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# A high-throughout put sequencing SSR markers development and its validation in three developmental stages of fenugreek (*Trigonella foenum-graecum* L.)

**Shubha R and Rukam S Tomar**

**Abstract**
Fenugreek (*Trigonella foenum-graecum* L.) popularly known as "Methi" belongs to family fabaceae and subfamily papilionaceae. Gujarat Methi Variety 2 was selected for transcritome study from the three developmental stages that were vegetative stage (20-30 DAS), reproductive stage (50-60 DAS) and maturity stage (80-90 DAS) which were sequenced using Ion S5 genome sequencing machine. SSR molecular markers were identified individually for each transcript after assembly, by BatchPrimer3 software. A total of 189 primers were developed, out of which 78 from vegetative stage, 64 from reproductive stage and 47 from maturity stage. For validation purpose 30 primers were selected, 10 primers from each developmental stages. The 10 primers for each stage were checked on different stages of cDNA for validation analysis. The primers generated from the vegetative stage were also checked in other two stage of growth. Similarly the primers of reproductive and maturity stages were checked in other two stages of growth. The SSRs containing sequences (Fasta file) which were generated after assembly were also analyzed in NCBI-BLASTn to get the hits for the gene. All the three samples were most related to *Medicago trucatula*, *Arachis duranensis*, *Arachis hypogea*, *Arachis ipaensis*, *Cicer arisetinum*, *Cajanus cajan* and *Glycine max*.

**Keywords:** fenugreek, developmental stages, SSR markers, validation

## 1. Introduction
Fenugreek (*Trigonella foenum-graecum* L.) popularly known as "Methi" belongs to family fabaceae and subfamily papilionaceae. It is an important spice crop largely grown in the northern India during rabi season. Fenugreek is used as an herb (dried or fresh leaves), spice (seeds), and vegetable (fresh leaves, sprouts, and microgreens). Cuboid-shaped, yellow- to amber-colored fenugreek seeds are frequently encountered in the cuisines of the Indian subcontinent, used both whole and powdered in the preparation of pickles, vegetable dishes, dal, and spice mixes such as panch phoron and sambar powder. They are often roasted to reduce bitterness and enhance flavour (BBC – 2017) [1].

Fruit is a curved seed-pod, with ten to twenty flat and hard, yellowish-brown seeds. They are angular- rhomboid, oblong or even cubic, and have a deep furrow dividing them into two unequal lobes. It contains three important chemical constituents with medicinal value; *i.e.*, 1) steroidal sapogenins, 2) galactomannans and 3) isoleucine. These constituents have placed fenugreek among the most commonlyss recognized "nutraceutical" or health food products. It has been reported that fenugreek contains 81 phyconutrients and diosgenin, a steroid saponin found in fenugreek seeds, is the most bioactive component. Diosgenin is often used as a raw precursor for the production of steroidal drugs and hormones such as testosterone, glucocorticoids and progesterone. Studies reveal that a maximum level of diosgenin [(25R)-5-spirosten- 3h-ol] is found to be in young leaves (20mg/g dry weight) and in mature seeds with the percentage range from 0.28% - 0.92%. Reported that steroidal sapogenins were effective agents for the treatment of hypocholesterolemia, a disorder often associated with diabetes (Raju *et al*., 2004) [2].

SSR markers (Co-dominant) are widely used for high-throughput genotyping and map construction as they are advantageous due to high abundance, random distribution within the genome and stable co-dominance. The reproducibility, co-dominance, relative abundance and complete genome coverage of SSR markers have made them one of the most useful tools for

detecting genetic diversity, genetic linkage mapping, association mapping and evolution analysis. Genomic SSRs and expressed sequence tag (EST)-SSRs, which are considered complementary to plant genome mapping. During this study a few SSRs were developed. However, their use is limited due to relatively low polymorphism and high possibility of no gene-rich regions in the genome. In contrast, genomic SSRs are highly polymorphic and tend to be widely distributed throughout the genome, resulting in more accurate detection of genetic diversity.

In current study, Gujarat Methi Variety 2 was selected for development of SSR molecular markers and validation in the three developmental stages that were vegetative stage (20-30 DAS), reproductive stage (50-60 DAS) and maturity stage (80-90 DAS).

## 2. Materials and Methods

Present investigation on "A high-throughput sequencing SSR markers development and its validation in three developmental stages of fenugreek (*Trigonella foenum-graecum* L.)" was under taken at the Junagadh Agricultural University (JAU), Junagadh during 2015-16 and 2016-17. Laboratory studies on various aspects were carried out at Biotech Cell, Department of Biotechnology, College of Agriculture, Junagadh. Details of the materials and method followed are described here under.

### Sample collection

Fenugreek seeds of GMV-2 (Gujarat Methi Variety-2) were sown in plot, under the natural environmental condition, for the collection of the samples from three developmental stages *i.e.,* vegetative stage (20-30 DAS), reproductive stage (50-60 DAS) and maturity stage (70-80 DAS) (Figure 3.1). All the tissues were kept in RNA *later* at -20ºC till they were used for isolation of RNA.

### Extraction of RNA, mRNA and cDNA preparation

RNA was extracted from three developmental stages of fenugreek that were vegetative stage (20-30 DAS), reproductive stage (50-60 DAS) and maturity stage (80-90 DAS) by using trizol RNA isolation protocol method, isolation steps followed under as,

### A. Homogenization of Tissues

To obtain high yield of RNA, grinding is a critical point in the extraction of genetic material. So, grinding should be fine as much as possible and temperature should be low as much as possible to avoid degradation. Took 50 mg of sample and grinded it with the help of liquid nitrogen in mortar and pestle that was sterilized and baked, then add 1 ml of Trizol/Tri reagent/Tri extract per 50 - 100 mg of tissue in 2 ml microcentrifuge tube. Sample volume should be less than 100 µl. Mixed the sample by vigorously shaking by briefly vortexing until the sample is thoroughly resuspended.

### B. Phase Separation

In this step, incubated the samples for 5 minutes at room temperature. After that added 0.2 ml of chloroform to each tube and shaked vigorously by hand for 15 seconds, then incubated the samples for 5 minutes at room temperature. After the incubation centrifuge for 15 minutes at 12,000 ×g (RCF) at 4˚C or room temperature.

### C. RNA Precipitation

Transfered the upper aqueous phase to a fresh tube. Then added 0.5 ml of isopropyl alcohol to precipitate RNA (If this RNA will be used for RT-PCR, first add 50 µl isopropyl alcohol to precipitate RNA, mixed it and incubate samples at room temperature for 5 min and centrifuge at 12,000 ×g (RCF) for 10 minutes at 4˚C or room temperature), Mix it gently, incubate the samples at room temperature for 10 minutes and centrifuge at 12,000 ×g (RCF) for 10 minutes at 4˚C or room temperature. The RNA will form a white minute pellet on side or bottom of the tube.

### D. RNA Washing

Discard the supernatant gently and wash the pellet with 1 ml of chilled 75% ethanol (Freshly prepared in DEPC water). Mix samples by vortexing for 15 secs, then centrifuge at 12,000 ×g (RCF) for 5 minutes at 4˚C or room temperature. Repeat this step twice.

### E. Dissolving and storage of RNA

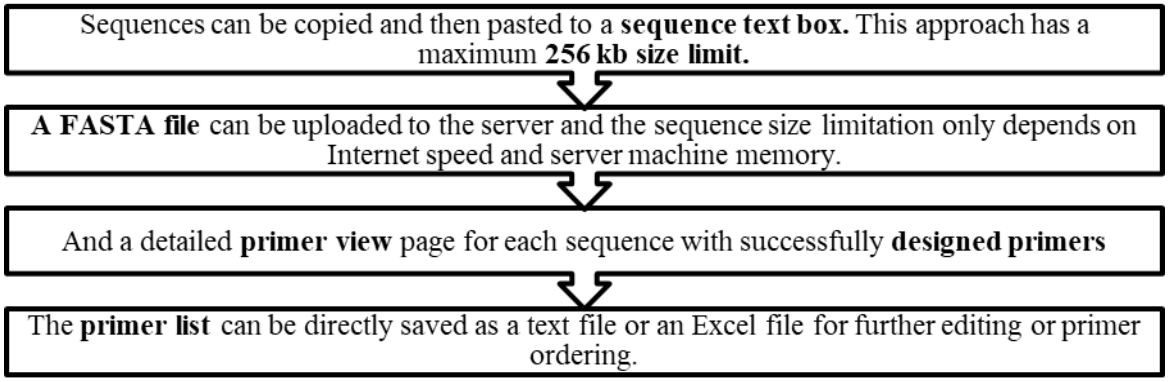
Removed the supernatant gently, then air dry the pellet not more than 5 minutes. Dissolved the pellet in 60 µl RNase free water (Commercial) or autoclaved DEPC treated water. For the short time storage we can put it in −20˚C and for long time storage put it in −80˚C.

### mRNA isolation and cDNA preparation

In present study fragment library was constructed for Ion S5 sequencing platform size selection and purification of fragmented. The library was constructed using enzymatic fragmentation. The enzymatic time was for 3 min to make a library with average read of 200 bp. and mRNA was purified with Magnetic Oligo (dT) beads in accordance with the manufacturer's instructions by Life technologie's. mRNA was isolated by using Protocol for Dynabeads® mRNA DIRECT™ Micro Kit. cDNA library was constructed using reverse transcription process with reverse transcriptase enzyme.

### SSR marker development

SSR marker was designed by using a high through output web tool Batch Primer3 (v 1.0). It is a comprehensive web primer design program using Primer3 core program as a major primer design engine to design different types of PCR primers and sequencing primers in a high-through manner. A batch input of large number of sequences and a tab-delimited result output greatly facilitates rapid primer design, steps are followed under as.




**SSR markers validation in fenugreek**

**PCR Protocol**

The master mix was prepared in a microfuge tube in which the buffer was added first followed by sterile water, Primer, dNTPs mix followed by Taq DNA polymerase. At the last DNA was added in each tube separately.

**Preparation of reaction mixture for SSR**

| Index | Reagents | Quantity |
|---|---|---|
| 1 | PCR buffer (10X) | 2 µl |
| 2 | Taq polymerase (3 U.µl$^{-1}$) | 0.3 µl |
| 3 | dNTPs mix (2.5 mM each) | 0.06 µl |
| 4 | Primer-F (25 pmoles.µl$^{-1}$) | 1 µl |
| 5 | Primer-R (25 pmoles.µl$^{-1}$) | 1 µl |
| 6 | Template cDNA (50 ng.µl$^{-1}$) | 1 µl |
| 7 | Millipore sterile distilled water | 14.74 µl |
| Total | | 20 µl |

The reagents were mixed gently by tapping against the tube. The tubes were then placed in the Thermal Cycler for amplification. The PCR condition for thermal cycler is given below.

**PCR conditions for SSR**

| Index | Steps | Temperature (ºC) | Duration |
|---|---|---|---|
| 1 | Initial denaturation | 94 | 4.0 min |
| 2 | Denaturation | 94 | 45 sec |
| 3 | Annealing | 52 | 30 sec |
| 4 | Extension | 72 | 45 sec |
| Repeat the steps 2 to 4 for 40 times | | | |
| 5 | Final extension | 72 | 10 min |
| 6 | Hold | 4 | -- |

**Agarose gel electrophoresis of amplified product**

PCR products were subjected to electrophoresis with marker DNA of known molecular weight in 2.2% agarose gel. After electrophoresis, the gel was carefully taken out of the casting tray and photographed in Gene Sys gel documentation system.

**3. Results and Discussion**

Total RNA isolated was 10; 15 and 2µg/ml, followed by mRNA isolation was 6.1; 7.63 and 1.52µg/ml and construction of cDNA library 25.4; 38.7 and 5.35µg/ml amplification of cDNA library, from all the three developmental stages of fenugreek GMV-2 that were vegetative stage (20-30 DAS), reproductive stage (50-60 DAS) and maturity stage (70-80 DAS) respectively. SSR marker was designed by using a high through output web tool Batch Primer3 (v1.0). BatchPrimer3 is a comprehensive web primer design program using Batchprimer3 core program as a major primer design engine to design different types of PCR primers and sequencing primers in a high-through manner. In this study, a total 100 contigs were mined for SSR marker identification in each stages that are vegetative, reproductive and maturity stages. Among this 10 markers from each stages were used for validation purpose by using polymerase chain reaction.

For the identification of SSRs FASTA files of 100 contigs from vegetative stage, 100 contigs from reproductive stage and 100 contigs from maturity stage were used. Total 78 primers were developed from vegetative stage (Table 1). Of fenugreek these developed primers met the following parameters: primer size from 18-27 bp (optimal 20bp), GC content 33%-55% (optimal 45% and 55%) and the annealing temperature was set at 58ºC- 60ºC (optimal 59ºC). In reproductive stage 64 primers were developed with the parameters of primer size was 20-22 bp (optimal 20bp), GC content 40%-60% (optimal 45% and 50%) and the annealing temperature was 59ºC-60ºC) (Table 2). 47 primers were developed from maturity stage of fenugreek with the primer size of 20-27bp (optimal 20), GC content 33%-60% (optimal 50%) and the annealing temperature was 58ºC-60ºC (optimal 59ºC) (Table 3).

Total 30 primers were selected for validation purpose 10 primers each from each developmental stage that are vegetative stage, reproductive and maturity stage (Figure 1) with the basic primer parameters: primer size 20bp, GC content 45%-60% (optimal 50% and 55%) and the annealing temperature 59ºC-60ºC. During the SSR marker identification and primer designing some information was generated that is summarized here, Tm and GC % was found between 59ºC-60ºC and 45%-60% respectively and length of SSR primers was between 20-23 bp.

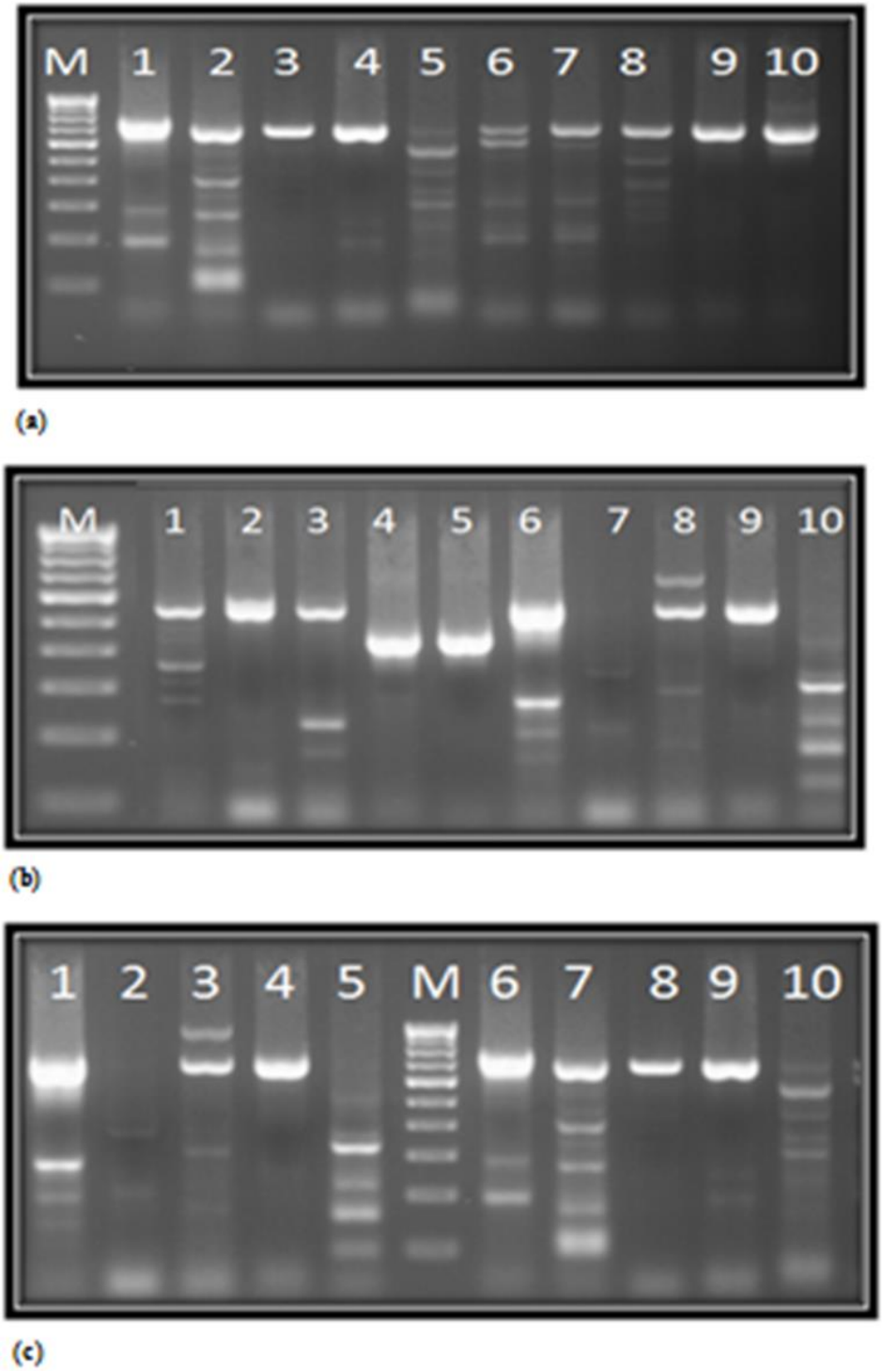



**Fig 1:** SSR markers screening images (a) vegetative stage, (b) reproductive stage and (c) maturity stage

**Table 1:** List of SSR markers developed by using BatchPrimer3 web tool from vegetative stage.

| Index | Location or Gene ID | O | PL | Temp(°C) | GC(%) | Primer sequence |
|---|---|---|---|---|---|---|
| 1 | Vegetative_(single)_trimmed_contig_6 | F | 20 | 59.96 | 55 | CTGGTCCAACCACTGGATCT |
| | | R | 20 | 60.08 | 55 | GTGGAGGAGGATGACAGCAT |
| 2 | Vegetative_(single)_trimmed_contig_7 | F | 20 | 59.80 | 45 | TCATCACAAAATGGCTCTCG |
| | | R | 20 | 59.74 | 55 | ATCACAGTGCCTGTCTGGTG |
| 3 | Vegetative_(single)_trimmed_contig_8 | F | 20 | 60.47 | 50 | TCCAGAAATGAGCCTTCTCG |
| | | R | 20 | 59.87 | 45 | AATAGCATCTTTGCGCCTGT |
| 4 | Vegetative_(single)_trimmed_contig_9 | F | 20 | 59.99 | 50 | TTGCTGACCATCTTGCTGAC |
| | | R | 20 | 59.94 | 45 | TCCAACTGTTCATCCAACCA |
| 5 | Vegetative_(single)_trimmed_contig_10 | F | 20 | 59.87 | 50 | AAGTTGGAGCTGGATGCTGT |
| | | R | 18 | 60.14 | 50 | TGGAAATGTTGGGGTTGG |
| 6 | Vegetative_(single)_trimmed_contig_11 | F | 20 | 59.41 | 40 | TTTGTTCCGGAGGAAAATGT |
| | | R | 22 | 59.39 | 31.82 | TTTCCATCATCATTTTCCAAGA |
| 7 | Vegetative_(single)_trimmed_contig_12 | F | 21 | 59.57 | 47.62 | TCTTGGGGAAGTAGGAAAAGG |
| | | R | 20 | 58.90 | 40 | TCCCCTTTGATGAATGAACA |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | Vegetative_(single)_trimmed_contig_13 | F | 21 | 59.18 | 47.62 | TCTCCTCCAAAGTACGGATTG |
| | | R | 20 | 59.75 | 55 | TCTTCCTCTTCCTCCCTTCC |
| 9 | Vegetative_(single)_trimmed_contig_14 | F | 20 | 60.19 | 50 | AGGGGTAAAGATGGCAAACC |
| | | R | 20 | 60.08 | 45 | GTTCTGCAAAATGCCTCCAT |
| 10 | Vegetative_(single)_trimmed_contig_15 | F | 21 | 58.76 | 42.86 | TGTTTGAGACCTTCAATGTGC |
| | | R | 20 | 61.08 | 55 | CTGGTGGAGCCACAACCTTA |
| 11 | Vegetative_(single)_trimmed_contig_16 | F | 20 | 60.11 | 50 | CATCAGTGGATTGGTTGCAG |
| | | R | 20 | 59.80 | 45 | CATCATGACGAAGCTTGGAA |
| 12 | Vegetative_(single)_trimmed_contig_17 | F | 21 | 60.21 | 38.10 | TAACCGGAAACATTGGTTTGA |
| | | R | 20 | 59.79 | 45 | CCACCAATTCTCGGTTCAAT |
| 13 | Vegetative_(single)_trimmed_contig_18 | F | 20 | 60.00 | 55 | GCAACAAGGTCTGCTCCTTC |
| | | R | 20 | 59.82 | 55 | GGGACCTTCACCTGTGACAT |
| 14 | Vegetative_(single)_trimmed_contig_19 | F | 20 | 59.65 | 50 | TCCTTCCACCGCATCTCTAT |
| | | R | 20 | 60.68 | 50 | GCTGCCACTTCAAACAAACC |
| 15 | Vegetative_(single)_trimmed_contig_20 | F | 20 | 59.28 | 50 | CAGATGACCCAGAGGCTTTT |
| | | R | 20 | 57.39 | 45 | AGCCCCACATTTATTGTGTC |
| 16 | Vegetative_(single)_trimmed_contig_21 | F | 20 | 59.96 | 45 | TGTCATGCATTAGGCCATGT |
| | | R | 20 | 60.15 | 45 | TATGCTGCTTTTGCTTGTCG |
| 17 | Vegetative_(single)_trimmed_contig_22 | F | 20 | 59.18 | 50 | GATAAGCGGAGAACCCGTAA |
| | | R | 21 | 59.98 | 42.86 | TGAATTTTGGGCAACACTAGG |
| 18 | Vegetative_(single)_trimmed_contig_24 | F | 20 | 60.08 | 50 | TTGGACTCAAACAGGGGAAG |
| | | R | 20 | 59.66 | 50 | TAGCCTCACCTCGGGAAATA |
| 19 | Vegetative_(single)_trimmed_contig_29 | F | 20 | 59.99 | 55 | CTCCGTCTTCGTGGTGGTAT |
| | | R | 20 | 60.00 | 55 | CACAAGGTGAAGGGTGGACT |
| 20 | Vegetative_(single)_trimmed_contig_31 | F | 20 | 60.62 | 50 | GAGCCGATTTGTGCGATTAC |
| | | R | 21 | 57.49 | 33.33 | TTGGCATATTTCATCACAACA |
| 21 | Vegetative_(single)_trimmed_contig_32 | F | 20 | 59.83 | 50 | GAACCGGGACATTGACTGTT |
| | | R | 20 | 60.24 | 45 | TGTTGCCACCTTCATCTTGA |
| 22 | Vegetative_(single)_trimmed_contig_33 | F | 20 | 59.74 | 50 | CAAGTTCCCTGGGGATGATA |
| | | R | 20 | 60.19 | 55 | CACTTTCGACACCACACCTG |
| 23 | Vegetative_(single)_trimmed_contig_37 | F | 20 | 60.24 | 55 | GGACGTGGACCTTCTTCTGA |
| | | R | 20 | 60.03 | 45 | TCGAGATTTGCCCCTAATTG |
| 24 | Vegetative_(single)_trimmed_contig_38 | F | 20 | 60.03 | 50 | GGCTCAACAACTGACAAGCA |
| | | R | 20 | 59.18 | 50 | TCTGGACTGTTCCTCATCCA |
| 25 | Vegetative_(single)_trimmed_contig_40 | F | 20 | 59.94 | 40 | GCATTTGCCAAGGATGTTTT |
| | | R | 20 | 59.99 | 55 | GTACAAAGGGCAGGGACGTA |
| 26 | Vegetative_(single)_trimmed_contig_41 | F | 20 | 60.15 | 50 | CAATGGTGGTGCTGAAACTG |
| | | R | 20 | 61.10 | 50 | GGTGAATCGCTTGATTCGTT |
| 27 | Vegetative_(single)_trimmed_contig_42 | F | 20 | 60.08 | 45 | TGCAAGAAGCAAGATGGCTA |
| | | R | 20 | 59.58 | 45 | TTCAGAATGACCAGCACTCG |
| 28 | Vegetative_(single)_trimmed_contig_43 | F | 20 | 59.71 | 45 | TCAGGGAGGAGAAATCCTGA |
| | | R | 20 | 59.98 | 50 | GTTAGCATGGCCAGGTTGTT |
| 29 | Vegetative_(single)_trimmed_contig_44 | F | 20 | 59.73 | 50 | AATCCATCCTCGGAATCCTT |
| | | R | 20 | 60.00 | 50 | TTTTTAACACATCAAGCTATGCAG |
| 30 | Vegetative_(single)_trimmed_contig_45 | F | 20 | 59.73 | 45 | CTCAGACGCAGATTCCAACA |
| | | R | 24 | 58.58 | 33 | ACGAACATGTTTCAGCCACA |
| 31 | Vegetative_(single)_trimmed_contig_46 | F | 20 | 59.98 | 50 | GTCAAGGCAGCTGCTACTCC |
| | | R | 20 | 60.16 | 45 | GTTGACAGGGTCAGCCAAAT |
| 32 | Vegetative_(single)_trimmed_contig_47 | F | 20 | 60.16 | 60 | CTTCAACCGATGGACCTTTG |
| | | R | 20 | 59.97 | 50 | GCAGCAAAATTGTGCATAACA |
| 33 | Vegetative_(single)_trimmed_contig_48 | F | 20 | 60.49 | 50 | GGCTCATCTCAGTCGGAGTC |
| | | R | 21 | 59.76 | 38 | AAAGGTCCCTGCCTTGAAAT |
| 34 | Vegetative_(single)_trimmed_contig_49 | F | 20 | 59.95 | 60 | TGGAGCTTAGGACCAGTTTCA |
| | | R | 20 | 59.94 | 45 | GCATGAGAAGACCCACCAAC |
| 35 | Vegetative_(single)_trimmed_contig_50 | F | 21 | 59.86 | 47.62 | TGGAGCTTAGGACCAGTTTCA |
| | | R | 20 | 60.52 | 56 | GCATGAGAAGACCCACCAAC |
| 36 | Vegetative_(single)_trimmed_contig_51 | F | 20 | 60.25 | 45 | CCAGCTTTGTGCAGGAAAAT |
| | | R | 26 | 59.84 | 26.92 | TCCAAGATTTTAACAAACATTTGAA |
| 37 | Vegetative_(single)_trimmed_contig_53 | F | 25 | 59.52 | 32 | TGGATTCTTATCCATAAACCAATTC |
| | | R | 22 | 59.27 | 40.91 | AAGAGCAGAATCAGCAGAACAA |
| 38 | Vegetative_(single)_trimmed_contig_54 | F | 25 | 57.03 | 28 | TGAATTGAAGACTACATTGGATAAA |
| | | R | 27 | 59.06 | 33.33 | CAACTAGTACTATAATTGCCCATTCAA |
| 39 | Vegetative_(single)_trimmed_contig_55 | F | 20 | 58.75 | 50 | TGGAGAAGATCTGGCATCAC |
| | | R | 20 | 59.81 | 50 | AGCAATACCAGGGAACATGG |
| 40 | Vegetative_(single)_trimmed_contig_56 | F | 20 | 60.15 | 50 | TCAACCACTACGACCGAACA |
| | | R | 20 | 59.93 | 50 | GGTGGTTATGGCAAAGAGGA |
| 41 | Vegetative_(single)_trimmed_contig_57 | F | 20 | 59.85 | 45 | TTTGGAAGCACATGCTGAAC |
| | | R | 20 | 59.96 | 45 | CGCGTTAGAATCCCAACAAT |
| 42 | Vegetative_(single)_trimmed_contig_59 | F | 21 | 59.86 | 52.38 | GGTCGTGATGAGAGTTCTTCG |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | R | 25 | 59.86 | 28 | AAACTTGAAAACTTTTGGACCTTTT |
| 43 | Vegetative_(single)_trimmed_contig_60 | F | 20 | 60.07 | 55 | CAGAGCTTGCAGCTTCACTG |
| | | R | 20 | 60.19 | 50 | CCACCCTATTGGCATCAAAC |
| 44 | Vegetative_(single)_trimmed_contig_61 | F | 20 | 59.95 | 50 | CCTTCGATCCTTTTGCTGAG |
| | | R | 23 | 58.78 | 39.13 | CTTGCAGTAATCATTCAATCCAG |
| 45 | Vegetative_(single)_trimmed_contig_62 | F | 20 | 60.12 | 40 | ACATGTTTCATTGGCAAGCA |
| | | R | 24 | 59.39 | 29.17 | TTTGTTTAAAAGCACCAAAAGAGA |
| 46 | Vegetative_(single)_trimmed_contig_63 | F | 20 | 60.21 | 50 | CAAAACCACTCCCACCAATC |
| | | R | 20 | 60.84 | 50 | GCTGCTGCAATGGTACATGA |
| 47 | Vegetative_(single)_trimmed_contig_65 | F | 20 | 59.43 | 55 | GAGACGGGAACAGAAGGCTA |
| | | R | 21 | 59.38 | 47.62 | TGCTCTATGCAACACACACCT |
| 48 | Vegetative_(single)_trimmed_contig_67 | F | 20 | 60.12 | 50 | GGTCAAAGTCGCTTCACCAT |
| | | R | 20 | 60.26 | 55 | CGGGGACGAAGTTAGTAGCA |
| 49 | Vegetative_(single)_trimmed_contig_68 | F | 20 | 60.44 | 35 | TTTTTCCATGGCTTGCATTT |
| | | R | 20 | 58.95 | 40 | TTTTCCTGTTCATCCAACCA |
| 2050 | Vegetative_(single)_trimmed_contig_69 | F | 20 | 59.84 | 45 | GGATTTGTTGTTGGGCTTGT |
| | | R | 20 | 59.98 | 55 | CCTTCACAGCTTCTCCTTGG |
| 51 | Vegetative_(single)_trimmed_contig_70 | F | 20 | 60.06 | 55 | GGGATGACCGTATTGGTGTC |
| | | R | 20 | 60.07 | 50 | ATGGGCTTGAGTTGATCAGG |
| 52 | Vegetative_(single)_trimmed_contig_74 | F | 20 | 60.25 | 55 | AACGACCTCGTTCTCCTCCT |
| | | R | 20 | 60.06 | 50 | CATCTGCCAATATGGCTCCT |
| 53 | Vegetative_(single)_trimmed_contig_75 | F | 20 | 59.97 | 55 | CTAGGCTTGGCTTGATGGAG |
| | | R | 20 | 59.87 | 50 | ATTCACACTGCTGGGAGCTT |
| 54 | Vegetative_(single)_trimmed_contig_76 | F | 20 | 60.37 | 55 | CCACGTGGAAGATAGGGTTG |
| | | R | 23 | 59.22 | 43.48 | AAGCAGTGAGTTCTTCTTCCT |
| 55 | Vegetative_(single)_trimmed_contig_77 | F | 20 | 59.84 | 45 | GCTTATGAATGTGGCAAGCA |
| | | R | 20 | 59.72 | 50 | TGGTATGCGAACTTGTCTGG |
| 56 | Vegetative_(single)_trimmed_contig_78 | F | 20 | 59.96 | 45 | AAGAACGCTTCGAAGATGGA |
| | | R | 20 | 60.07 | 50 | ATCCCAGTCCTCAGGCTTTT |
| 57 | Vegetative_(single)_trimmed_contig_79 | F | 20 | 60.01 | 55 | AGCGAGTCGAGGTGGTAGAA |
| | | R | 20 | 59.72 | 60 | CCCAGCTACTCTTGGTCCAC |
| 58 | Vegetative_(single)_trimmed_contig_80 | F | 20 | 59.90 | 45 | CCCAAATCCAGAACCTTCAA |
| | | R | 20 | 59.81 | 45 | TGACCATCAACGGTGACAAT |
| 59 | Vegetative_(single)_trimmed_contig_81 | F | 20 | 60.88 | 50 | AAACCAGCCTGGCATTAGGT |
| | | R | 20 | 60.09 | 55 | GAAGAGAAGTGGTGGGTGGA |
| 60 | Vegetative_(single)_trimmed_contig_82 | F | 20 | 59.60 | 50 | CTTTTTGTGGGTAGCCCTTG |
| | | R | 20 | 60.59 | 40 | TTCCGAAAGATTGCGTTCAT |
| 61 | Vegetative_(single)_trimmed_contig_84 | F | 20 | 60.02 | 50 | AGCCGACAGAGAAGCAATGT |
| | | R | 20 | 59.97 | 50 | GGTGGCAGACATGAATTGTG |
| 62 | Vegetative_(single)_trimmed_contig_85 | F | 20 | 60.36 | 55 | CACTCCACTCCAAACCCATC |
| | | R | 20 | 59.99 | 45 | CCAAATCAAGTTGAGCAGCA |
| 63 | Vegetative_(single)_trimmed_contig_88 | F | 20 | 59.85 | 50 | CCAAAAGCGACAAAGACCTC |
| | | R | 20 | 59.92 | 45 | ATGGATTCATTCGCCGTTAG |
| 64 | Vegetative_(single)_trimmed_contig_89 | F | 20 | 60.24 | 40 | TGGATTTGGAAGGATTGGAA |
| | | R | 20 | 59.86 | 40 | AAACATTTGGTGTTGGCACA |
| 65 | Vegetative_(single)_trimmed_contig_90 | F | 23 | 57.35 | 30.43 | TTCAGATGATTTTCATTCTGTCA |
| | | R | 23 | 58.80 | 39.13 | CATTGACACCAACCACATAAGTT |
| 66 | Vegetative_(single)_trimmed_contig_92 | F | 20 | 60.12 | 55 | CAGATCATCACGACGACCAC |
| | | R | 20 | 59.60 | 55 | GAACTCAGCCAGTGCCTTCT |
| 67 | Vegetative_(single)_trimmed_contig_93 | F | 20 | 60.22 | 55 | GGAGCTCGGGACTGTAATGA |
| | | R | 21 | 60.02 | 42 | TCACCCACATGTAAAAGCACA |
| 68 | Vegetative_(single)_trimmed_contig_94 | F | 20 | 59.14 | 50 | GCTCCAGGTGACTTTGGATT |
| | | R | 21 | 60.15 | 47.62 | CCGATAACATGCTGTCGACTT |
| 69 | Vegetative_(single)_trimmed_contig_95 | F | 20 | 61.51 | 55 | GACCATCCTCTCCTCCGAAA |
| | | R | 23 | 59.76 | 39.13 | CATGGCATTTCTGGTTAATAAGG |
| 70 | Vegetative_(single)_trimmed_contig_96 | F | 20 | 59.81 | 50 | CATTCCCTTCGTTCTTCAGC |
| | | R | 20 | 60.36 | 45 | GATGCCTTGCAAGCTCAAAT |
| 71 | Vegetative_(single)_trimmed_contig_97 | F | 20 | 59.53 | 50 | CCCATAACAGGTCCCACATT |
| | | R | 24 | 59.49 | 41.67 | AACTTGTGTACAGACCAGGATTTG |
| 72 | Vegetative_(single)_trimmed_contig_100 | F | 20 | 60.29 | 50 | AAGAAGCCACACCGTTTCAG |
| | | R | 22 | 58.97 | 40.91 | TTCAAATAGAGTCCCTTGCTCA |
| 73 | Vegetative_(single)_trimmed_contig_110 | F | 20 | 60.23 | 50 | GCAGCCACTTCATCCTTCAT |
| | | R | 20 | 59.85 | 45 | TCAGCCAATTGAAGTGTTGC |
| 74 | Vegetative_(single)_trimmed_contig_111 | F | 20 | 60.24 | 40 | ACCAAATCCAAATCCCCATT |
| | | R | 20 | 59.80 | 60 | GATCGACCGGTCTCTCTGTC |
| 75 | Vegetative_(single)_trimmed_contig_112 | F | 20 | 59.68 | 50 | TGGACCTGTCAATGTCAAGC |
| | | R | 20 | 60.14 | 50 | GTAGCAACGCCAAATCCTGT |
| 76 | Vegetative_(single)_trimmed_contig_113 | F | 20 | 61.00 | 55 | GAACGGTCTCGGAAAACTCC |
| | | R | 20 | 59.26 | 40 | TCCCAAATTGTCCAACCTTT |

| | | | | | | |
|---|---|---|---|---|---|---|
| 77 | Vegetative_(single)_trimmed_contig_114 | F | 20 | 59.74 | 45 | GTTACGCCAATGCAGTTCAA |
| | | R | 20 | 59.99 | 50 | TGCACACTCCATTCTTGCTC |
| 78 | Vegetative_(single)_trimmed_contig_115 | F | 20 | 60.12 | 45 | ATTCGCCAAACGTGAGAAAC |
| | | R | 20 | 60.30 | 55 | CACCAGAAGTGCAGGAGGTT |

* O: Orientation, PL: Primer length, Temp: Temperature

**Table 2:** List of SSR markers developed by using BatchPrimer3 web tool from reproductive stage

| Index | Location or Gene ID | O | PL | Temp (ºC) | GC (%) | Primer sequence |
|---|---|---|---|---|---|---|
| 1 | Reproductive_(single)_trimmed_contig_6 | F | 20 | 59.55 | 45 | TCAAACAATATGGCCACAGC |
| | | R | 20 | 60.05 | 55 | AGGAGGTACCAATCCCCAAC |
| 2 | Reproductive_(single)_trimmed_contig_7 | F | 20 | 60.07 | 35 | TATTGCAATTCGCAAAACCA |
| | | R | 20 | 59.86 | 50 | CAGTCGAAACGTAGCCATCA |
| 3 | Reproductive_(single)_trimmed_contig_8 | F | 20 | 60.17 | 50 | CAGCTCATGCTGTTGATGCT |
| | | R | 20 | 59.98 | 55 | GCCGCTCTATCAAGTTCAGG |
| 4 | Reproductive_(single)_trimmed_contig_10 | F | 20 | 59.86 | 36.36 | TGAGAAGGAAAGCAAATTGTGA |
| | | R | 20 | 60.16 | 50 | AGGCTGAAGCTCAGTTGCAT |
| 5 | Reproductive_(single)_trimmed_contig_11 | F | 20 | 60.12 | 50 | GGTCAAAGTCGCTTCACCAT |
| | | R | 20 | 60.26 | 55 | CGGGGACGAAGTTAGTAGCA |
| 6 | Reproductive_(single)_trimmed_contig_12 | F | 20 | 58.99 | 50 | GATCTGGTGGACCGAGTTTT |
| | | R | 20 | 59.05 | 45 | GGCTGGCCAAACTTATTCAT |
| 7 | Reproductive_(single)_trimmed_contig_13 | F | 20 | 60.60 | 50 | CATCCAATGGAGATGGTGGT |
| | | R | 20 | 59.91 | 47.37 | GCCGGCCAAACTTATTCAT |
| 8 | Reproductive_(single)_trimmed_contig_14 | F | 20 | 59.93 | 45 | TCACATGCCATCTTCACCAT |
| | | R | 20 | 59.69 | 50 | AGGCTCCTTTTAAGCCCATC |
| 9 | Reproductive_(single)_trimmed_contig_15 | F | 20 | 59.99 | 50 | GCCACTTGCACTTCTCATCA |
| | | R | 20 | 59.94 | 50 | CATCAGCATCTCCGTCAAGA |
| 1 | Reproductive_(single)_trimmed_contig_16 | F | 20 | 58.86 | 45 | CTGGAGCCATTTATCCCAAT |
| | | R | 20 | 61.14 | 50 | GCCATCTCGCTATCCAACAA |
| 2 | Reproductive_(single)_trimmed_contig_20 | F | 20 | 60.36 | 55 | GCTGAGGATCCGAACGAGTA |
| | | R | 20 | 60.34 | 55 | GGGTGGTAGCATCCATCTTG |
| 3 | Reproductive_(single)_trimmed_contig_22 | F | 26 | 59.48 | 30.77 | AAAATGAAGGTGAGTTATGAGAATGA |
| | | R | 20 | 59.01 | 50 | TGCACTTTGGAGTTCTCAGG |
| 4 | Reproductive_(single)_trimmed_contig_23 | F | 20 | 59.68 | 40 | AATTTTAGGGGACGGCATTT |
| | | R | 20 | 59.95 | 55 | GCTCTGAGAATCCCTCAACG |
| 5 | Reproductive_(single)_trimmed_contig_25 | F | 20 | 59.80 | 45 | CAAGAAGCAATCGGATGTGA |
| | | R | 20 | 59.10 | 50 | AGGCAAAAACACAGGTAGGG |
| 6 | Reproductive_(single)_trimmed_contig_26 | F | 20 | 60.02 | 50 | AGATTCATGCAGCGTGTGAG |
| | | R | 22 | 59.66 | 50 | GGCAAAGACACACACAGATAGG |
| 7 | Reproductive_(single)_trimmed_contig_27 | F | 20 | 60.04 | 50 | TGAGGATTCCCAGAACAAGG |
| | | R | 20 | 59.84 | 45 | CGAAAGTGTTTGGCTCATCA |
| 8 | Reproductive_(single)_trimmed_contig_28 | F | 20 | 59.98 | 45 | TTGGTCAGTTTGGTGTTGGA |
| | | R | 20 | 60.02 | 50 | AGCTCTTCGCAGTTGTCCAT |
| 9 | Reproductive_(single)_trimmed_contig_29 | F | 20 | 60.44 | 50 | GGAGCCAATTATGCATCTGG |
| | | R | 20 | 60.11 | 50 | CTCAGTTGGGTGGAAAGCAT |
| 10 | Reproductive_(single)_trimmed_contig_30 | F | 20 | 60.07 | 50 | ACGATTCCCTTGATGAGACG |
| | | R | 20 | 60.39 | 55 | CAAGAGGAGGCAACGTAGGA |
| 11 | Reproductive_(single)_trimmed_contig_31 | F | 20 | 59.84 | 45 | GAATAGCAATCGTCGTGCAA |
| | | R | 20 | 60.03 | 45 | AGAAAGCCGGAAGGGAATTA |
| 12 | Reproductive_(single)_trimmed_contig_32 | F | 20 | 59.97 | 40 | AAAGCGTTTTGGGAACATTG |
| | | R | 20 | 59.84 | 55 | GCCACTAATCGCTCCTCAAC |
| 13 | Reproductive_(single)_trimmed_contig_34 | F | 20 | 60.03 | 40 | ACCCAACCCATTTCATTTCA |
| | | R | 21 | 59.79 | 47.62 | GAATGCTGAATAACCCCTTCC |
| 14 | Reproductive_(single)_trimmed_contig_35 | F | 20 | 60.02 | 55 | GCAAGCCAGTCAAGCTAACC |
| | | R | 20 | 59.87 | 50 | AGCAAGATGGTCAGCAAGGT |
| 15 | Reproductive_(single)_trimmed_contig_36 | F | 20 | 59.96 | 50 | ACAGGGCAATCGACCATAAG |
| | | R | 20 | 59.80 | 45 | AATGCAACAAACCCAGATCC |
| 16 | Reproductive_(single)_trimmed_contig_37 | F | 20 | 59.87 | 50 | ACGAGTTTGCAGACCGAGAT |
| | | R | 21 | 59.43 | 42.86 | TTTGAGAGTGATGGTGCTGAA |
| 17 | Reproductive_(single)_trimmed_contig_38 | F | 21 | 59.19 | 38.10 | TTCTGTTTTTCCCAAGAATGC |
| | | R | 20 | 60.47 | 45 | AAACCCATGAATGCAGGCTA |
| 18 | Reproductive_(single)_trimmed_contig_39 | F | 20 | 59.79 | 45 | GCCTGCATTCATGGGTTTAT |
| | | R | 20 | 59.07 | 45 | TAACATTTCAGCTGCCTTGC |
| 19 | Reproductive_(single)_trimmed_contig_40 | F | 20 | 59.34 | 50 | CACAAGAAGTTCCACGTTGC |
| | | R | 20 | 60.28 | 40 | CATGAATGCCATTTCCATCA |
| 20 | Reproductive_(single)_trimmed_contig_42 | F | 20 | 59.93 | 50 | CGGACCTCAAGTTTGGAATC |
| | | R | 20 | 59.97 | 50 | AGTTGTGTTGATGCCCTTCC |
| 21 | Reproductive_(single)_trimmed_contig_43 | F | 20 | 60.13 | 40 | TTTTTGCTGGTGTTGGTTCA |
| | | R | 20 | 59.12 | 50 | GCAAGAGGAGCATGTTCAGA |

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|---|---|---|---|---|---|---|
| 22 | Reproductive_(single)_trimmed_contig_45 | F | 22 | 58.82 | 40.91 | GGTTGATGTGTTGTTGGATTCT |
| | | R | 21 | 59.21 | 42.86 | CCCACTCTCAATAAAGGCAAA |
| 23 | Reproductive_(single)_trimmed_contig_51 | F | 20 | 60.15 | 50 | GGTGGGCATTTAGGGAGATT |
| | | R | 20 | 60.44 | 50 | AAATCGAGTGGGGCTAAAGG |
| 24 | Reproductive_(single)_trimmed_contig_52 | F | 20 | 59.82 | 45 | GGTTGCAATTATGTGCATGG |
| | | R | 20 | 59.36 | 50 | TTAGGATCCGGGATGTCTGT |
| 25 | Reproductive_(single)_trimmed_contig_55 | F | 20 | 58.91 | 50 | CATGGCAGAGGAGAATCAGA |
| | | R | 20 | 60.01 | 45 | ACAAAGCCCCAAAACAACAG |
| 26 | Reproductive_(single)_trimmed_contig_56 | F | 20 | 60.21 | 45 | TTGAAACCTGGAATGGTGGT |
| | | R | 20 | 60.26 | 45 | GCGCAGACTTCAAACCAAAT |
| 27 | Reproductive_(single)_trimmed_contig_57 | F | 20 | 59.87 | 50 | CCAACTGGACTGCAAACTGA |
| | | R | 20 | 59.94 | 50 | GAACCCAGTTCTGGGAACAA |
| 28 | Reproductive_(single)_trimmed_contig_59 | F | 20 | 59.97 | 55 | TTCTCCTCCACCGTAACACC |
| | | R | 20 | 60.39 | 45 | GCATGCAACTTTTTCCTGCT |
| 29 | Reproductive_(single)_trimmed_contig_61 | F | 20 | 60.05 | 45 | TGGTCATTTCCAAAGGAAGC |
| | | R | 20 | 59.92 | 50 | GAACACCCCCAGGGTAAAAT |
| 30 | Reproductive_(single)_trimmed_contig_62 | F | 20 | 59.75 | 55 | ACCGAGACAGCTGAGAAAGC |
| | | R | 20 | 60.25 | 55 | CTTCCCTGTGCCCTTGTCTA |
| 31 | Reproductive_(single)_trimmed_contig_63 | F | 20 | 59.85 | 55 | GCGTCAACCCTTTCACTCTC |
| | | R | 21 | 59.68 | 47.62 | TCAGACGGGGATAATAGCTGA |
| 32 | Reproductive_(single)_trimmed_contig_65 | F | 20 | 60.86 | 55 | TGTCGAAGAGAGGTCGTGGT |
| | | R | 18 | 60.06 | 61.11 | CGTACCATGGGCTTCCTG |
| 33 | Reproductive_(single)_trimmed_contig_66 | F | 20 | 59.96 | 45 | TGTCATGCATTAGGCCATGT |
| | | R | 20 | 60.15 | 45 | TATGCTGCTTTTGCTTGTCG |
| 34 | Reproductive_(single)_trimmed_contig_67 | F | 20 | 60.17 | 45 | ACCCCCACAAAGATGATGAA |
| | | R | 20 | 60.08 | 45 | CATGTGCCATCAATTCAAGC |
| 35 | Reproductive_(single)_trimmed_contig_68 | F | 20 | 59.75 | 55 | ACGCACTCTCTTTCCTCTCG |
| | | R | 20 | 59.06 | 50 | TGAAATCCTCCTCCACTTCC |
| 36 | Reproductive_(single)_trimmed_contig_69 | F | 20 | 59.55 | 50 | TTCCTCTTCAGCTGTGACCA |
| | | R | 21 | 60.67 | 42.86 | GGTTCTTCCAAATTCCAATGC |
| 37 | Reproductive_(single)_trimmed_contig_70 | F | 20 | 59.79 | 50 | GAACCGTTTATCCACCCTCA |
| | | R | 20 | 60.03 | 55 | GGCCTTCCTCCCAATCTAAG |
| 38 | Reproductive_(single)_trimmed_contig_80 | F | 20 | 59.76 | 50 | AAGGAAGGTATTCCCCCAGA |
| | | R | 20 | 59.87 | 50 | CACCAACAGCTCAGAAACCA |
| 39 | Reproductive_(single)_trimmed_contig_82 | F | 20 | 60.15 | 45 | AAAAGCCACAAACTCCAACG |
| | | R | 20 | 59.74 | 45 | TTTGTTGCTGAACCAACGTC |
| 40 | Reproductive_(single)_trimmed_contig_83 | F | 20 | 60.16 | 60 | CCTTACTGCTCCTCGTCAGC |
| | | R | 20 | 59.67 | 45 | TGGCAATCTCTGGAGGTTTT |
| 41 | Reproductive_(single)_trimmed_contig_84 | F | 20 | 60.05 | 40 | GTTTCGGCCTTTGATTTTCA |
| | | R | 25 | 57.59 | 28 | AGGAATGAAACAAGAATTTTCTACA |
| 42 | Reproductive_(single)_trimmed_contig_85 | F | 20 | 60.07 | 55 | CCAACCTCCCTTTCCTAAGC |
| | | R | 20 | 60.06 | 55 | AGGAGAGCCACACAAGCACT |
| 43 | Reproductive_(single)_trimmed_contig_86 | F | 20 | 59.13 | 35 | TTTTCAAGAAATGGCACCAA |
| | | R | 20 | 59.76 | 45 | GTTGCCTCATCCCCAAAATA |
| 44 | Reproductive_(single)_trimmed_contig_87 | F | 20 | 59.88 | 45 | ACCAGCAGCGTCTCATTTTT |
| | | R | 20 | 59.54 | 45 | CGAAAATCTGCATACCACCA |
| 45 | Reproductive_(single)_trimmed_contig_88 | F | 20 | 60.28 | 45 | TGTGACAAGGGTTTTGCTGA |
| | | R | 20 | 59.93 | 50 | AATCCACCATGTGCTCTTCC |
| 46 | Reproductive_(single)_trimmed_contig_89 | F | 20 | 60.26 | 45 | GCCAGCGATGAAACTTTGTT |
| | | R | 20 | 60.12 | 40 | ATCCGATCCATTTGGCATTA |
| 47 | Reproductive_(single)_trimmed_contig_91 | F | 20 | 60.46 | 50 | TAGTTGGGATGCGACCCTTA |
| | | R | 20 | 59.94 | 45 | TACAATTTTCTGCGGGTTCC |
| 48 | Reproductive_(single)_trimmed_contig_92 | F | 20 | 60.04 | 50 | TTGGGACTTGGAGATCTTGG |
| | | R | 20 | 59.90 | 50 | GTTTTCCCCACTCCTGATGA |
| 49 | Reproductive_(single)_trimmed_contig_93 | F | 20 | 60.02 | 50 | AGCAGCAACAGCATCATCAC |
| | | R | 20 | 59.78 | 55 | CCAGCTGCTAGAAGGTTGCT |
| 50 | Reproductive_(single)_trimmed_contig_94 | F | 20 | 60.21 | 50 | GGCGATTACTCGGAAGTGAA |
| | | R | 20 | 60.06 | 45 | ACCACGACAATCCATTCCAT |
| 60 | Reproductive_(single)_trimmed_contig_95 | F | 20 | 59.99 | 45 | CTGAATTGAAAGCCACAGCA |
| | | R | 20 | 59.55 | 45 | CAAATTCAAGTCCCCAAAGC |
| 61 | Reproductive_(single)_trimmed_contig_96 | F | 20 | 59.42 | 55 | GAAGATGTCGTCAGGTGCAG |
| | | R | 21 | 59.69 | 42.86 | TCCCTTTAAGCCAAGATGACA |
| 62 | Reproductive_(single)_trimmed_contig_97 | F | 19 | 60.30 | 57.89 | GCCGCACTCTTGCAGACTA |
| | | R | 20 | 60.88 | 50 | TAAAATCCACCACGGAGACG |
| 63 | Reproductive_(single)_trimmed_contig_98 | F | 20 | 58.89 | 40 | TGCTTTCTTCGTTTTCATGG |
| | | R | 20 | 59.67 | 50 | GCATGACGGAAAGGATAAGC |
| 64 | Reproductive_(single)_trimmed_contig_100 | F | 25 | 60.09 | 48 | TCTCTCTCTCTCTCTCTTTCTGTGC |
| | | R | 22 | 59.36 | 36.36 | TTGCTAAACCACCAATAATCCA |

* O: Orientation, PL: Primer length, Temp: Temperature

**Table 3:** List of SSR markers developed by using BatchPrimer3 web tool from maturity stage.

| Index | Location or Gene ID | O | PL | Temp (ºC) | GC (%) | Primer sequence |
|---|---|---|---|---|---|---|
| 1 | Maturity_(single)_trimmed_contig_1 | F | 20 | 59.66 | 40 | TGCATTTATTTCTGCGGATG |
| | | R | 20 | 59.55 | 50 | ATAATCAGACGGAGGCGGTA |
| 2 | Maturity_(single)_trimmed_contig_2 | F | 20 | 59.99 | 50 | TCCAACGAGCTTCCACTTCT |
| | | R | 20 | 59.74 | 50 | TGGAAGATGGGTTGATAGGG |
| 3 | Maturity_(single)_trimmed_contig_3 | F | 20 | 59.99 | 60 | CGGTATAGAGGCTGCTGAGG |
| | | R | 20 | 60.42 | 50 | ACAGTTTTGCAGCGCCTATC |
| 4 | Maturity_(single)_trimmed_contig_4 | F | 20 | 59.99 | 50 | TGTTGATGCTGCAACTCCTC |
| | | R | 20 | 59.97 | 45 | CACAAGGGCCTAAACCAAAA |
| 5 | Maturity_(single)_trimmed_contig_5 | F | 20 | 60.15 | 55 | GCTGCAGTTAAGGGAAGCTG |
| | | R | 20 | 60.09 | 55 | TGCATTACCGGGACTAGGAG |
| 6 | Maturity_(single)_trimmed_contig_6 | F | 20 | 59.96 | 45 | ATTCGAGAACCTCGCAAGAA |
| | | R | 20 | 58.88 | 33.33 | CCAACCACAAAGTTATCATTTTTC |
| 7 | Maturity_(single)_trimmed_contig_7 | F | 20 | 60.15 | 55 | GCTGCAGTTAAGGGAAGCTG |
| | | R | 20 | 60.09 | 55 | TGCATTACCGGGACTAGGAG |
| 7 | Maturity_(single)_trimmed_contig_11 | F | 20 | 59.18 | 50 | TCCCAACTCATCAGTCTCCA |
| | | R | 20 | 60.47 | 43.48 | CAAGTTGCAATAGACATGTGGTG |
| 8 | Maturity_(single)_trimmed_contig_12 | F | 20 | 60.29 | 50 | GTCCATTGCTTCCCATCATC |
| | | R | 21 | 59.97 | 50 | GGACATGCACCAGGAAGTTT |
| 9 | Maturity_(single)_trimmed_contig_13 | F | 20 | 60.04 | 45 | CCATTTCTGATTGCCTCGAT |
| | | R | 20 | 60.35 | 50 | TCCGATCTTAAAGCCACTGC |
| 10 | Maturity_(single)_trimmed_contig_14 | F | 20 | 59.90 | 50 | TGCCACCTTTGCTGTAAGTG |
| | | R | 20 | 59.69 | 45 | CAAAGCAAAATCTCCGTTCC |
| 11 | Maturity_(single)_trimmed_contig_18 | F | 27 | 58.86 | 33.33 | GGATCAACATTTATAACTCCTGTATGA |
| | | R | 20 | 59.50 | 40 | TGATTGCTTCTTTCCCCAAT |
| 12 | Maturity_(single)_trimmed_contig_19 | F | 20 | 59.86 | 50 | TCAGCTTCTGTGGCTCTTCA |
| | | R | 20 | 59.96 | 55 | CCAGGTCCACCTCATCACTT |
| 13 | Maturity_(single)_trimmed_contig_21 | F | 20 | 59.86 | 50 | CCACAAGACAAGGCTGCATA |
| | | R | 20 | 58.68 | 45 | ATCTTGCTTGTGCAGTTGCT |
| 14 | Maturity_(single)_trimmed_contig_23 | F | 20 | 60.15 | 50 | GCATCCAAAAGGGAGGGTAT |
| | | R | 20 | 59.90 | 60 | GCTACTACCAGAGGCCAACG |
| 15 | Maturity_(single)_trimmed_contig_24 | F | 20 | 60.38 | 50 | TGGCTCTTCTTCGACAAACC |
| | | R | 20 | 59.71 | 45 | AGGACAAAGAAAACCCAGCA |
| 16 | Maturity_(single)_trimmed_contig_27 | F | 20 | 60.48 | 50 | AGGACCGAAACGAAGAAACC |
| | | R | 20 | 58.17 | 50 | CACGCATAATCCTTCACCTC |
| 17 | Maturity_(single)_trimmed_contig_28 | F | 20 | 59.93 | 45 | TAAGGGGTCAGCAAAATTGG |
| | | R | 20 | 59.90 | 45 | AGGAATTTGCCCAGGAATCT |
| 18 | Maturity_(single)_trimmed_contig_29 | F | 20 | 59.65 | 45 | CCCCAAATCATTCCAACAGT |
| | | R | 20 | 60.05 | 45 | GCCTTCCAAGTTCAAATCCA |
| 19 | Maturity_(single)_trimmed_contig_32 | F | 20 | 59.97 | 50 | GTGAAAGGTCCATTGCCAGT |
| | | R | 20 | 59.96 | 40 | TCAATGCTGCTGCAAAAATC |
| 20 | Maturity_(single)_trimmed_contig_33 | F | 20 | 59.67 | 50 | CAATGAAGCCTGGAAAGACC |
| | | R | 20 | 60.29 | 60 | GAGCGAGCTTGAGTGGAGTC |
| 21 | Maturity_(single)_trimmed_contig_34 | F | 20 | 59.95 | 60 | CTCCGACCTCTCTTCCCTCT |
| | | R | 20 | 59.97 | 50 | GGGTTCATGTTGTGATGCTG |
| 22 | Maturity_(single)_trimmed_contig_38 | F | 21 | 60.31 | 52.38 | TCTCCCCTAATTCCCACACTC |
| | | R | 20 | 59.64 | 50 | GCATCAGGATCTGGTGTTGA |
| 23 | Maturity_(single)_trimmed_contig_43 | F | 20 | 60.10 | 50 | AGATCTGGATTACGGCAACG |
| | | R | 20 | 60.15 | 50 | AGTTGGCACTGGGAAACTTG |
| 24 | Maturity_(single)_trimmed_contig_44 | F | 22 | 58.95 | 36.36 | TGGCATTTCCATATGTTTCTTC |
| | | R | 20 | 59.36 | 45 | TCCCACTAAAATTCCCTCCA |
| 25 | Maturity_(single)_trimmed_contig_47 | F | 21 | 59.61 | 52.38 | GGTGTTGCTAATGGAGGTCTG |
| | | R | 20 | 59.96 | 45 | AGGCCTTTCTGAAGCATGAA |
| 26 | Maturity_(single)_trimmed_contig_48 | F | 23 | 59.25 | 39.13 | GAGATAGAAAAGGAAAGGGGATT |
| | | R | 20 | 59.95 | 55 | GGATCAAGAGACGGCTGAAG |
| 27 | Maturity_(single)_trimmed_contig_50 | F | 20 | 60.29 | 50 | CCACAGTTTCCTTCGTTGCT |
| | | R | 21 | 57.33 | 33.33 | TCAACCAAGAAAATTCCTTCA |
| 28 | Maturity_(single)_trimmed_contig_57 | F | 20 | 59.99 | 50 | CGGCACCACAGTTTAGGAAT |
| | | R | 20 | 59.98 | 50 | AGCAGTAATGGCGGAGAAGA |
| 29 | Maturity_(single)_trimmed_contig_59 | F | 20 | 60.13 | 45 | AGAAGCACGAGGCAAAGAAA |
| | | R | 20 | 60.64 | 45 | GGATTCACATCAGCATGCAA |
| 30 | Maturity_(single)_trimmed_contig_60 | F | 20 | 59.54 | 50 | ACTCTGTTGCCAGGGATGAT |
| | | R | 20 | 59.25 | 50 | TCCCTCAAGGACTTTTCTGG |
| 31 | Maturity_(single)_trimmed_contig_61 | F | 20 | 60.28 | 55 | CTGTTGCCAGGGATGCTAGT |
| | | R | 20 | 59.98 | 45 | TCAGGGTTGAACAACAACCA |
| 32 | Maturity_(single)_trimmed_contig_63 | F | 20 | 58.96 | 40 | GCAAAAGCGTAATTGTCGAA |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | R | 20 | 60.23 | 40 | TCATTGGCGAAAGTGTTTCA |
| 33 | Maturity_(single)_trimmed_contig_64 | F | 20 | 60.30 | 45 | TTCCCGATACCAAAGGAACA |
| | | R | 20 | 60.74 | 50 | TTGGACCACGAATAGGTTGG |
| 34 | Maturity_(single)_trimmed_contig_66 | F | 21 | 59.63 | 52.38 | TGCTACAACTGAGGCTCCTTC |
| | | R | 20 | 60.40 | 55 | TTGGAGCAGTTCCAGTAGCC |
| 35 | Maturity_(single)_trimmed_contig_68 | F | 20 | 60.07 | 50 | AGAATGGGGTTGATGAGCAG |
| | | R | 20 | 60.12 | 50 | GCAGTTTCCACCGAGTTGAT |
| 36 | Maturity_(single)_trimmed_contig_70 | F | 20 | 60.06 | 50 | TAGTGCAAACCAGTGCAAGC |
| | | R | 20 | 59.93 | 50 | GGTGAAGGTGGCTCATCATT |
| 37 | Maturity_(single)_trimmed_contig_73 | F | 20 | 60.07 | 50 | AAGAGCCTCCTTCCAATGGT |
| | | R | 20 | 60.07 | 45 | ATTTCCTTGGAGCGGAAAGT |
| 38 | Maturity_(single)_trimmed_contig_74 | F | 20 | 60.26 | 55 | ACAAGGAGACATGCCCTCAG |
| | | R | 20 | 59.82 | 50 | CCCCTTGTTGTCCAAGATGT |
| 39 | Maturity_(single)_trimmed_contig_75 | F | 20 | 60.01 | 50 | GGATCAGGACGAGATTCCAA |
| | | R | 20 | 59.94 | 55 | CCCTCTGCTCCTTGATTCTG |
| 40 | Maturity_(single)_trimmed_contig_77 | F | 20 | 59.99 | 50 | AGGGAACTTCTCGCTGAACA |
| | | R | 20 | 60.07 | 55 | CACCCCCAACAGGATATACG |
| 41 | Maturity_(single)_trimmed_contig_78 | F | 20 | 60.05 | 55 | GTGGTCCCATGGGTAGAATG |
| | | R | 20 | 59.41 | 45 | TCCCATTCATCATCATGCTC |
| 42 | Maturity_(single)_trimmed_contig_79 | F | 20 | 60.04 | 45 | CAAACCGAGAGGGAATTTCA |
| | | R | 20 | 59.97 | 45 | CATTTTGGTTTGGCCACTCT |
| 43 | Maturity_(single)_trimmed_contig_83 | F | 20 | 60.41 | 55 | ACAGGAGTGGCAGATCGAAG |
| | | R | 20 | 59.93 | 50 | ACGCACATTACAAGCTGACG |
| 44 | Maturity_(single)_trimmed_contig_85 | F | 20 | 60.07 | 45 | AAAACCCTCGCTCAAATCCT |
| | | R | 20 | 60.13 | 45 | AAGCTTCGCCTTCAACTCAA |
| 45 | Maturity_(single)_trimmed_contig_87 | F | 19 | 60.56 | 47.37 | AAAAGCTGCTGATGGCACA |
| | | R | 20 | 59.30 | 55 | GACTTGTCCAGAGCCAACCT |
| 46 | Maturity_(single)_trimmed_contig_88 | F | 20 | 60.11 | 50 | TGGTGGTGCTCTACCATTGA |
| | | R | 20 | 58.85 | 45 | TGTTGCATTTGGAAGAGGAG |
| 47 | Maturity_(single)_trimmed_contig_91 | F | 20 | 59.96 | 55 | CCACTCTTTGGGAGGTGGTA |
| | | R | 20 | 60.07 | 50 | AAGGTGGATGCCATCTTGAG |

* O: Orientation, PL: Primer length, Temp: Temperature

**Validation of SSR markers in three developmental stages**

In order to validate the SSR primers, 30 SSRs were selected manually out of 189 primers for their validation in genotypes of fenugreek (GMV- 2). Criteria used to select the SSR primers for validation purpose was based on the melting temperature (Tm) and GC content (GC%) of the primers. Those primers were selected which were having melting temperature above 55ºC and GC% above 40% and primers were choosen from those contigs which were involved in either biological or cellular or molecular process. Details of primers was given in (Table 4).

The agarose gel electrophoresis was used to separate amplified product of SSR primers. The performance of individual primer to amplify cDNA of different developmental stages that were vegetative, reproductive and maturity stages of GMV-2 genotype is discussed as under.

30 selected SSR markers from all the three developmental stages of fenugreek. 10 from each vegetative stage, reproductive stage and maturity stage were validated in different developmental stages of GMV-2 *viz.*, vegetative stage, reproductive stage and maturity stage for this 6 different combinations were made that are represented as follows.

1. Primers developed from reproductive stage were checked in cDNA of vegetative stage.
2. Primers developed from vegetative stage were checked in cDNA of reproductive stage.
3. Primers developed from maturity stage were checked in cDNA of vegetative stage.
4. Primers developed from maturity stage were checked in cDNA of reproductive stage.
5. Primers developed from reproductive stage were checked in cDNA of maturity stage.
6. Primers developed from vegetative stage were checked in cDNA of maturity stage.

In first combination, (reproductive primers + vegetative stage cDNA) (Figure 2) only nine primers of reproductive stage amplified the cDNA of vegetative stage, and those primers were involved in carbohydrate metabolic process, cellular amino acid metabolic process, oxidation-reduction process, translational initiation, protein metabolic process, single-organism process, cytoplasm, cell wall and integral component of membrane. These functions are common in both the stages. It is concluded that among 10 primers of reproductive stage, nine primers gave results in both stages and remaining one primer were specific to reproductive stage only.

In second combination, (vegetative stage primers + reproductive stage cDNA) (Figure 3) among 10 primers used only five primers of vegetative stage amplified the cDNA of reproductive stage. It means from those sequences vegetative primers were developed responsible for functions like, casein kinase, LOV-domain containing, protein binding, catalytic activity, proteolysis and metal ion binding are present in both vegetative stage and reproductive stage. The remaining five primers which did not gave any bands in reproductive stage cDNA. It shows that the remaining five primers performing functions like zinc ion binding, translation initiation factor activity, GT Pase activity, translational initiation, ubiquitin-protein transferase activity and membrane activity these are perticularly for vegetative stage and not in reproductive stage. It is concluded that among 10 primers of vegetative stage, five primers were given results in both stages and remaining five primers were specific to vegetative stage only.

In third combination, (maturity stage primers + vegetative stage cDNA) (Figure 4) among 10 primers used only three

primers of maturity stage amplified the cDNA of vegetative stage. It means from those sequences maturity primers were developed responsible for functions like, RNA– dependent RNA polymerase, RNA binding, hydrolase activity, RNA processing, transcription and UBP1-aasociated 2A-like are present in both maturity stage and vegetative stage. The remaining seven primers which does not gave any bands in vegetative stage cDNA. It shows that the remaining seven primers performing functions like RNA–dependent RNA polymersase, methyl transferase, acting on acid anhydrides, mRNA methylation and purine nucleobase metabolic process these are perticularly for maturity stage and not in vegetative stage. It is concluded that among 10 primers of maturity stage, three primers were given results in both stages and remaining seven primers were specific to maturity stage only.

In fourth combination, (maturity stage primers + reproductive stage cDNA) (Figure 5) among 10 primers used only three primers of maturity stage amplified the cDNA of reproductive stage, it means from those sequences maturity primers were developed responsible for functions like, RNA –dependent RNA polymerase, RNA binding, hydrolase activity, RNA processing, transcription are present in both maturity stage and reproductive stage. The remaining seven primers which did not gave any bands in reproductive stage cDNA. It shows that the remaining seven primers performing functions like RNA–dependent RNA polymersase, methyl transferase, acting on acid anhydrides, mRNA methylation these are perticularly for maturity stage and not in vegetative stage. It is concluded that among 10 primers of maturity stage, three primers were gave results in both stages and remaining seven primers were specific to maturity stage only.

In fifth (reproductive stage primers + maturity stage cDNA) and sixth (vegetative stage primers + maturity stage cDNA) did not gave any amplifications in maturity stage cDNA. It revealed that primers of these stages which were specific to reproductive and vegetative stage not in maturity stage.

Zheng *et al*. (2013) [4] they got a total of 10,754 primer pairs were designed for marker development out of these they used 320 primers were synthesized and used for validation of amplification and assessment of polymorphisms in 25 individual plants. The total of 275 primer pairs yielded PCR amplification products. In present study, total 189 primers developed from all the three developmental stage, out of which 10 primers from each developmental stage used for validation purpose in fenugreek variety GMV- 2. Parmar *et al*. (2015) [3] they developed 11 EST–SSR markers from 742 available ESTs of *Withania somnifera* L. EST sequences and 95 SSR primer pairs derived from other solanaceous crops (tomato, eggplant, chili, and tobacco) were utilized for their amplification and validation. Out of 11, 10 EST–SSRs showed good amplification quality and produced 13 loci with a product size ranging between 167 and 291 bp. In present study, validation of SSR markers in fenugreek variety GMV-2, 10 primers from one developmental stage used for amplification of another developmental stage (Primers developed from reproductive stage were checked in cDNA of vegetative stage) in this case, only nine primers of reproductive stage amplified the cDNA of vegetative stage, the remaining one primer did not amplified the cDNA of vegetative stage, means that one primer specific to reproductive stage only.

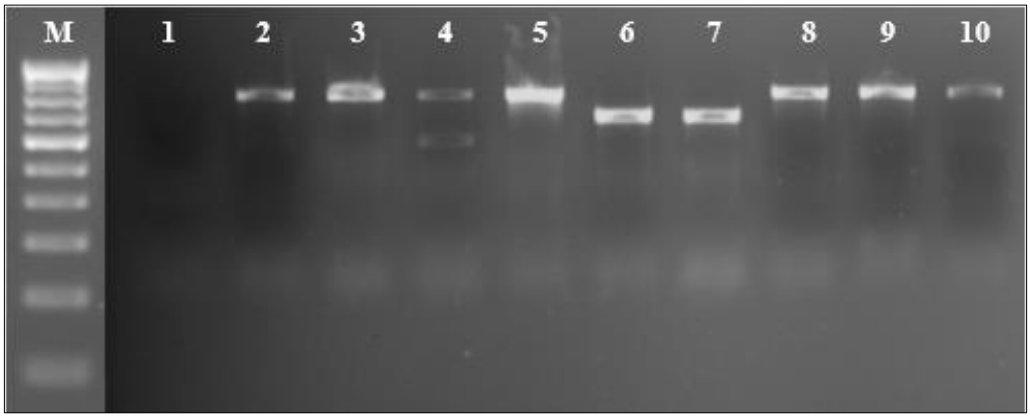


**Fig 2:** Validation image of primers of reproductive stage + cDNA of vegetative stage

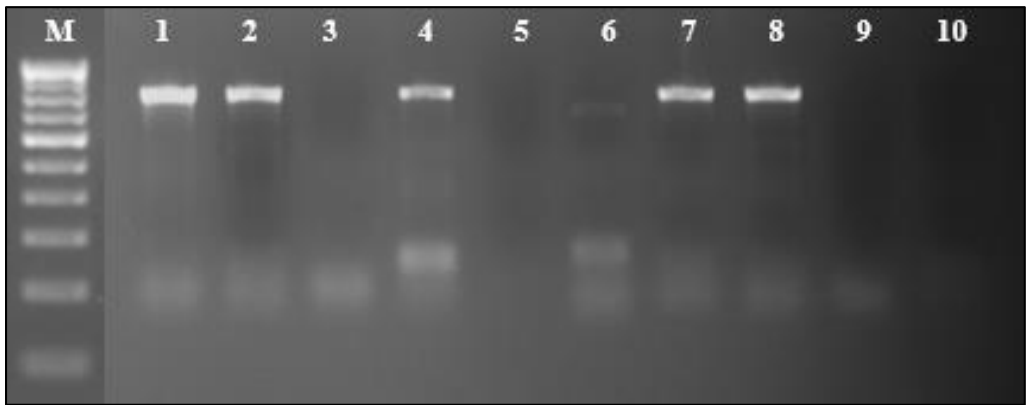


**Fig 3:** Validation image of primers of vegetative stage + cDNA of reproductive stage

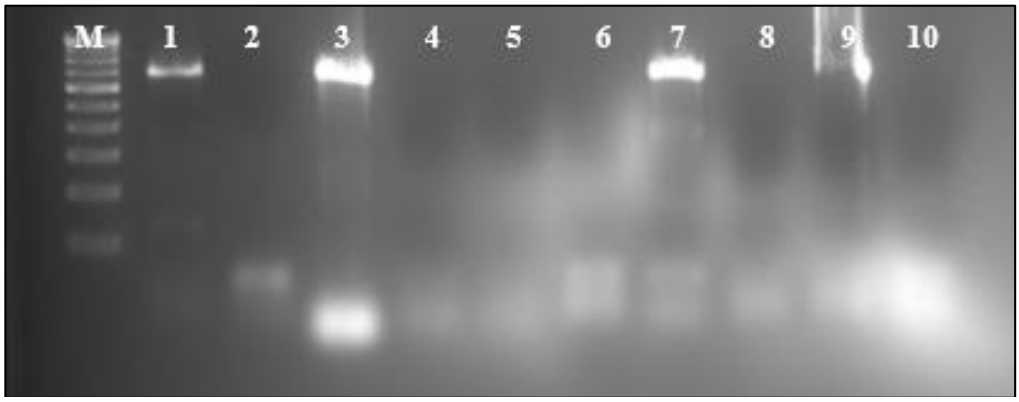


**Fig 4:** Validation image of primers of maturity stage+ cDNA of vegetative stage

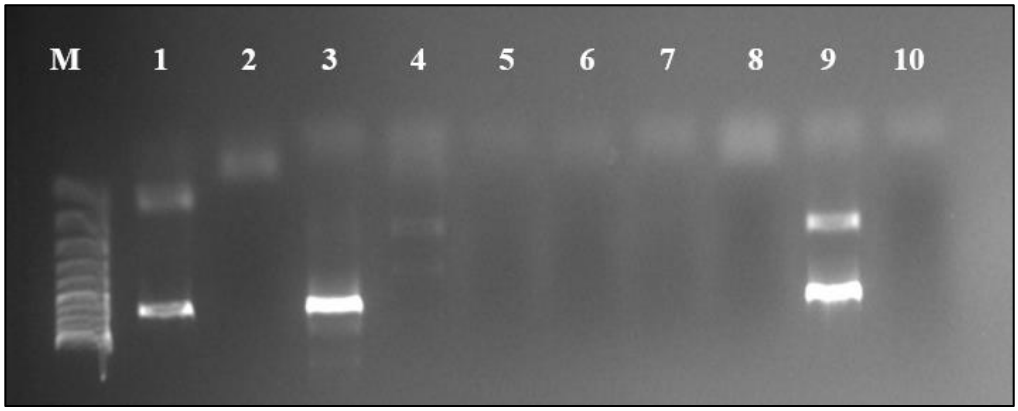


**Fig 5:** Validation image of primers of maturity stage+ cDNA of reproductive stage

**Table 4:** List of SSR markers used for validation purpose from different developmental stages of fenugreek.

| Index | Location or Gene ID | Primer sequence | PL | Temp (ºC) | GC (%) |
|---|---|---|---|---|---|
| **(a) Vegetative stage** | | | | | |
| 1 | Vegetative_(single)_trimmed_contig_32 | GAACCGGGACATTGACTGTT | 20 | 59.83 | 50 |
| | | TGTTGCCACCTTCATCTTGA | 20 | 60.24 | 45 |
| 2 | Vegetative_(single)_trimmed_contig_33 | CAAGTTCCCTGGGGATGATA | 20 | 59.74 | 50 |
| | | CACTTTCGACACCACACCTG | 20 | 60.19 | 55 |
| 3 | Vegetative_(single)_trimmed_contig_37 | GGACGTGGACCTTCTTCTGA | 20 | 60.24 | 55 |
| | | TCGAGATTTGCCCCTAATTG | 20 | 60.03 | 45 |
| 4 | Vegetative_(single)_trimmed_contig_38 | GGCTCAACAACTGACAAGCA | 20 | 60.03 | 50 |
| | | TCTGGACTGTTCCTCATCCA | 20 | 59.18 | 50 |
| 5 | Vegetative_(single)_trimmed_contig_40 | GCATTTGCCAAGGATGTTTT | 20 | 59.94 | 40 |
| | | GTACAAAGGGCAGGGACGTA | 20 | 59.99 | 55 |
| 6 | Vegetative_(single)_trimmed_contig_41 | CAATGGTGGTGCTGAAACTG | 20 | 60.15 | 50 |
| | | CGTGGGCAATGACTCAAAGT | 20 | 61.10 | 50 |
| 7 | Vegetative_(single)_trimmed_contig_42 | GGTGAATCGCTTGATTCGTT | 20 | 60.08 | 45 |
| | | CACTTCAAAACGCCCATACA | 20 | 59.58 | 45 |
| 8 | Vegetative_(single)_trimmed_contig_43 | TGCAAGAAGCAAGATGGCTA | 20 | 59.71 | 45 |
| | | TTCAGAATGACCAGCACTCG | 20 | 59.98 | 50 |
| 9 | Vegetative_(single)_trimmed_contig_44 | TCAGGGAGGAGAAATCCTGA | 20 | 59.73 | 50 |
| | | GTTAGCATGGCCAGGTTGTT | 20 | 60.00 | 50 |
| 10 | Vegetative_(single)_trimmed_contig_46 | CTCAGACGCAGATTCCAACA | 20 | 59.98 | 50 |
| | | ACGAACATGTTTCAGCCACA | 20 | 60.16 | 45 |
| **(b) Reproductive stage** | | | | | |
| 11 | Reproductive_(single)_trimmed_contig_6 | TCAAACAATATGGCCACAGC | 20 | 59.55 | 45 |
| | | AGGAGGTACCAATCCCCAAC | 20 | 60.05 | 55 |
| 12 | Reproductive_(single)_trimmed_contig_7 | TATTGCAATTCGCAAAACCA | 20 | 60.07 | 35 |
| | | CAGTCGAAACGTAGCCATCA | 20 | 59.86 | 50 |
| 13 | Reproductive_(single)_trimmed_contig_8 | CAGCTCATGCTGTTGATGCT | 20 | 60.17 | 50 |
| | | GCCGCTCTATCAAGTTCAGG | 20 | 59.98 | 55 |
| 14 | Reproductive_(single)_trimmed_contig_10 | TGAGAAGGAAAGCAAATTGTGA | 20 | 59.86 | 36.36 |
| | | AGGCTGAAGCTCAGTTGCAT | 20 | 60.16 | 50 |
| 15 | Reproductive_(single)_trimmed_contig_11 | GGTCAAAGTCGCTTCACCAT | 20 | 60.12 | 50 |
| | | CGGGGACGAAGTTAGTAGCA | 20 | 60.26 | 55 |
| 16 | Reproductive_(single)_trimmed_contig_12 | GATCTGGTGGACCGAGTTTT | 20 | 58.99 | 50 |
| | | GGCTGGCCAAACTTATTCAT | 20 | 59.05 | 45 |

| | | | | | |
|---|---|---|---|---|---|
| 17 | Reproductive_(single)_trimmed_contig_13 | CATCCAATGGAGATGGTGGT | 20 | 60.60 | 50 |
| | | GCCGGCCAAACTTATTCAT | 20 | 59.91 | 47.37 |
| 18 | Reproductive_(single)_trimmed_contig_14 | TCACATGCCATCTTCACCAT | 20 | 59.93 | 45 |
| | | AGGCTCCTTTTAAGCCCATC | 20 | 59.69 | 50 |
| 19 | Reproductive_(single)_trimmed_contig_15 | GCCACTTGCACTTCTCATCA | 20 | 59.99 | 50 |
| | | CATCAGCATCTCCGTCAAGA | 20 | 59.94 | 50 |
| 20 | Reproductive_(single)_trimmed_contig_23 | AATTTTAGGGGACGGCATTT | 20 | 59.68 | 40 |
| | | GCTCTGAGAATCCCTCAACG | 20 | 59.95 | 55 |
| **(c) Maturity stage** | | | | | |
| 21 | Maturity_(single)_trimmed_contig_2 | TCCAACGAGCTTCCACTTCT | 20 | 59.99 | 50 |
| | | TGGAAGATGGGTTGATAGGG | 20 | 59.74 | 50 |
| 22 | Maturity_(single)_trimmed_contig_3 | CGGTATAGAGGCTGCTGAGG | 20 | 59.99 | 60 |
| | | ACAGTTTTGCAGCGCCTATC | 20 | 60.42 | 50 |
| 23 | Maturity_(single)_trimmed_contig_7 | GCTGCAGTTAAGGGAAGCTG | 20 | 60.15 | 55 |
| | | TGCATTACCGGGACTAGGAG | 20 | 60.09 | 55 |
| 24 | Maturity_(single)_trimmed_contig_23 | GCATCCAAAAGGGAGGGTAT | 20 | 60.15 | 50 |
| | | GCTACTACCAGAGGCCAACG | 20 | 59.90 | 60 |
| 25 | Maturity_(single)_trimmed_contig_64 | TTCCCGATACCAAAGGAACA | 20 | 60.30 | 45 |
| | | TTGGACCACGAATAGGTTGG | 20 | 60.74 | 50 |
| 26 | Maturity_(single)_trimmed_contig_73 | AAGAGCCTCCTTCCAATGGT | 20 | 60.07 | 50 |
| | | ATTTCCTTGGAGCGGAAAGT | 20 | 60.07 | 45 |
| 27 | Maturity_(single)_trimmed_contig_74 | ACAAGGAGACATGCCCTCAG | 20 | 60.26 | 55 |
| | | CCCCTTGTTGTCCAAGATGT | 20 | 59.82 | 50 |
| 28 | Maturity_(single)_trimmed_contig_75 | GGATCAGGACGAGATTCCAA | 20 | 60.01 | 50 |
| | | CCCTCTGCTCCTTGATTCTG | 20 | 59.94 | 55 |
| 29 | Maturity_(single)_trimmed_contig_77 | AGGGAACTTCTCGCTGAACA | 20 | 59.99 | 50 |
| | | CACCCCCAACAGGATATACG | 20 | 60.07 | 55 |
| 30 | Maturity_(single)_trimmed_contig_78 | GTGGTCCCATGGGTAGAATG | 20 | 60.05 | 55 |
| | | TCCCATTCATCATCATGCTC | 20 | 59.41 | 45 |

* PL: Primer length, Temp: Temperature

## 4. Conclusion

Fenugreek (*Trigonella foenum-graecum* L.) is extensively used as a spice crop in India and the Mediterranean region and is known to possess a number of medicinal properties. Steroidal sapogenins and mucilaginous fibers present in the seed and leaves of this legume plant contribute to anti-diabetic and hypocholesterolaemic properties attributed to the plant. Microsatellites are the important resource for determining functional genetic variation. SSRs were identified by using Batchprimer3 bioinformatic tool. The SSRs containing sequences (fasta file) which generated after assembly were analyzed for NCBI-BLASTn to get the hits. The developed molecular markers are foundation for further genetic linkage analysis and gene localization and they will be essential to accelerate the process of breeding.

The data generated from present study will be usefull to accelerate the breeding programme in *Trigonella foenum-graecum* L. It is a viable alternative source for different metabolites like, isoleucine, sapogenins and galactomannans production. Transcripts generated in the present study will be useful in the understanding different metabolites processes and formation. The similarity distribution showed, maximum similarity of *Trigonella foenum-graecum* L. with *Medicago truncatula*, *Medicago ipaensis*, *Cicer arietinum*, *Arachis ipaensis*, *Arachis duranensis* and *Glycine max*. This transcriptomic information will be useful in genetic engineering of *Trigonella foenum-graecum* L.

## 5. References


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