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Molecular Marker Based Genetic Diversity Analysis in Cape Gooseberry (*Physalis peruviana* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cape gooseberry is an important minor fruit crop of India. In the present study, genetic diversity of 12 genotypes of Cape gooseberry was analysed using Random Amplified Polymorphic DNA (RAPD) markers. For this purpose, twenty RAPD primers were employed to screen out the polymorphic primer(s) for use in the genetic diversity assessment. Results showed that genotypes, CITH Sel-7 and CITH Sel- 9, and CITH Sel-1 and CITH Sel-15 as the least diverged genotypes. Though, all the genotypes of CITH were found to be more diverged than the local SS/VK genotypes, as they clustered separately. The most promising genotypes from a genetic diversity point of view were CITH Sel-7, CITH Sel-9, CITH Sel-16, CITH Sel-5, SS/VK/501 and SS/VK/601. These genotypes could be utilised as a diverse parent in hybridisation programme or as direct selection and may yield useful segregants by virtue of the high level of heterosis.

Keywords: Genetic diversity; Cape gooseberry; RAPD; Solanaceae; minor fruit.

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ABBREVIATIONS

CITH Sel: *Central Institute of Temperate Horticulture Selection*
SS/VK: *Sanjay Sahay/Vikash Kumar*
IU: *International unit*

1. INTRODUCTION

Cape gooseberry **(***Physalis peruviana* L.**)** is one of the important minor fruit crops which belong to the Solanaceae family. The fruit type is berry, like a small globe having colour from green to yellowish, with the diameter around 12.5 to 25.0 millimetres and weight ranges from 4 to 10 g, containing around 100 to 300 seeds. These fruits are very attractive in colour at maturity contains a high level of ascorbic acid (36 mg $100g^{-1}$ pulp), rich in Vitamin A (1730 IU. $100g^{-1}$ of pulp), iron (38 mg $100g^{-1}$ of pulp) and phosphorus (1.2 mg $100g^{-1}$ of pulp) [1]. *Physalis* have economic and horticultural importance due to their high nutritional value. They are rich in vitamin A, B complex and C along with minerals and antioxidants. Also they have potential medicinal properties such as, anti-inflammatory, anti-bacterial, and anti-cancer properties [2].

The demand for this crop is increasing day by day among the health conscious urban people due to its immense nutraceutical and pharmaceutical values as it is a rich source of vitamins, antioxidants, minerals and flavonoids. This crop has been paid little attention earlier on the aspect of its germplasm evaluation and genetic divergence study, for use in varietal improvement programmes. It is also felt that the genotype should be evaluated before recommending any genotypes for harshening maximum yield with high nutritive value.

Molecular markers based genetic diversity analysis is being widely used nowadays along with morphological analysis to get the most authenticated picture of diversity among genotypes, which otherwise, will be difficult to distinguish only by one method alone. Molecular markers are reported to be a reliable method for estimating phylogenetic relationships among genotypes of any organism [3]. DNA sequences from few genes and ISSR markers have been used to investigate the phylogeny of *Physalis* along with their relationship to other Solanaceae members. Recently, 5971 SSR markers were discovered by analysing the assembled *P. peruviana* leaf transcriptome sequences [4]. However, only 30 markers have publically available primer information. The unavailability of markers prevents the detailed study of the genetic diversity and phylogeny in *Physalis* at a molecular level.

Therefore, RAPD markers-based analysis was conducted to assess the genetic diversity among genotypes of cape gooseberry, which is a fast and simple method that requires no prior knowledge of nucleotide sequence information.

2. MATERIALS AND METHODS

The experimental material consists of twelve genotypes of Cape gooseberry (*Physalis peruviana* L.) collected from CITH, Srinagar (CITH Sel 1, CITH Sel 3, CITH Sel 5, CITH Sel 7, CITH Sel 9, CITH Sel 11, CITH Sel 15, CITH Sel 16) and various district of Bihar (SS/VK/301, SS/VK/401, SS/VK/501 and SS/VK/601) in India.

2.1 DNA Isolation

The young leaf (100 mg) of each genotype was used for DNA extraction following CTAB method [5]. The quality and quantity of extracted DNA were estimated by UV/VIS spectrophotometer and gel electrophoresis.

2.2 RAPD Analysis

Initially, twenty RAPD primers were employed in PCR amplification to screen out the panel of primer(s) which generates maximum polymorphic bands (Table 1).

The PCR amplified products were electrophoresed on 1.5% agarose gel and viewed under UV-trans-illuminator. The gel picture was taken using a gel documentation system. Bands obtained in this analysis were scored visually as "1" for the presence and "0" for the absence. To represent the genetic distance among genotypes, a dendrogram was generated using a window-based tool TFPGA: A Tool For Population Genetic Analysis [6]. The clustering was done from Genetic Distance Similarity Matrix [7]. Initially, twenty RAPD primers were employed in PCR amplification to screen out the primer(s) which generates maximum polymorphic bands. Out of twenty RAPD primers, one primer was found to be polymorphic, therefore, with this primer, all the genotypes were analyzed. The PCR amplified products were electrophoresed on 1.5% agarose gel and viewed under UV-trans-illuminator.

Table 1. List of RAPD primers used for screening of germplasm in the present study

S.N.	Name of primer	Sequence	Melting temperature (T_m, °C)
1	Rapid1	5'- CGTACTGCAG -3'	32
2	Rapid2	5'- CGTCACAATG -3'	30
3	Rapid3	5'- GGTGCGAGCT -3'	34
4	Rapid4	5'- CTCTGACGGC -3'	34
5	Rapid5	5'- GGATTACGTG -3'	30
6	Rapid6	5'- CACCGAAACA -3'	30
7	Rapid7	5'- GGCACCGTCA -3'	34
8	Rapid8	5'- CATGGCACTG -3'	32
9	Rapid9	5'- TCCACACAGA -3'	30
10	Rapid10	5'- GCCGACGATG -3'	34
11	Rapid11	5'- TGTTTGCGCC -3'	32
12	Rapid12	5'- AAGCTAGCCC -3'	32
13	Rapid13	5'- GGGCCCACAC -3'	36
14	Rapid14	5'- TGCCGAGACG -3'	34
15	Rapid15	5'- TGCCGAGACG -3'	34
16	Rapid16	5'- CAGAAGTGGG -3'	32
17	Rapid17	5'- CGTGGACACT -3'	32
18	Rapid18	5'- GTTTAGCGCA -3'	30
19	Rapid19	5'- AAACGGTTCG -3'	30
20	Rapid20	5'- AGTCCAGCTA -3'	30

The gel picture was taken using a gel documentation system. Bands obtained in this analysis were scored visually as "1" for the presence and "0" for the absence. To represent the genetic distance among genotypes, a dendrogram was generated using a window-based tool TFPGA: A Tool For Population Genetic Analysis [6].

3. RESULTS AND DISCUSSION

The crop improvement programme mainly depends on the choice of elite parents for hybridisation. As the accessions are poorly distinguished it is essential at a time of the beginning of a breeding program to discriminate among available genotypes to establish the level of genetic diversity and thereby, identify the most suitable materials for crossing. In general, genetic diversity among genotypes is determined using morphological and molecular markers. The molecular markers based on DNA sequence polymorphism, are independent of environmental conditions. Molecular markers have elucidated the structure of genetic diversity in a broad range of the crops. Molecular markers provide a quick and reliable method for estimating phylogenetic relationships among genotypes of any organism [3].

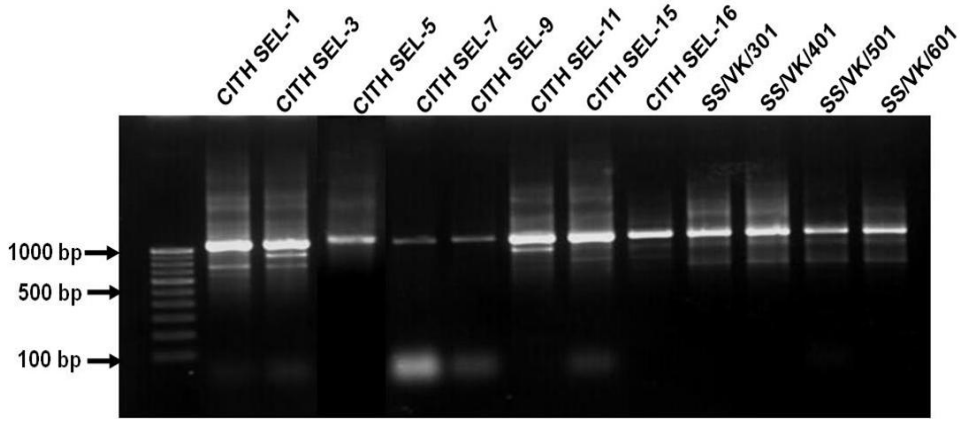

Fig. 1. Representative RAPD profile generated by primer Rapid 13 in twelve genotypes of Cape gooseberry (Marker: 100 bp DNA ladder)

Table 2. Genetic distance similarity matrix [Based on Nei's [7] identities/Distance]

Genotypes	CITH Sel-1	CITH Sel-3	CITH Sel-5	CITH Sel-7	CITH Sel-9	CITH Sel-11	CITH Sel-15	CITH Sel-16	SS/VK/301	SS/VK/401	SS/VK/501	SS/VK/601
CITH Sel-1	****											
CITH Sel-3	0.857	****										
CITH Sel-5	0.428	0.571	****									
CITH Sel-7	0.428	0.285	0.714	****								
CITH Sel-9	0.428	0.285	0.714	1.000	****							
CITH Sel-11	0.714	0.857	0.714	0.428	0.428	****						
CITH Sel-15	1.000	0.857	0.428	0.428	0.428	0.714	****					
CITH Sel-16	0.428	0.571	0.714	0.714	0.714	0.428	0.428	****				
SS/VK/301	0.428	0.571	0.714	0.428	0.428	0.714	0.428	0.428	****			
SS/VK/401	0.571	0.714	0.571	0.285	0.285	0.857	0.571	0.285	0.857	****		
SS/VK/501	0.285	0.428	0.571	0.571	0.571	0.571	0.285	0.571	0.571	0.714	****	
SS/VK/601	0.428	0.571	0.428	0.428	0.428	0714	0.428	0.428	0.714	0.857	0.857	****

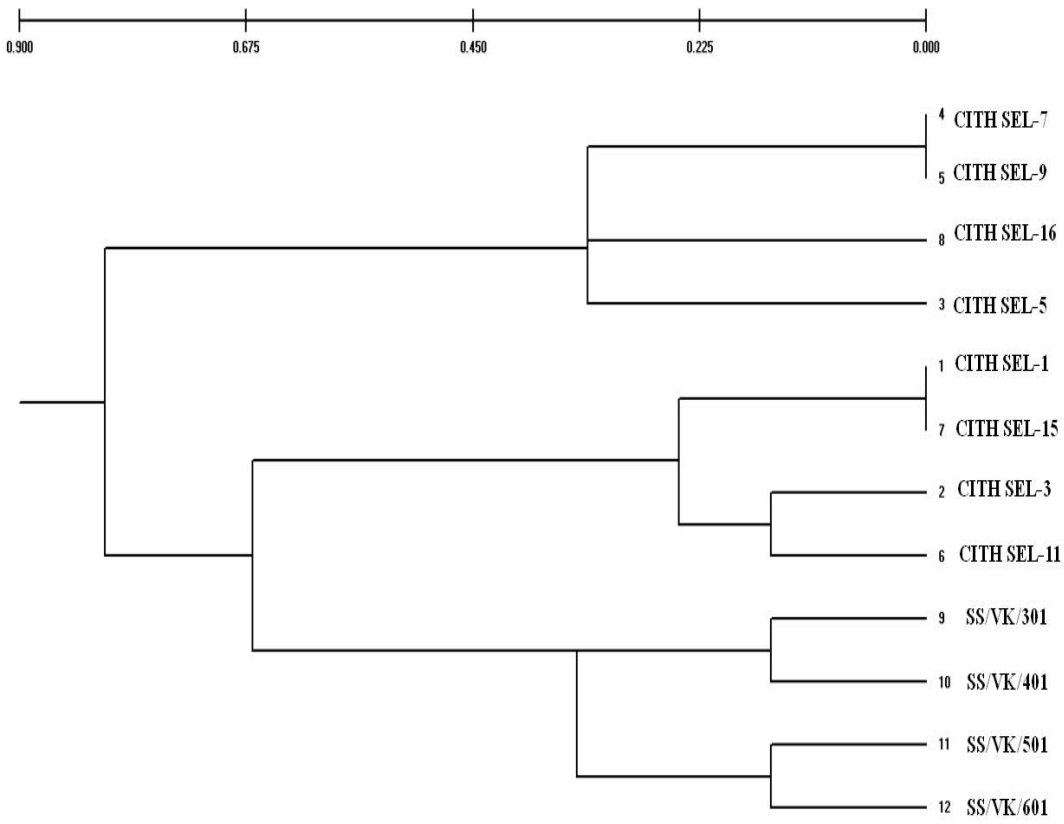

Fig. 2. Phylogenetic analysis of Cape gooseberry twelve genotypes based on TFPGA tool

The least diverged genotypes CITH Sel-7 and CITH Sel-9 and CITH Sel-1 and CITH Sel-15. All the genotypes of CITH were found to be divergent as they clustered separately than the local SS/VK genotypes

The RAPD analysis employed in the present study for the assessment of genetic diversity was for their simplicity, fast and easy to perform and comparatively cheaper and requirement of no awareness of DNA sequences [8,9]. RAPD analysis showed the diverse nature of some of the genotypes. The unique banding pattern was helpful in categorisation of genotypes as evident from the gel picture of RAPD analysis in Fig. 1. Several bands were observed in all the genotypes except CITH Sel-7 and CITH Sel-9 (Single band). The number of bands observed in all the twelve genotypes ranged from 1-6. These bands were used for generation of dendrogram shown in Fig. 2 and for the similarity matrix shown in Table 2.

4. CONCLUSION

On the basis of RAPD based genetic diversity analysis, the genotypes such as CITH Sel-7 and/or CITH Sel-9, and CITH Sel-16 and CITH Sel-5 and SS/VK/501 and SS/VK/601 were found to be the most divergent. These genotypes could be utilised as a diverse parent in hybridisation programme and may yield useful segregants by virtue of the high level of heterosis. Substantial genetic diversity was found within Cape gooseberry genotypes as revealed by RAPD markers. The information obtained from this study may be useful for better management of genetic resources of this species, identification of promising genotypes for popularisation and understanding of the extent of genetic diversity present in this Cape gooseberry species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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