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# Diversity in snake gourd (*Trichosanthes anguina* L.) genotypes based on morphological traits and EST-SSR markers

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## ABSTRACT

*A study was conducted to assess both the morphological and molecular diversity of thirty-two snake gourd genotypes. Mahalanobis $D^2$ analysis were used to group the genotypes into four major clusters. Out of four clusters, cluster I was the largest, comprising seventeen genotypes followed by cluster II with ten genotypes, cluster III with four genotypes and cluster IV with only one genotype, suggesting the existence of divergence. The highest inter-cluster distance was found in clusters III and IV followed by clusters I and III. Genetic divergence using Expressed Sequence Tags derived Simple Sequence Repeats (EST-SSR) markers revealed that, a high level of genetic diversity was recorded with a total of 21 alleles with a mean of 2.33 alleles per locus. Polymorphic Information Content (PIC) varied from 0.94 to 0.97 with an average of 0.96. The polymorphic EST-SSR markers could be useful in genetic fingerprinting and association analysis of yield and yield-related characters in snake gourd. The complexity of the genetic makeup of snake gourd, coupled with the limited availability of genetic resources for this crop, made accurate assessment of a demanding task. However, despite these challenges, the study yielded insightful results. The findings of this study provide valuable information on genetic structure of snake gourd population and offering a foundation for future breeding programs aimed at enhancing yield and yield attributing traits.*

***Keywords:*** *Snake gourd, Genetic diversity, Molecular marker, Polymorphic information content, Dendrograms.*

## INTRODUCTION

Snake gourd (*Trichosanthes anguina* (L.) 2n=2x=22) is an annual, day-neutral, herbaceous and climbing-type vegetable crop. It belongs to family Cucurbitaceae, sub-family cucurbitoideae tribe Trichosantheae. It is originated in Indo-Malayan region. The wild species of *Trichosanthes* is considered as the progenitor of *Trichosanthes anguina*. It is widely distributed in Asia, extending through Malaya to North Australia in one direction and through China and Japan in another. In India, snake gourd is commonly cultivated in South India and also grown in Punjab, Delhi, Uttar Pradesh, Bihar, Gujarat and other parts of the country. It is a good source of minerals, fibre and nutrients to make the food wholesome and healthy [1]. It contains a considerable amount of protein (0.5%), fat (0.3%), minerals (0.5%), fibre (0.5%) and carbohydrates (3.3%). The predominant mineral elements are potassium (121.6mg/100g), phosphorus (135mg/100g), sodium, magnesium and zinc [9]. Genetic diversity is an important factor for heritable improvement in any crop.

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The knowledge about nature and degree of divergence in existing germplasm is extremely valuable in identifying suitable parental combinations to create heterotic hybrids [7]. It is well recognized that the use of diverse parents results in superior hybrids and desirable recombination. DNA based markers have been developed and accepted for the assessment of genetic diversity in crop species. Hence, the marker system reveals difference at the DNA level and they are not affected by environmental conditions. The genetic distances among the germplasm could be estimated with high precision by using DNA-based markers. Several molecular markers viz., RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism) and Simple Sequence Repeats (SSR). SSR is an excellent molecular marker system with the advantage of being codominant, abundant, highly reproducible, highly polymorphic and easy to assay. Expressed sequence tag derived simple sequence repeat (EST-SSRs) markers were selected for the present study as they have high levels of polymorphism and easily adaptable to automation. In this context, the present study was carried out with the objective to study the magnitude of morphological and molecular diversity among the snake gourd genotypes.

## MATERIAL AND METHODS

An experiment was conducted at Dr. YSRHU - College of Horticulture, Venkataramannagudem during *Kharif* season

2021-22. The experimental site was geographically situated at 27° 12' 16.6" N latitude and 77° 29' 51.7" E longitude. Soil in the experimental site is red sandy loamy.

A total of thirty-two snake gourd genotypes were collected from National Bureau of Plant Genetic Resources (Thrissur), Kerala Agricultural University (Thrissur), Tamilnadu Agricultural Univesrity (Coimbatore and Periyakulam) and local collections from Andhra Pradesh. Genotypes were sown at a spacing of 2 m x 1.5 m in a randomized block design with two replications. Standard cultural practices and plant protection measures were adopted uniformly to all the treatments to raise a healthy crop. Five plants were selected randomly from each genotype to record observations on twenty characters viz., days to first male flower opening, days to first female flower opening, node to first male flower, node to first female flower, number of male flowers per vine, number of female flowers per vine, sex ratio (%), fruit set (%), fruit length (cm), fruit girth (cm), average fruit weight (g), flesh thickness (cm), number of seeds per fruit, number of fruits per vine, fruit yield per vine (kg), vitamin C content (mg/100g), acidity content (%), potassium (mg/100g), TSS ($^{0}$ Brix) and fibre content (g/100g).

**Genomic DNA extraction and quantification**

Genomic DNA was isolated from the leaf tissue of each genotype using CTAB method. The detailed protocol and the steps involved in CTAB method of DNA isolation was initially given by [8] with slight modifications. A 200 mg of leaf sample was made into small pieces and placed in a pestle and mortar and added 400l of extraction buffer (4 ml 0.5 M EDTA (pH8.0), 10 ml 1 M Tris (pH 8.0), 28 ml 5 M NaCl, 1g PVP-40 and 2 g CTAB). The contents were crushed using an alcohol sterilized blunt-ended glass rod to ensure that the leaf pieces were completely grounded. Another 400l of extraction buffer was also added into it. Entire extract was transferred into a 1.5ml capacity micro centrifuge tube and incubated at 60°C for 40 minutes and cooled at room temperature. An equal volume (800l) of chloroform was added to the contents and well mixed by inverting the tube for about 5min and centrifuge at 12,000 rpm for 15min at room temperature.

After centrifugation, the supernatant was separated from the micro-centrifuge tube and was transferred into another micro-centrifuge tube (1.5ml) ensured that the intermediate layer of insoluble proteins has not disturbed. Equal volume of chloroform:iso amyl alcohol (24:1) was added to the micro centrifuge tube. Again, Centrifugation has done at 12,000 rpm for 10min at room temperature. Top aqueous phase was transferred to a fresh centrifuge tube and 0.6 µl of iso-propanol was added into it. Later, centrifugation has done at 12,000 rpm for 5 min at room temperature. Two volumes of cold 95 % ethanol were added until DNA strands begin to appear. Centrifugation had done at 12,000 rpm for 10 min at room temperature. The supernatant was decanted and washed the pellet with cold 70 % ethanol and air dried for 15-20 minutes. After air-drying, the pellet was dissolved in 50l of sterile 1X TE buffer at room temperature and DNA was stored at -20ºC for long-term preservation. The spectrophotometer (Nanodrop 8000, Thermo Scientific) reading was taken at 260 and 280 nm for each sample to estimate the concentration of DNA.

**PCR amplification based on EST-SSR markers**

DNA samples were subjected to PCR amplification using snake gourd specific EST-SSR markers. PCR reaction mixture was prepared in 25µl volumes containing 16.55µl of deionized nuclease free water, 2.0 µl of 10X PCR buffer, 0.5 µl of 2 mM dNTPs, 0.75µl of 50mM $MgCl_2$, 1.0µl of forward primer (15pmol/µl), 1.0µl of reverse primer (15pmol/µl), 0.2µl of Taq DNA polymerase (5U/µl) and 3.0µl sample DNA template (50ng). PCR tubes containing the master mix and DNA template with each set of primers were thoroughly mixed and subjected to master cycler (nexus gradient, Eppendorf). Initial denaturation was carried out at 94°C for 2 minutes followed by a loop of 35 cycles each consisting of denaturation (at 94ºC for 30seconds), annealing (specific annealing temperatures for each primer (Table 1) for 1 minute) and extension (72 °C for 2 minute). The final extension was performed at 72 °C for 7 minutes. Then, the PCR products were stored at 4 ºC.

Amplified DNA fragments were electrophoresed in 3 per cent agarose gel as per the procedure outlined by [13].The frame of the gel casting unit was cleaned and sealed with tape to form a mold. The frame was placed on a flat platform to ensure a flat and level base and then the comb was positioned parallel to the open edge of the frame about 2 mm above the surface. Agarose powder was added to TBE buffer (1X) and then dissolved by melting at 100°C. Later, the solution was cooled to 50°C and 10µl of ethidium bromide (10 mg/ml final concentration) was added to the gel and then poured into the gel frame and allowed to solidify. After solidification of the gel, it was transferred to the gel tank such that the wells were towards the negative pole. The gel tank was filled with TBE buffer just enough to cover the surface of the gel. Then, 10µl of the PCR product was mixed with 2µl of (6X) loading dye and loaded to the wells of submerged gel along with marker DNA using a micropipette. The electrophoresis apparatus connected to the power supply and electrophoresis was carried out with the help of at 70 V for 2 hours or up to deep blue dye migrated to the end of the gel. It was then visualized and documented by gel documentation system (Major science).

**Statistical analysis**

Genetic diversity in thirty two genotypes were analysed through Mahalanobis $D^2$ analysis and genotypes were grouped into various clusters by following Tocher's method. The average inter-cluster and intra-cluster distances were calculated by the formula suggested by [15]. The cluster analysis was performed for SSR similarity matrix using UPGMA algorithm, from which dendrograms depicting similarity among accessions were drawn and plotted using NTSYS-pc.2.11 a Software [12].

## RESULTS AND DISCUSSION

One of the current methodologies of determining genetic divergence is by Mahalanobis $D^2$ stastic. This technique calculates the force of differentiation at intra and inters cluster levels and thus provides a reasonable basis for selection of genetically divergent parents in breeding programmes. However, it is desirable to select suitable genetically divergent parents based on information about the genetic variability and diversity present in the available germplasm.

Thirty-two snake gourd genotypes were subjected to $D^2$ statistic based on twenty characters. These genotypes were grouped into four clusters (Fig 1 and Table 2) based on $D^2$ values using Tocher's method [11]. Out of four clusters, cluster I was the largest, comprising 17 genotypes followed by cluster II with 10 genotypes, cluster III with four genotypes and clusters IV with only one genotype (mono-genotypic cluster), suggesting the existence of heterogeneity among genotypes. The inter-cluster distance was higher than the intracluster distance indicating the

presence of average genetic diversity among the genotypes under study. The intra-cluster $D^2$ values varied from 0.00 to 695.66 (Fig 2 and Table 3). The intra-cluster distance of cluster IV was zero because only one genotype was included. The maximum inter-cluster distance was found in clusters III and IV (1638.06) followed by clusters I and III (1566.77). The minimum inter-cluster distance was recorded between cluster II and cluster III (917.70) [16].

The cluster means for various quantitative characters have been presented in Table 4. Cluster I included the genotypes with the lowest days to first female flower opening (59.32). Cluster II included the genotypes with the highest fruit set percentage (75.83) and lowest sex ratio (16.01). Cluster III included the genotypes with the highest fruit length (52.26), number of seeds per fruit (63.38) and fruit yield per vine (6.15). Cluster IV included the genotypes with the highest number of female flowers per vine (28.10) and lowest node to first female flower (8.20). The trait's Potassium content showed the highest contribution towards divergence by ranking first with a contribution of 63.91% followed by vitamin C content (10.69%), number of seeds per fruit (6.85%) and fruit yield per vine (6.05%). Thus, the traits *viz*., potassium content, vitamin C content, number of seeds per fruit and fruit yield per plant alone contributed ninety percent of diversity (Table 5) [17].

**Molecular Diversity**

EST-SSRs offer many benefits for identifying variation because of their high degree of polymorphism and random distribution across the genome [1]. Thirty two snake gourd genotypes were evaluated for their genetic diversity at the molecular level using 20 Expressed Sequence Tags derived Simple Sequence Repeat (EST-SSR) markers. Out of 20 primers used, 18 primers amplified (90.00%) while two primers (EST-SSR-3 and EST-SSR-4) were failed to amplify any product (10.00%) and therefore were not considered for further analysis. Among 18 amplified primers, 09 primers (45.00%) amplified with varying degrees of polymorphism, while nine primers (45.00%) were monomorphic. The amplification and banding pattern of EST-SSR markers in all the 32 snake gourd genotypes are illustrated in figure3. The bands were scored by using 100bp ladder for assessing the genetic diversity based on the polymorphism. Banding profiles obtained with EST-SSR primers were analyzed based on the presence or absence of the band. The nine EST- SSR primers detected a total of 21 amplicons (alleles) with a mean of 2.33 alleles per locus which were comparable with the values obtained by [3] in bitter gourd. A study [10] in ridge gourd found a greater number of alleles per primer (with a mean of 3.52 alleles per locus) than our results.

The major allele frequency ranged from 23.43% to 96.87% with a mean of 62.78%. Genetic diversity/relatedness among the genotypes was assessed based on Polymorphic information content (PIC) value. PIC value for each EST-SSR primer was calculated and it was ranged from 0.94 to 0.97 with an average of 0.96 (Table 6). Similar results were found by [10] in ridge gourd, [4] in bitter gourd and [2] in sponge gourd. The average PIC is an ideal index to measure polymorphism. A PIC value was greater than 0.50 indicates high polymorphism, values between 0.25 and 0.50 indicates loci of intermediate polymorphism and less than 0.25 indicate loci of low polymorphism [5].

The scored data were used for the estimation of pair-wise Jaccard's similarity coefficients [6] among these genotypes using NTSYS-pc version 2.11a to establish the genetic relationship (Table 7). The average genetic similarity among thirty-two snake gourd genotypes ranged from a coefficient of 0.22 to 0.93. The wide range of Jaccard's similarity coefficient values (0.22 to 0.93) suggested that the germplasm collection represents a genetically diverse population. Among the genotypes studied, PKM-1 was found to have the lowest similarity index (0.22) with IC-410160 indicated the maximum genetic divergence.

The similarity matrix values obtained for thirty two snake gourd genotypes were subjected to the cluster analysis using the Unweighted Paired Group Method using Arithmetic Averages (UPGMA) approach and a dendrogram was generated based on genotypic differences (Table 8 and Figure 4). These genotypes were grouped into eight clusters. The genotype (PKM-1) was found to be distinct with a low similarity coefficient value from all the other genotypes and was accommodated in cluster II.

The present study of EST-SSR markers in the diversity analysis of snake gourd genotypes observed that 9 polymorphic EST-SSR markers were found to be effective to differentiating some significant accessions and certainly could be useful for certification of varieties and development of high-yielding varieties.

The genotypes *viz*., IC-418496, IC-202157 and IC-212513; Madiki local, IC-277390, IC-426984, IC-284875 and Kaumudhi; IC-284753, IC-410142 and IC-347377 and Manusree and IC-212483 were closely placed in dendrograms generated by both morphological and molecular diversity analysis whereas the remaining genotypes did not match. The majority of the characters are regulated by large number of genes (polygenes) and these are heavily modified by the environment, which may be the primary cause of the discrepancy between clustering based on molecular markers and morphological traits. The clustering pattern obtained in this study would be stable even in the addition of newer markers and there is a less chance of change in this grouping pattern. The present data provide adequate evidence of the applicability of EST-SSR markers for diversity analysis, cultivar identification and characterization of the snake gourd germplasm.

## CONCLUSION

In the present study, dendrograms revealed that the close placement of certain genotypes (IC-418496, IC-202157, IC-212513, Madiki local, IC-277390, IC-426984, IC-284875, Kaumudhi, IC-284753, IC-410142, IC-347377, Manusree, IC-212483) based on both morphological and molecular traits. However, other genotypes did not align. Environmental influences on polygenic traits may explain discrepancies between molecular and morphological clustering. Notably, genotypes IC-308557, Manusree, IC-212513, IC-347377 and PKM-1 exhibited desirable characteristics at molecular and phenotypic levels, indicating potential for snake gourd improvement. The study underscores the utility of EST-SSR markers for snake gourd diversity analysis and germplasm characterization, suggesting the avenues for varietal or hybrid development.

**Statements & Declarations**

This work was supported by [Dr. G Kranthi Rekha]. Author M. Jayanth kumar has received research support from Dr. Y.S.R Horticultural University. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [M.jayanth kumar] and [Dr.G. Kranthi Rekha]. The first draft of the manuscript was written by [M.jayanth kumar] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

***Table 3. Cluster distances of snake gourd genotypes***

| Cluster | I | II | III | IV |
|---|---|---|---|---|
| I | **421.54** | 1114.39 | 1566.77 | 924.49 |
| II | | **325.33** | 917.70 | 1308.65 |
| III | | | **695.66** | 1638.06 |
| IV | | | | **0.00** |

**Future scope of the study**

Screening of genotypes can be undertaken against important pests and diseases. The genotypes of cluster III and cluster IV showed maximum genetic diversity. Hence, crosses between these genotypes are likely to produce new recombinants with desired traits. Among 20 EST-SSR markers, 09 were found to be polymorphic. Probably these markers could be useful for certification of varieties and development of high yielding varieties, diversity analysis and characterization of snake gourd improvement.

**Conflict of Interest**

The authors declare that there is no conflict of interest for this study.

**Acknowledgements**

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***Table 1. Sequence information expected product size and annealing temperature of the EST-SSRprimers used in the present study.***

| S.No. | Forward primer | Reverse primer | Product size (bp) | Annealing Temperature (°C) |
|---|---|---|---|---|
| 1 | CTCCCACTCTCTCTCTCTCTGTCT | ACTCCCATTCACAAGTTTACCG | 275 | 55.1 |
| 2 | CTGAGAATGTGGGAGCAAAAC | GATACCAAGCATAGCAGAACCC | 386 | 56.5 |
| 3 | GAGAAGAGAGCAAGAC CGAGAC | AGGCACTTGACAGAGACGACTT | 386 | - |
| 4 | GTTGGAGTTCCTGTGGTCTTTC | TCTCTCTCTCCCTTACCGTTCA | 205 | - |
| 5 | TCACAACACCTCCTCTCTCTCTC | TCACGGTCAATCACTTTCAACT | 262 | 64.2 |
| 6 | TCTATCTGGTCTACTGGGGAGC | CATACACACACAAAACCACCCT | 357 | 55.1 |
| 7 | CCAACACTCAAAATCACCAAGA | ACTCCTATCAAATGGGGTTCCT | 155 | 64.2 |
| 8 | CACACACACACACACA CACTCTC | GTCGTCATCTCCTTCTTGCTCT | 175 | 52.9 |
| 9 | CATCTTCGTCCTCAACCTCATC | GGTCGTCATTATCGTCGTCTTT | 135 | 55.1 |
| 10 | CCAGATTGGAAATAGAGGAGGA | AAGCGTGGAGAAGAGACAAAAG | 302 | 56.5 |
| 11 | AGAAAGGGAAATGGAAGAGGAG | AGATGATGGTGATGAGATGACG | 237 | 53.3 |
| 12 | CGATTGAGGAGAAG GAGAAGAA | AACAAACAACACCAACATCGTC | 115 | 53.5 |
| 13 | AGGAGGATTTGAGTTGGGATGT | CCTCTTTCACTGTCTCACCGAT | 114 | 55.8 |
| 14 | CTGATGGATTTGGTTCTGGTTT | TCCTGATTTGGGTTCTTCTTGT | 400 | 54.4 |
| 15 | CTGTTTCTTTTCTGTTTTGGGG | ACCACCTTTGTTGTTGTTGTTG | 355 | 53.5 |
| 16 | TCAAAGAGAGAGAGC GAAATCC | TTCGGAGAAGAAGAAGACAAGG | 163 | 53.5 |
| 17 | AAGAAGAAGAAGGGGAAAATGG | CGTGGAGTCTGAAACCGAAT | 223 | 56.9 |
| 18 | GACGAGACAACGCAGATAGGA | CAACCTAAAATCTCAAATCCCG | 109 | 53.5 |
| 19 | GATTCCAGATTCATCGGTTCAC | ATCAGGGAGGGAGGAAGGA | 124 | 57.7 |
| 20 | TCTGCTTGGAGTTGGTATTCG | CTGAGAAGATAAGATTGATTACGGG | 194 | 52.5 |

***Table 2. Distribution of snake gourd genotypes into clusters as per Mahalanobis D2values.***

| Cluster | Count | Genotype |
|---|---|---|
| | | IC284753 |
| | | KAUMUDHI |
| | | IC212513 |
| | | IC410142 |
| | | IC212517 |
| | | IC212483 |
| | | IC202157 |
| | | IC212416 |
| | | IC277390 |
| | | IC347377 |
| | | IC202155 |
| I | 17 | IC284875 |
| | | MANUSREE |
| | | IC426984 |

| | | |
|---|---|---|
| | | IC264713 |
| | | IC418496 |
| | | MADIKI LOCAL |
| | | IC212527 |
| | | IC539812 |
| II | 10 | IC212475 |
| | | CO-2 |
| | | IC202158 |
| | | IC410160 |
| | | PKM-1 |
| | | BABY |
| | | GUDEM LOCAL |
| | | IC212465 |
| | | IC546082 |
| | | IC212474 |
| | | IC308557 |
| III | 4 | IC333314 |
| IV | 1 | GUNTUR LOCAL |

***Table 4. Cluster mean values for different characters of snake gourd genotypes.***

| Characters | Clusters | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Days to first male flower opening | 38.45 | 40.74 | 33.73 | 34.40 |
| Days to first female flower opening | 59.32 | 61.18 | 60.46 | 63.80 |
| Node to first male flower | 4.95 | 4.65 | 5.56 | 4.30 |
| Node to first female flower | 10.11 | 11.66 | 11.16 | 8.20 |
| Number of male flowers per vine | 431.81 | 431.19 | 432.63 | 434.25 |
| Number of female flowers per vine | 26.13 | 27.05 | 27.13 | 28.10 |
| Sex ratio (%) | 16.57 | 16.01 | 16.73 | 16.30 |
| Fruit set (%) | 75.19 | 75.83 | 69.28 | 63.50 |
| Fruit length (cm) | 39.60 | 39.16 | 52.26 | 37.29 |
| Fruit girth (cm) | 13.81 | 13.66 | 13.29 | 11.65 |
| Average fruit weight (g) | 275.76 | 266.90 | 312.74 | 164.13 |
| Flesh thickness (cm) | 0.46 | 0.42 | 0.54 | 0.35 |
| Number of seeds per fruit | 41.38 | 28.13 | 63.38 | 45.75 |
| Number of fruits per vine | 19.56 | 20.53 | 18.50 | 17.82 |
| Fruit yield per vine (kg) | 5.37 | 5.30 | 6.15 | 2.93 |
| Vitamin C content (mg/100 g) | 4.35 | 3.61 | 4.16 | 4.28 |
| Acidity content (per cent) | 0.15 | 0.14 | 0.17 | 0.16 |
| Potassium (mg/100 g) | 177.32 | 279.15 | 294.63 | 190.50 |
| TSS ($^{o}$Brix) | 3.36 | 3.50 | 3.36 | 2.63 |
| Fibre content (g/100 g) | 0.46 | 0.39 | 0.45 | 0.55 |

***Table 5 Per cent contribution of different characters towards diversity in snake gourd genotypes.***

| S.NO | Character | Contribution (%) | Times Ranked first |
|---|---|---|---|
| 1 | Days to first male flower opening | 0.81 | 4.00 |
| 2 | Days to first female flower opening | 0.20 | 1.00 |
| 3 | Node to first male flower | 0.00 | 0.00 |
| 4 | Node to first female flower | 0.00 | 0.00 |
| 5 | Number of male flowers per vine | 0.00 | 0.00 |
| 6 | Number of female flowers per vine | 0.00 | 0.00 |
| 7 | Sex ratio (%) | 0.00 | 0.00 |
| 8 | Fruit set (%) | 0.20 | 1.00 |

| | | | |
|---|---|---|---|
| 9 | Fruit length (cm) | 1.01 | 5.00 |
| 10 | Fruit girth (cm) | 0.00 | 0.00 |
| 11 | Average fruit weight (g) | 0.81 | 4.00 |
| 12 | Flesh thickness (cm) | 0.00 | 0.00 |
| 13 | Number of seeds per fruit | 6.85 | 34.00 |
| 14 | Number of fruits per vine | 0.00 | 0.00 |
| 15 | Fruit yield per vine (kg) | 6.05 | 30.00 |
| 16 | Vitamin C content (mg/100 g) | 10.69 | 53.00 |
| 17 | Acidity content (per cent) | 5.44 | 27.00 |
| 18 | Potassium (mg/100 g) | 63.91 | 317.00 |
| 19 | TSS ($^{o}$Brix) | 1.81 | 9.00 |
| 20 | Fiber content (g/100 g) | 2.22 | 11.00 |

***Table 6 Allele frequency, heterozygosity and PIC values of polymorphic EST-SSR markers obtained through NTSYS 2.11 a.***

| Sr.No | Marker | Number of alleles | Majorallelefrequency (%) | Observed heterozygosity | Expected heterozygosity | PIC |
|---|---|---|---|---|---|---|
| 1 | EST-SSR-06 | 2 | 84.37 | 0.00 | 0.96 | 0.94 |
| 2 | EST-SSR-10 | 2 | 51.56 | 1.00 | 0.97 | 0.96 |
| 3 | EST-SSR-11 | 2 | 96.87 | 1.00 | 0.98 | 0.96 |
| 4 | EST-SSR-13 | 2 | 48.43 | 1.00 | 0.99 | 0.97 |
| 5 | EST-SSR-15 | 2 | 95.31 | 0.43 | 0.97 | 0.95 |
| 6 | EST-SSR-16 | 2 | 50.00 | 0.43 | 0.98 | 0.96 |
| 7 | EST-SSR-17 | 2 | 48.43 | 1.00 | 0.98 | 0.96 |
| 8 | EST-SSR-18 | 3 | 66.66 | 1.00 | 0.98 | 0.96 |
| 9 | EST-SSR-20 | 4 | 23.43 | 0.56 | 0.97 | 0.95 |
| | | **21(T)** | **62.78(M)** | **0.71(M)** | **0.97(M)** | **0.96(M)** |

***Table 8. Distribution of genotypes into different clusters based on EST- SSR analysis.***

| S.No | Cluster no. | Number of genotypes | Genotypesincluded in the cluster |
|---|---|---|---|
| 1 | I | 2 | IC546082 and IC 202155 |
| 2 | II | 1 | PKM-1 |
| 3 | III | 4 | IC 418496, IC 202157, IC 212513 and IC 410160 |
| 4 | IV | 4 | IC 308557, IC 212517, IC 212416 and IC 212475 |
| 5 | V | 15 | MADIKI LOCAL, IC 277390, IC 426984, IC 539812, IC 212465, GUDEM LOCAL, CO-2, GUNTUR LOCAL, IC 202158, IC 212527, IC 284875, IC 212474, IC 333314, KAUMUDHI and BABY |
| 6 | VI | 3 | IC 284753, IC 410142 and IC 347377 |
| 7 | VII | 1 | IC 264713 |
| 8 | VIII | 2 | MANUSREE and IC 212483 |

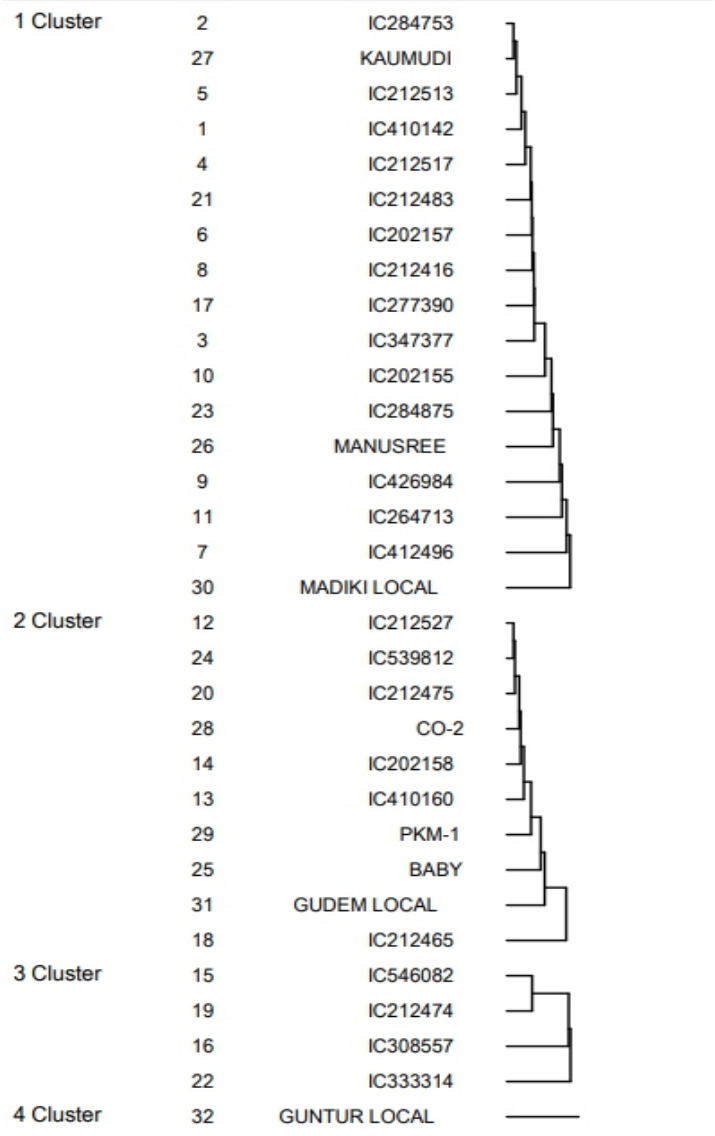
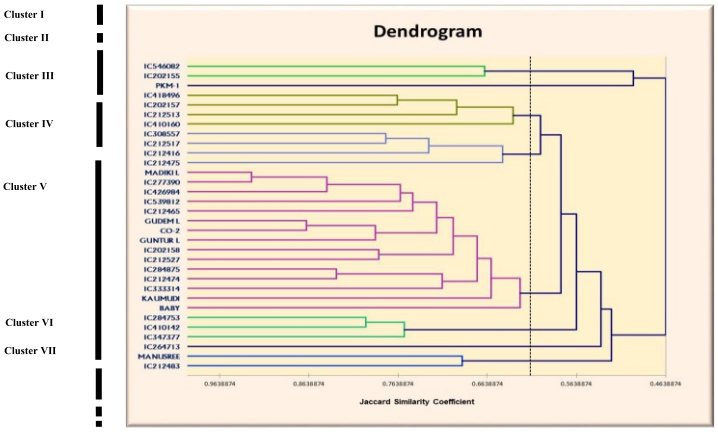



*Figure 1 Diagram illustrating the clustering pattern in snake gourd genotypes by Tocher's method.*



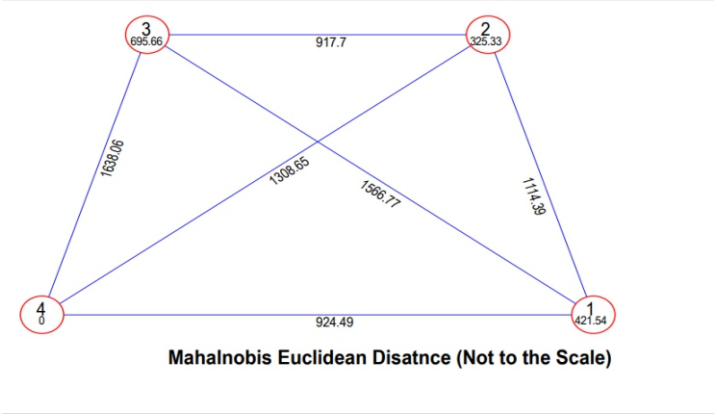




*Figure 2 Diagram representation of cluster distances of snake gourd genotypes by Tocher's method.*

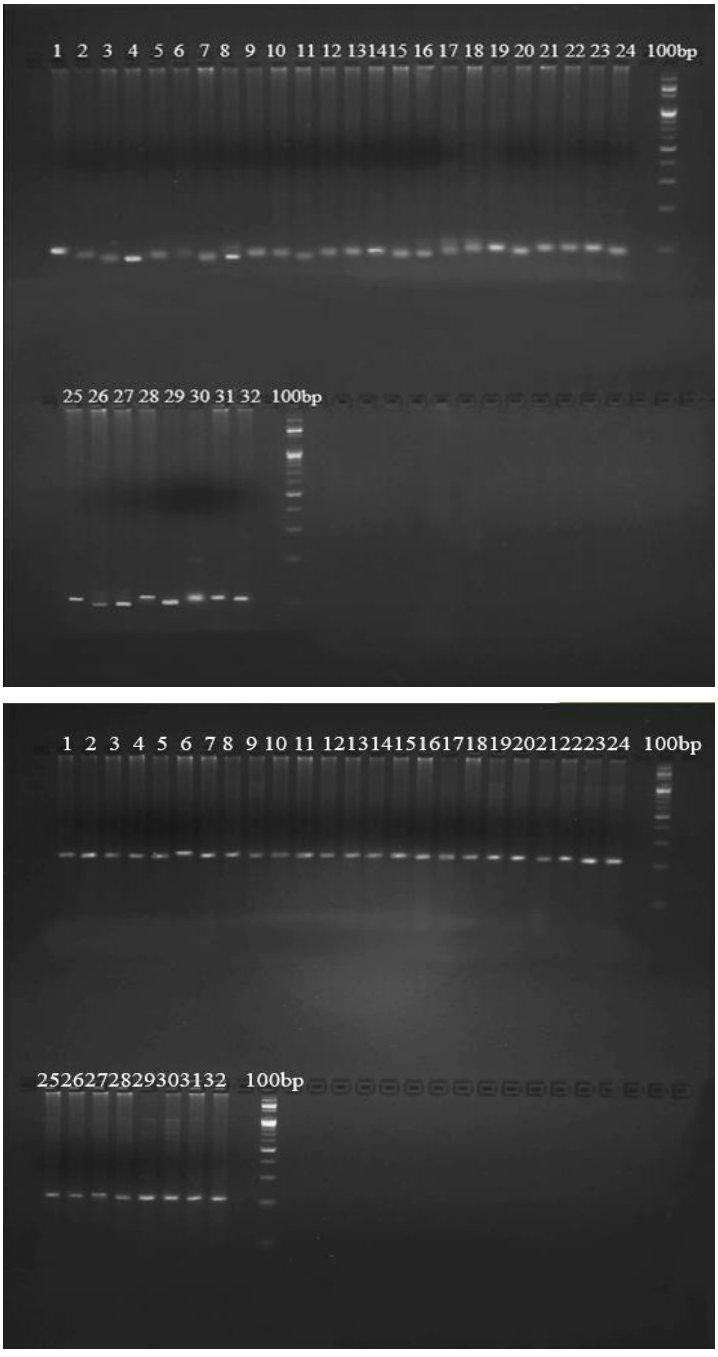



*Figure3.Gel profile of snake gourd genotypes with EST-SSR primer 13 and 17*

***Table 7. Similarity index of snake gourd genotypes based on Jaccard's similarity coefficient.***

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **2** | 0.80 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **3** | 0.80 | 0.71 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **4** | 0.58 | 0.50 | 0.59 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **5** | 0.65 | 0.67 | 0.56 | 0.56 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **6** | 0.58 | 0.59 | 0.50 | 0.58 | 0.65 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **7** | 0.67 | 0.69 | 0.59 | 0.76 | 0.75 | 0.76 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | | |
| **8** | 0.63 | 0.56 | 0.56 | 0.72 | 0.61 | 0.63 | 0.72 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | | |
| **9** | 0.72 | 0.65 | 0.56 | 0.63 | 0.71 | 0.72 | 0.82 | 0.78 | 1.00 | | | | | | | | | | | | | | | | | | | | | | | |
| **10** | 0.39 | 0.29 | 0.38 | 0.56 | 0.35 | 0.32 | 0.47 | 0.44 | 0.53 | 1.00 | | | | | | | | | | | | | | | | | | | | | | |
| **11** | 0.44 | 0.53 | 0.44 | 0.44 | 0.41 | 0.44 | 0.44 | 0.50 | 0.50 | 0.31 | 1.00 | | | | | | | | | | | | | | | | | | | | | |
| **12** | 0.56 | 0.56 | 0.47 | 0.47 | 0.44 | 0.56 | 0.56 | 0.61 | 0.71 | 0.53 | 0.71 | 1.00 | | | | | | | | | | | | | | | | | | | | |
| **13** | 0.61 | 0.63 | 0.44 | 0.45 | 0.59 | 0.71 | 0.61 | 0.58 | 0.67 | 0.33 | 0.47 | 0.69 | 1.00 | | | | | | | | | | | | | | | | | | | |
| **14** | 0.50 | 0.50 | 0.41 | 0.50 | 0.39 | 0.50 | 0.59 | 0.56 | 0.65 | 0.57 | 0.53 | 0.79 | 0.53 | 1.00 | | | | | | | | | | | | | | | | | | |
| **15** | 0.32 | 0.38 | 0.38 | 0.47 | 0.44 | 0.47 | 0.47 | 0.44 | 0.53 | 0.67 | 0.50 | 0.64 | 0.41 | 0.57 | 1.00 | | | | | | | | | | | | | | | | | |
| **16** | 0.60 | 0.53 | 0.61 | 0.78 | 0.58 | 0.68 | 0.68 | 0.74 | 0.74 | 0.50 | 0.65 | 0.67 | 0.55 | 0.61 | 0.59 | 1.00 | | | | | | | | | | | | | | | | |
| **17** | 0.61 | 0.63 | 0.44 | 0.53 | 0.59 | 0.61 | 0.71 | 0.67 | 0.88 | 0.60 | 0.56 | 0.80 | 0.65 | 0.73 | 0.60 | 0.63 | 1.00 | | | | | | | | | | | | | | | |
| **18** | 0.65 | 0.67 | 0.56 | 0.56 | 0.63 | 0.56 | 0.65 | 0.71 | 0.71 | 0.53 | 0.60 | 0.73 | 0.59 | 0.67 | 0.53 | 0.58 | 0.80 | 1.00 | | | | | | | | | | | | | | |
| **19** | 0.47 | 0.47 | 0.38 | 0.39 | 0.44 | 0.39 | 0.47 | 0.53 | 0.63 | 0.54 | 0.62 | 0.77 | 0.50 | 0.57 | 0.54 | 0.50 | 0.71 | 0.64 | 1.00 | | | | | | | | | | | | | |
| **20** | 0.50 | 0.60 | 0.50 | 0.59 | 0.56 | 0.50 | 0.59 | 0.65 | 0.65 | 0.38 | 0.64 | 0.67 | 0.63 | 0.50 | 0.47 | 0.71 | 0.63 | 0.56 | 0.57 | 1.00 | | | | | | | | | | | | |
| **21** | 0.47 | 0.38 | 0.38 | 0.47 | 0.35 | 0.47 | 0.56 | 0.44 | 0.63 | 0.54 | 0.31 | 0.53 | 0.50 | 0.47 | 0.43 | 0.42 | 0.60 | 0.44 | 0.43 | 0.38 | 1.00 | | | | | | | | | | | |
| **22** | 0.65 | 0.56 | 0.47 | 0.47 | 0.44 | 0.47 | 0.56 | 0.61 | 0.71 | 0.53 | 0.50 | 0.73 | 0.59 | 0.56 | 0.44 | 0.58 | 0.80 | 0.63 | 0.64 | 0.56 | 0.53 | 1.00 | | | | | | | | | | |
| **23** | 0.59 | 0.60 | 0.41 | 0.42 | 0.56 | 0.50 | 0.59 | 0.56 | 0.75 | 0.57 | 0.53 | 0.79 | 0.63 | 0.60 | 0.57 | 0.53 | 0.86 | 0.67 | 0.83 | 0.60 | 0.57 | 0.79 | 1.00 | | | | | | | | | |
| **24** | 0.58 | 0.69 | 0.50 | 0.67 | 0.65 | 0.67 | 0.76 | 0.72 | 0.72 | 0.47 | 0.63 | 0.65 | 0.61 | 0.59 | 0.56 | 0.68 | 0.81 | 0.75 | 0.56 | 0.69 | 0.47 | 0.65 | 0.69 | 1.00 | | | | | | | | |
| **25** | 0.63 | 0.64 | 0.53 | 0.53 | 0.50 | 0.44 | 0.63 | 0.59 | 0.69 | 0.50 | 0.57 | 0.71 | 0.56 | 0.64 | 0.40 | 0.56 | 0.67 | 0.71 | 0.62 | 0.64 | 0.50 | 0.60 | 0.64 | 0.53 | 1.00 | | | | | | | |
| **26** | 0.59 | 0.41 | 0.50 | 0.59 | 0.39 | 0.50 | 0.50 | 0.56 | 0.65 | 0.57 | 0.44 | 0.67 | 0.53 | 0.50 | 0.47 | 0.61 | 0.63 | 0.56 | 0.57 | 0.50 | 0.69 | 0.67 | 0.60 | 0.50 | 0.53 | 1.00 | | | | | | |
| **27** | 0.53 | 0.44 | 0.53 | 0.44 | 0.41 | 0.44 | 0.53 | 0.59 | 0.69 | 0.50 | 0.57 | 0.71 | 0.47 | 0.53 | 0.50 | 0.56 | 0.67 | 0.60 | 0.75 | 0.53 | 0.62 | 0.60 | 0.64 | 0.53 | 0.57 | 0.64 | 1.00 | | | | | |
| **28** | 0.58 | 0.50 | 0.50 | 0.67 | 0.56 | 0.58 | 0.67 | 0.82 | 0.82 | 0.56 | 0.63 | 0.75 | 0.53 | 0.69 | 0.56 | 0.78 | 0.81 | 0.75 | 0.67 | 0.69 | 0.56 | 0.65 | 0.69 | 0.76 | 0.63 | 0.69 | 0.73 | 1.00 | | | | |
| **29** | 0.35 | 0.33 | 0.33 | 0.53 | 0.40 | 0.44 | 0.53 | 0.41 | 0.50 | 0.50 | 0.27 | 0.40 | **0.22** | 0.54 | 0.50 | 0.47 | 0.47 | 0.40 | 0.38 | 0.33 | 0.38 | 0.31 | 0.43 | 0.44 | 0.36 | 0.43 | 0.36 | 0.53 | 1.00 | | | |
| **30** | 0.56 | 0.56 | 0.47 | 0.47 | 0.53 | 0.56 | 0.65 | 0.61 | 0.81 | 0.64 | 0.50 | 0.73 | 0.59 | 0.67 | 0.64 | 0.58 | **0.93** | 0.73 | 0.64 | 0.56 | 0.64 | 0.73 | 0.79 | 0.75 | 0.60 | 0.56 | 0.71 | 0.75 | 0.40 | 1.00 | | |
| **31** | 0.65 | 0.56 | 0.56 | 0.56 | 0.53 | 0.56 | 0.65 | 0.71 | 0.81 | 0.53 | 0.50 | 0.73 | 0.50 | 0.79 | 0.53 | 0.67 | 0.80 | 0.73 | 0.64 | 0.56 | 0.53 | 0.63 | 0.67 | 0.65 | 0.60 | 0.67 | 0.71 | 0.87 | 0.62 | 0.73 | 1.00 | |
| **32** | 0.63 | 0.64 | 0.64 | 0.53 | 0.50 | 0.53 | 0.63 | 0.59 | 0.69 | 0.40 | 0.57 | 0.71 | 0.47 | 0.64 | 0.50 | 0.65 | 0.67 | 0.60 | 0.62 | 0.64 | 0.50 | 0.60 | 0.64 | 0.63 | 0.57 | 0.64 | 0.69 | 0.73 | 0.58 | 0.60 | 0.85 | 1.00 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | IC 410142 | **5** | IC 212513 | **9** | IC 426984 | **13** | IC 410160 | **17** | IC 277390 | **21** | IC 212483 | **25** | BABY | **29** | PKM-1 |
| **2** | IC 284753 | **6** | IC 202157 | **10** | IC 202155 | **14** | IC 202158 | **18** | IC 212465 | **22** | IC 333314 | **26** | MANUSREE | **30** | MADIKI LOCAL |
| **3** | IC 347377 | **7** | IC 418496 | **11** | IC 264713 | **15** | IC 546082 | **19** | IC 212474 | **23** | IC 284875 | **27** | KAUMUDHI | **31** | GUDEM LOCAL |
| **4** | IC 212517 | **8** | IC 212416 | **12** | IC 212527 | **16** | IC 308557 | **20** | IC 212475 | **24** | IC 539812 | **28** | CO-2 | **32** | GUNTURLOCAL |

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