGGGACATAA
6	VM40(F)	TATTACGAGAGGCTATTTATTGCA
	VM40(R)	CTCTAACACCTCAAGTTAGTGATC
7	DMB SSR160(F)	TAGAGCCTTCTGGTTTTTCACA
	DMB SSR160(R)	AGGAGGAGGATTTTGATGATGA
8	CEDG026(F)	TCAGCAATCACTCATGTGGG
	CEDG026(R)	TGGGACAAACCTCATGGTTG
9	CEDG286(F)	CGAGCAGAACACTGATCATG
	CEDG286(R)	CCTCTTAGAGGTCATTGCTC
10	CEDG006(F)	AATTGCTCTCGAACCAGCTC
	CEDG006(R)	GGTGTACAAGTGTGTGCAAG
11	CEDG008(F)	AGGCGAGGTTTCGTTTCAAG
	CEDG008(R)	GCCCATATTTTTACGCCCAC
12	CEDG271(F)	GCACTAAAGTTAGACGTGGTTC
	CEDG271(R)	CACTCCCACTGCCAAACAAGG
13	CEDG198(F)	CAAGGAAGATGGAGAGAATC
	CEDG198(R)	CCTTCTAAGAACAGTGACATG
14	CEDG048(F)	TCTCTTCCTCTATGGCTTGG
	CEDG048(R)	GCTCCTCTTTTTGCTGCATC
15	CEDG016(F)	TTAGTTCACTCCGCTTGGTC
	CEDG016(R)	CACGTCATCCTCTGTTAGAC

	CEDG018(F)	AGCGTGTTTGTGGTGATAGC
16	CEDG018(R)	ACACAGGAACGAACAAACCC
	CEDG253(F)	CACTTCCATGATGATGACTCACC
17	CEDG253(R)	CACCCTTCTTTATCCTCTTCG
	CEDG021(F)	GCAGAATTTTAGCCACCGAG
18	CEDG021(R)	AAAGGATGCGAGAGTGTAGC
	VR1(F)	AGCCCTTCGTGCTAGGAAAT
19	VR1(R)	CCCTACCGGTTGGTTGGT
20	VR155(F)	AAGATCACACACAACCAACCC
	VR155(R)	AATTAGTTCCACAGGCCAGATT
	CEDG204(F)	CCTTGGTTGGAGCAGCAGC
21	CEDG204(R)	CACAGACACCCTCGCGATG
	CEDG139(F)	CAAACTTCCGATCGAAAGCGCTTG
22	CEDG139(R)	GTTTCTCCTCAATCTCAAGCTCCG
	CEDG268(F)	CATCTCCCTGAAACTTGTG
23	CEDG268(R)	GCTATCAATCGAGTGCAG
	CEDG030(F)	TGAGGGAATGGGAGAGAGGC
24	CEDG030(R)	TCCGCAGATAGAGGCTCACG
	CEDG092(F)	TCTTTTGGTTGTAGCAGGATGAAC
25	CEDG092(R)	TACAAGTGATATGCAACGGTTAGG
	CEDG022(F)	AGGAATGTGAGATTTG
26	CEDG022(R)	AATCGCTTCAAGGTCAAGCC
	CEDG024(F)	CATCTTCCTCACCTGCATTC
27	CEDG024(R)	TTTGGTGAAGATGACAGCCC
	CEDG013(F)	CGTTCGAGTTTCTTCGATCG
28	CEDG013(R)	ACCATCCATCCATTCGCATC
	DMB-SSR182(F)	TAGAGCCTTCTGGTTTTTCACA
29	DMB-SSR182(R)	AGGAGGAGGATTTTGATGATGA
	CEDG133(F)	GCATACATAATGTGGTGAGATG
30	CEDG133(R)	GTCTCGTGCCTTTCACAC
	CEDG141(F)	CCAGGCATCCATGATGACC
31	CEDG141(R)	GAAGTTGTTGGTAATGGTTGCCTC
	CEDG225(F)	GAGGAAGTGTTGCAGCACC
32	CEDG225(R)	GAGGAAGTGTTGCAGCACC
	CEDG284(F)	GGTGCTAACGTTGGAAACTGAG
33	CEDG284(R)	CACTCCATTCTGAGGATCAATCC
	CEDG127(F)	GGTTAGCATCTGAGCTTCTTCGTC
34	CEDG127(R)	CTCCTCACTTGGTCTGAAACTC
	CEDG014(F)	GCTTGCATCACCCATGATTC
35	CEDG014(R)	AAGTGATACGGTCTGGTTCC
36	CEDG020(F)	TATCCATACCCAGCTCAAGG

	CEDG020(R)	GCCATACCAAGAAAGAGG
37	CEDG067(F)	AGACTAAGTTACTTGGGCAACCAG
	CEDG067(R)	TGACGGCCCGGCTCTCC
38	CEDG059(F)	AGAAAAGGGTGGCCTCGTTG
	CEDG059(R)	GCAGGCATTTCCATCGCAG
39	CEDG112(F)	GCAATATTCGCATTATTCATTCA
	CEDG112(R)	GTGTTTCAAAGCACTATACTTAA
40	CEDG269(F)	CTGTTACGGCACCTGGAAAG
	CEDG269(R)	GCAGAGACACACCTTAACCTTG
41	CEDG011(F)	GTCCGACTTTATGTGTGGAG
	CEDG011(R)	TTTCTAGTTCCAGCCCCGAC
42	CEDG056(F)	TTCCATCTATAGGGGAAGGGAG
	CEDG056(R)	GCTATGATGGAAGAGGGCATGG
43	CEDG044(F)	TCAGCAACCTTGCATTGCAG
	CEDG044(R)	TTTCCCGTCACTCTTCTAGG
44	VrCSSSR3(F)	GCAGACACAACCATAAATCC
	VrCSSSR3(R)	GGTCTTTGACGGCAATCTC
45	CEDG180 (F)	GGTATGGAGCAAAACAATC
	CEDG180 (R)	GTGCGTGAAGTTGTCTTATC
46	CEDG073(F)	CCCCGAAATTCCCCTACAC
	CEDG073(R)	AACACCCGCCTCTTTCTCC
47	CEDG075(F)	CGACCTCGAAAATGGTGGTTT
	CEDG075(R)	CACCAACTCACTCGCTCACTG
48	CEDG091(F)	CTGGTGGAACAAAGCAAAAGAGT
	CEDG091(R)	TGGGTCTTGGTGCAAAGAAGAAA
49	CEDG097 (F)	GTAAGCCGCATCCATAATTCCA
	CEDG097 (R)	TGCGAAAGAGCCGTTAGTAGAA
50	CEDG116 (F)	AACATCAACTCCAGTCTCACCAAA
	CEDG116 (R)	CTGCCAAAGATGGACAACTTGGAC