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# Identification of SSR Markers Linked to Mung Bean Yellow Mosaic Virus Resistance in Blackgram (*Vigna mungo* (L.) Hepper) Using Bulked Segregant Analysis in $F_{2:3}$ Population

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author DS designed the study, wrote the protocol and performed the statistical analysis. Author AA wrote the first draft of the manuscript and managed the literature searches. Author MAP, JLJ and SJH managed the analyses of the study. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** In blackgram (*Vigna mungo (L.)* Hepper), mung bean mung bean yellow mosaic virus (MYMV) disease causes severe yield reduction. MAS (Marker Assisted Selection) can be used to improve the selection efficiency for MYMV resistance**.** Hence the present study was carried out to use bulked segregant analysis (BSA) and simple sequence repeat (SSR) marker validation tests to find the simple sequence repeat (SSR) markers associated to 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 BSA using 50 simple sequence repeat (SSR) markers for mung bean mung bean yellow mosaic virus (MYMV) resistance studies in blackgram.

**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 the previous reports, it has been confirmed that CEDG 008 is a potential marker for MYMV resistance studies in different genetic backgrounds.


*Keywords: Blackgram; bulked segregant analysis; simple sequence repeat markers; mung bean 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 Mung bean yellow mosaic virus 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 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 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 $F_1$s 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 (scale 1.0 to scale 2.0); moderately resistant (scale 2.1 to scale 4); moderately susceptible (scale 4.1 to scale 5); susceptible (scale 5.1 to scale 7); highly susceptible (scale 7.1 to scale 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

**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 |

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. Field screening was carried out and the results are given in Table 2.

### 3.1 Parental Polymorphism

Among the studied fifty MYMV specific SSR markers, four markers *viz*., CEDG008, CEDG271, CEDG264 and VM6 showed polymorphism (8%) between the parents ADT 3 (Susceptible) x and IC343856 (resistant) while other markers were found to be monomorphic. Similarly, low levels of polymorphism have been reported by Chaitieng *et el.,* (2006), Ragul Subramaniyan *et al.,* (2021) and Nair *et al.,* (2024). Abhishek *et al.* (2024) reported that 20 accessions with MYMIV resistance in cowpea (*Vigna unguiculata* (L.) Walp.). Rolling circle amplification and complete DNA-A genome sequencing of MYMIV were also used by them to characterize the YMD-associated Begomovirus, which had a 99.02% identity overlap with the MYMIV isolate that infected cowpea in Pakistan. The 20 cowpea accessions shown in their study to be novel resistant sources to MYMIV may be used as donors in resistance breeding for yellow mosaic disease as well as for identifying resistance genes.

The identified polymorphic markers were used further in the MYMV resistance studies in the present study.

**Table 2. Field screening for MYMV disease resistance for the $F_{2:3}$ population of ADT 3 x IC 343856**

| Sl. No | Genotypes | MYMV Score | Response |
|---|---|---|---|
| 1 | ADT 3 | 7 | Susceptible |
| 2 | IC 343856 | 1 | Resistant |
| 3 | CO 5 (Susceptible check) | 9 | Highly susceptible |
| 4 | $F_{2:3}$ genotypes *viz*., 1, 5, 8, 9, 10 | 7 | Susceptible |
| 5 | $F_{2:3}$ genotypes *viz*., 2, 3, 4, 6, 7 | 6 | Susceptible |
| 6 | $F_{2:3}$ genotypes *viz*., 13, 14, 17, 18, 19, 20 | 1 | Resistant |
| 7 | $F_{2:3}$ genotypes *viz*., 11,12, 15, 16 | 2 | Resistant |

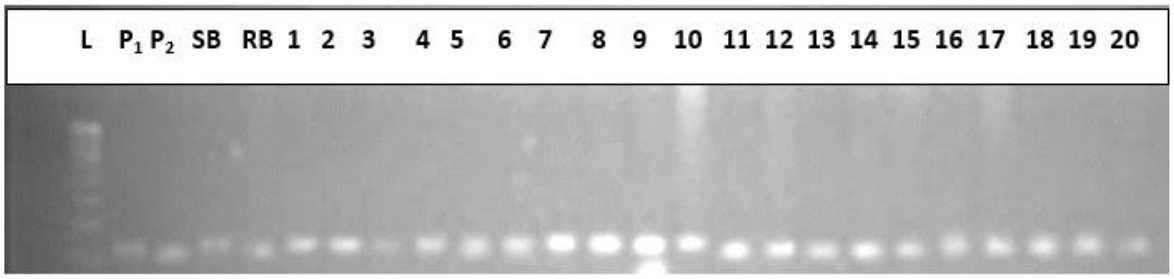


**Fig. 1. Bulked segregant analysis using the SSR marker CEDG008 in $F_{2:3}$ cross ADT 3 x IC343856**

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

## 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.

Godwin (2024) reported that the SSR markers *viz*., CEDG 141 and VrD1 were able to distinguish the MYMV susceptible parent KKM 1 and MYMV resistant parent VBN 9 through BSA in the $F_2$ population. Similarly, Godwin (2024) reported that, he SSR markers *viz*., CEDG 282 and VrD1 were able to distinguish the MYMV susceptible parent ADT 6 and MYMV resistant parent MASH 1008 through BSA in the $F_2$ population.

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. Prasanna (2019) reported that, the marker CEDG305 has the potential to use in identification of YMV resistant genotypes and the parent EC396117 can be successfully used in marker assisted breeding programmes as donor along with the identified marker source in greengram (*Vigna radiata* (L.) Wilczek). Naik *et al.* (2017) studied $F_2$ individuals of T9 (resistant) × LBG-759 (susceptible) cross to screen and identify the mung bean yellow mosaic virus 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 mung bean 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.

**Table 3. Marker validation studies for MYMV resistance in blackgram**

| Genotypes | Disease reaction to MYMV | MYMV Score | CEDG 008 | | Pedigree | Reference |
|---|---|---|---|---|---|---|
| | | | 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 |
| VBN 6 | Resistant | 1 | Present ($F_2$ and RILs) | Absent ($F_2$ and RILs) | VBN 1 × *Vigna mungo* var. *silvestris* | Narayanan (2021) |

*RILs – Recombinant Inbred Lines*

A draft reference-guided genome assembly of the black gram genotype "Uttara" (IPU 94-1), which is renowned for its high resistance to Mungbean Yellow Mosaic Disease, was developed by Ambreen *et al.,* (2022). It had a cumulative size of 454 Mb and 28,881 predicted genes, 444 Mb of which were anchored on 11 chromosomes. Karthikeyan et al. (2012) used 35 SSR primers and only 6 primers *viz.,* CEDG 243, CEDG 257, CEDG 115, CEDG 008, CEDG 269 and CEDG 201 (17.14%) showed polymorphism between the parents in greengram and none of these markers were able to distinguish the resistant and susceptible parents in BSA 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 3). 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).

## 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 used for MYMV resistance studies in blackgram. The identified potential marker in the present study, CEDG 008 can be used for MYMV resistance studies in blackgram breeding programmes. In order to increase the selection efficiency for MYMV resistance investigations, more molecular markers need to be investigated, and the current study has opened the door for further research in this field.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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## SUPPLEMENTARY

**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 |
| 16 | CEDG018(F) | AGCGTGTTTGTGGTGATAGC |
| | CEDG018(R) | ACACAGGAACGAACAAACCC |
| 17 | CEDG253(F) | CACTTCCATGATGATGACTCACC |
| | CEDG253(R) | CACCCTTCTTTATCCTCTTCG |
| 18 | CEDG021(F) | GCAGAATTTTAGCCACCGAG |
| | CEDG021(R) | AAAGGATGCGAGAGTGTAGC |
| 19 | VR1(F) | AGCCCTTCGTGCTAGGAAAT |
| | VR1(R) | CCCTACCGGTTGGTTGGT |
| 20 | VR155(F) | AAGATCACACACAACCAACCC |
| | VR155(R) | AATTAGTTCCACAGGCCAGATT |
| 21 | CEDG204(F) | CCTTGGTTGGAGCAGCAGC |
| | CEDG204(R) | CACAGACACCCTCGCGATG |
| 22 | CEDG139(F) | CAAACTTCCGATCGAAAGCGCTTG |
| | CEDG139(R) | GTTTCTCCTCAATCTCAAGCTCCG |
| 23 | CEDG268(F) | CATCTCCCTGAAACTTGTG |
| | CEDG268(R) | GCTATCAATCGAGTGCAG |
| 24 | CEDG030(F) | TGAGGGAATGGGAGAGAGGC |
| | CEDG030(R) | TCCGCAGATAGAGGCTCACG |
| 25 | CEDG092(F) | TCTTTTGGTTGTAGCAGGATGAAC |
| | CEDG092(R) | TACAAGTGATATGCAACGGTTAGG |
| 26 | CEDG022(F) | AGGAATGTGAGATTTG |
| | CEDG022(R) | AATCGCTTCAAGGTCAAGCC |
| 27 | CEDG024(F) | CATCTTCCTCACCTGCATTC |

| Sl No | SSR MARKERS | SEQUENCES |
|---|---|---|
| | CEDG024(R) | TTTGGTGAAGATGACAGCCC |
| 28 | CEDG013(F) | CGTTCGAGTTTCTTCGATCG |
| | CEDG013(R) | ACCATCCATCCATTCGCATC |
| 29 | DMB-SSR182(F) | TAGAGCCTTCTGGTTTTTCACA |
| | DMB-SSR182(R) | AGGAGGAGGATTTTGATGATGA |
| 30 | CEDG133(F) | GCATACATAATGTGGTGAGATG |
| | CEDG133(R) | GTCTCGTGCCTTTCACAC |
| 31 | CEDG141(F) | CCAGGCATCCATGATGACC |
| | CEDG141(R) | GAAGTTGTTGGTAATGGTTGCCTC |
| 32 | CEDG225(F) | GAGGAAGTGTTGCAGCACC |
| | CEDG225(R) | GAGGAAGTGTTGCAGCACC |
| 33 | CEDG284(F) | GGTGCTAACGTTGGAAACTGAG |
| | CEDG284(R) | CACTCCATTCTGAGGATCAATCC |
| 34 | CEDG127(F) | GGTTAGCATCTGAGCTTCTTCGTC |
| | CEDG127(R) | CTCCTCACTTGGTCTGAAACTC |
| 35 | CEDG014(F) | GCTTGCATCACCCATGATTC |
| | 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 |



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