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# DNA Fingerprinting of CUMS 17 (Suprava): A Newly Developed Variety of Sesame

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**ABSTRACT**

*The variety CUMS 17 (Suprava) has recently been developed in sesame by Genetics and Plant Breeding department, University of Calcutta. The variety is recommended by central varietal release committee in 2018. The variety CUMS 17 along with two check varieties namely GT 10 and Savitri were analysed by 21 SSR primers. Fourteen polymorphic SSR primers were finally used for DNA fingerprinting. The SSR primers produced a total of 46 alleles with an average number of 3.28 alleles with amplicon size ranging from 100 to 600 bp. Alleles were assigned with numeric numbers in the order of increasing size of amplicon that produced an allelic barcode that denotes the unique identification of the newly developed variety of sesame.*

***Key words:*** *DNA Fingerprinting, Microsatellite markers, Variety identification, Sesame.*

## INTRODUCTION

Sesame (*Sesamum indicum* L., Chromosome number is 2n=26) belonging to the family Pedaliaceae, is one of the oldest and important oilseed crops for its high quality of seed oil. It is known as "Queen of oil seed"[15] due to the presence its high seed oil content (44%–63%), and also excellent qualities of the seed oil like protein (18%–25%), and carbohydrates (13.5%) and meal[2]. Not only that, but also it is rich in minerals (copper, magnesium and calcium) niacin, and lignans (methylenedioxyphenyl compounds), such as sesamin, sesamol, sesamolin and tocopherols[4], and it has a great medicinal value. Sesame seeds are used in large number of confectionary products and sweets. Besides, sesame seeds and oil have industrial, nutraceutical and pharmaceutical uses. Molecular markers provide a way to identify unique DNA profiles without any biasness for the protection of new cultivars. Microsatellites or simple sequence repeat has proved its unequivocal supremacy because of high abundance and reproducibility, easy scoring, extensive coverage and co dominant nature[9]. SSR marker helps to detect multiple allele for a given locus which makes them ideal for genetic diversity dynamics[12].

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It showed polymorphism between sesame genotypes[5,7,13,1,9]. Expressed sequence tags-SSR (EST-SSR) is now also available to a considerable extent[16,17,3,9]. A newly variety named as CUMS 17 has been developed by inducing the genotype IC 21706 with Ethyl Methane Sulphonate (EMS) 0.5 %. The variety has ranked top in AICRIP on sesame trials in IVT, AVT 1 and AVT 2 over years. The average yield of the variety is 1.5 t/ha and oil content over 51%.

The objective of the present study is DNA fingerprinting of a new elite sesame variety CUMS 17 (Suprava) using SSR markers. The variety developed by Department of Genetics and Plant Breeding, Institute of Agricultural Science, University of Calcutta and recommended by Central Variety Release Committee of ICAR in 2017. The National Bureau of Plant Genetic Resource Centre has assigned the number IC 625735 for the variety in 2018. Suprava exhibited 25% seed yield superiority over the best national check TKG 22. It is identified for summer in the states of West Bengal, Odisha, Maharashtra, Chhattisgarh, Telangana, Tamil Nadu and Karnataka.

## MATERIAL AND METHODS

The national check variety GT 10 and the zonal check (West Bengal) of sesame variety Savitri along with induced mutant sesame variety CUMS 17 (Suprava) were used for microsatellite analysis and DNA fingerprinting. For genomic DNA we used leaf of germinated seeds (7-10 days) of three sesame genotypes. Using liquid nitrogen 0.5g of leaves was grounded and DNA was extracted by CTAB method Saghai-maroof *et al.*[12]. Twenty one SSR markers were tested for DNA fingerprinting of three sesame variety. DNA amplification was performed in 25 µl reaction mixture containing 50 ng genomic DNA, 0.2 µmolL−1 SSR primers, 10X PCR buffer, 50 mM MgCl2, 0.2mM dNTPs and 0.5 units *Taq* DNA polymerase. The PCR amplification was performed using a Eppendorp thermo cycler, with the following cycle profiles: denaturation at 95 °C for 5 min, in 38 cycles 30 seconds at 95°C, 45 seconds at the annealing temperature depending on particular primer pair (~55$^{o}$C), 1min at 72°C (extension) and final extension at 72°C for 5 min. After completion of PCR, the amplicons were separated through electrophoresis in 2% agarose gel prepared with 1x TAE buffer at 120 volts and stained with ethidium bromide (Biorad, USA). The size of amplified DNA was identified by comparing the band with respect to the molecular weight of a DNA ladder (50 base pair (bp) DNA ladder, Promega).

**Table 1: Sesame genotypes used in the present study**

| Variety | Origin | Release of year | Seed coat colour | Pedigree |
|---|---|---|---|---|
| GT-10 | Gujarat | 2002 | Black seed, | Selection from TNAU17 |
| SAVITRI | West bengal(PORS Govt of West Bengal) | 2008 | Light brown seed, | Selection from Germplasm SWB32 |
| CUMS17 (Suprava) | West Bengal(C.U Developed ) Identified by CVRC in 2018. | Recommended by Central varietal Identification Committee of ICAR in 2017. | Light brown seed, | Induced mutation by EMS ( 0.5%) on IC21706 |

**Table 2: List of primers used for SSR analysis used in the present study**

| Sl No. | Name | Primer Sequence | LG |
|---|---|---|---|
| 1 | CUSSR1 | F: CAAGCGTAGAAACAAATCAAC<br>R: AGCTCCCAATCTATTCACTTC | 1 |
| 2 | CUSSR16 | F: TTGTGGATTGTAAGCTATTCC<br>R: GTGACAATTCTTGCTCGTAAT | 2 |
| 3 | CUSSR17 | F: CTGCTTCTCTCTCATGCATAC<br>R: AACATGATCGAAAAGAAAACC | 2 |
| 4 | CUSSR30 | F: AGGAGAAAACACTCAAAGAGG<br>R: GTTTTGCAGAGCAGAGTAGAA | 3 |

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| | | | |
|---|---|---|---|
| 5 | CUSSR18 | F: CAAAACCCCCATCTATCTATC<br>R: TTAGTAGGACGTGGGTGAATA | 2 |
| 6 | CUSSR13 | F: AGAGGAATTCACAGTCCTTTC<br>R: CTTGTGTGCTTCTTTTTGAGT | 1 |
| 7 | CUSSR3 | F: TAACACTTCCACACACACACA<br>R: CACATGACCTTTCACCATAAT | 1 |
| 8 | CUSSR27 | F: AAGAAGAAAGCAAACCTTGAC<br>R: TATTCAGCATATTCCCTCTCC | 2 |
| 9 | ESTSSR 07 | F: AATTACCCACAAAAAGAATCC<br>R: AATTACCCACAAAAAGAATCC | Not available |
| 10 | ESTSSR 08 | F: ACTCTCCTCTTCAACCTTCAC<br>R: GAAGAGGTGGAGGAATTACG | Not available |
| 11 | ESTSSR 02 | F: AAGAAAGCTAAGAAGGCAGAG<br>R: GCTTGATAGAGAAGTTACGACA | Not available |
| 12 | ESTSSR 33 | F: TAATTCGCAAGGATTAAGAGA<br>R: GGTCCATGTGATATTCGTGTA | Not available |
| 13 | ESTSSR 11 | F:ATGCAAAAATACACACACACA<br>R: CGCCACATTTTATGCTTATT | Not available |
| 14 | ESTSSR 12 | F: TTGTCAAAGTCAAGAGTTCGT<br>R: TCTTATCCTTGCTAACAGCAG | Not available |

## RESULTS AND DISCUSSION

Twenty one SSR markers were tested for DNA fingerprinting of three sesame variety. The microsatellite markers displaying non-specific bands, without polymorphism or without bands were discarded. Finally, fourteen polymorphic primers were selected in the DNA profile analysis. Among fourteen polymorphic primers, six are EST-SSRs and rest eight are genomic SSR markers. All of these markers amplified successfully and showed polymorphism among three sesame genotypes (GT 10, Savitri, CUMS 17 (Suprava). The size of amplified DNA was estimated by using the DNA Ladder (50bp – 1500 bp. In total of 46 alleles with an average number of 3.28 alleles were observed in three sesame genotypes which suggesting a high range of polymorphism present. The number of alleles per microsatellite locus varied from 2 to 5. The overall size of the amplified product varied from 100 bp (SSR 1, SSR 6) to 600bp (SSR 12) for this experiment (table 2). Primers with allele numbers greater than the average (3.28) were SSR 1, SSR 10, SSR 12, SSR 13. Banding patterns generated by markers in three sesame genotypes are shown in table 2.

### Construction of DNA barcode

After genotyping the varieties with 14 SSR primer pairs, alleles produced at each locus were designated as 1, 2, 3…following the order of increasing size of the amplicons generated. In case of SSR 2 the alleles generated were of amplicon size 160, 170 and 190 bp which were assigned 1, 2, and 3, respectively. Based upon the amplicon profile produced by genotyping three varieties using 14 primer pairs, an allelic barcode for each variety was finally constructed (Table 4). For this purpose, the designated number of each allele for 14 primer pairs was placed from left to right and digits from left to right corroborated to the allele at loci SSR1, SSR2, SSR3, SSR4, SSR5, SSR6, SSR 7, SSR8, SSR 9, SSR10, SSR 11, SSR12, SSR13 and SSR14. This procedure of constructing barcodes was in line with the molecular identity code suggested by Gao *et al.*[6] and Rani *et al.*[11]. The barcode of two varieties itself would reflect how closely the varieties are related to each other[11]. GT 10, CUMS 17 and Savitri these three sesame varieties have different parentage which can be distinctly distinguished from each other by their barcodes.

**Table 3: DNA barcodes of three Sesame varieties using 14 SSR markers**

| Variety | Barcode |
|---|---|
| GT 10 | 12332233232231345142 |
| CUMS 17 | 23121312131114213523513 |
| Savitri | 421112123210124011 |

The variety CUMS 17 (Suprava) was clearly distinguishable from GT 10 and Savitri by the following four markers: the microsatellite marker SSR 3 amplified band of 170 bp in CUMS 17 while GT 10 and Savitri, the national and zonal check varieties produced 190 bp and 160 bp band respectively. The microsatellite primer, SSR 7 generated a single band of 150bp in CUMS 17 while the band size of GT 10 and Savitri were characterized by 168 bp and 140 bp respectively. The primer, SSR 10 produced 150 bp and 370 bp in the variety GT 10 while it generated two bands of 145 bp and 400 bp in the variety CUMS 17. Interestingly the variety Savitri shared the same 145 bp band of CUMS 17. In the loci SSR 13 exhibited three distinct bands were observed in CUMS 17 having the size 160bp, 220bp and 350 bp while in Savitri only one band of size 150bp was observed. In the variety GT 10, two clear bands (240bp and 150bp) were recorded.

**Table 4: Results of gel electrophoresis in allelic size (basepairs)**

| Sl No. | Primer name | Variety | | | No. of Allele(A) |
|---|---|---|---|---|---|
| | | GT 10 | CUMS 17 | Savitri | |
| 1 | CUSSR1 | 100,140 | 140,160 | 150 | 4 |
| 2 | CUSSR16 | 150 | 140 | 145 | 3 |
| 3 | CUSSR17* | 190 | 170 | 160 | 3 |
| 4 | CUSSR30 | 160 | 150 | 150 | 2 |
| 5 | CUSSR18 | 115 | 120 | 110 | 3 |
| 6 | CUSSR13 | 120 | 100 | 110 | 3 |
| 7 | CUSSR3* | 168 | 150 | 140 | 3 |
| 8 | CUSSR27 | 150,175 | 130,175 | 150,175 | 3 |
| 9 | ESTSSR 07 | 160 | 150 | 160 | 2 |
| 10 | ESTSSR 08* | 150,375 | 145, 400 | 145,0 | 4 |
| 11 | ESTSSR 02 | 250 | 280 | 250 | 3 |
| 12 | ESTSSR 33 | 160,300,600 | 140,300,600 | 150,300,0 | 5 |
| 13 | ESTSSR 11* | 240, 150 | 220, 160,350 | 150 | 5 |
| 14 | ESTSSR 12 | 300 | 290, 320 | 290 | 3 |

*Distinguishable polymorphic markers

The results demonstrated that microsatellite based fingerprinting can identify sesame genotypes unambiguously, which is needed for cultivar identification and notification. In sesame, still very few researches have been done on DNA fingerprinting[8]. Our results represent the attempts to find out a set of microsatellite makers to discriminate the sesame genotypes. The SSR markers under this study can lead towards marker-assisted selective breeding in future sesame breeding program. One of the major advantages of microsatellites is that microsatellite data can be easily compared among laboratories and are suitable for computer databases, which is not always the case with other markers, such as

RAPD[10]. Microsatellites are considered appropriate for variety identification because of their ability to detect large numbers of discrete alleles repeatedly, accurately and efficiently[14].

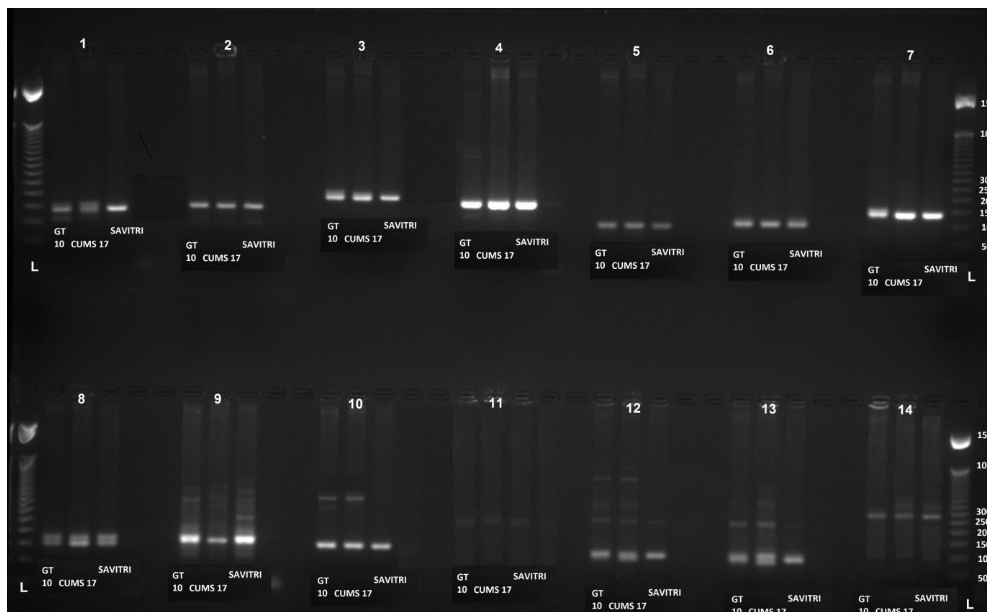


**Fig. 1: DNA fingerprinting profile of three sesame varieties based on fourteen microsatellite markers. (L=50bp DNA ladder)**

## CONCLUSION

In the analysis of microsatellite markers, three sesame genotypes revealed a high level of genetic polymorphism which allowed unique genotyping of the three genotypes. Thus the set of microsatellite markers used here provides a discernible assessment to the ability of SSR marker to produce unique DNA profiles of sesame genotypes and the variety CUMS17 (Suprava) was clearly distinguishable from GT 10 and Savitri by SSR markers under this study.

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