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Genetic Diversity Analysis of Bitter Gourd (*Momordica Charantia* l.) Germplasm Based on RAPD and SSR Markers

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***Abstract* – In this study, the genetic relatedness of 36 genotypes of bitter gourd (*Momordica Charantia* L.) collected from different parts of India was analysed by combination of RAPD and SSR primers. Among the 40 primers screened only 11 primers showed polymorphism. The average number of polymorphic bands per primer was 2.1. Dendrogram showed that all the accessions formed three main clusters with various degree of sub-clustering within the clusters. These results have implications for bitter gourd breeding and conservation in the future.**

***Keywords* – Genetic Diversity, Bitter Gourd, Microsatellites, RAPD.**

I. Introduction

Bitter gourd belongs to genus *Momordica* which includes approximately 59 species (Schaefer and Renner 2010). The highest species diversity is found in Africa and South East Asian countries. Recently it has been found that six species closely related to bitter gourd are found in India, of which four are dioecious and two monoecious in nature. *M. charantia* and *M. balsamina* are monoecious, while *M. dioica*, *M. sahyadrica*, *M. cochhinchinensis* and *M. subangulata* are dioecious (De Wilde and Duyfjer 2002, Joseph 2005, Joseph and Antony 2008). The origin of this crop is probably in India with secondary centre of diversity in China (Grubben 1977). It was domesticated in Asia, possibly in eastern India or southern China. Among the cucurbits, it is considered a prized vegetable because of its high nutritive value especially ascorbic acid and iron as well as its immense medicinal value, mainly due to its hypoglycemic properties (Behera 2004). Availability of germplasm is a pre-requisite for any crop improvement programme and has a crucial role in sustaining and strengthening the food, nutritional security and health of the increasing human population. The yield levels of most of the crop plants have reached a plateau due to narrow genetic base of these crops. Therefore, it becomes necessary to collect, characterize, evaluate, utilize and conserve germplasm. Detailed evaluation of germplasm lines for yield, its component traits and other economic attributes helps in the isolation of lines which can be used as such in the development of superior cultivars or as donors for the transfer of specific characters. Analysis of genetic diversity and relatedness between species and among genotypes is useful in plant breeding programs because it provides a tool for accurate organization of germplasm and efficient parent selection. Molecular markers have proved to be the powerful tool in the assessment of genetic variation and in elucidating the genetic relationships within and among species. Various types of molecular markers differing in their principles and methodologies are available. The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the most significant developments in the field of molecular genetics (Semagan *et al* 2006). Currently, the study of genetic diversity within and among the bitter gourd species is insufficient and limited, which makes the use of genetic markers in *M. charantia* important and necessary (Li et al., 2013). RAPD's (Randomly Amplified Polymorphic DNA) because of their simplicity, speed and relatively low cost and Microsatellites (simple sequence repeats, SSRs) due to the advantages of high variability, codominance and abiquity in eukaryotic genomes, have become a useful tool in population genetic analysis.

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In the present study, both marker systems (RAPD and SSR) were employed to provide the genetic information for 36 germplasm lines.

II. Materials and Methods

Plant Material and DNA Extraction

The experimental plant material comprised of 36 germplasm lines of bitter gourd (Table 1) originating from different agro-ecological regions. Molecular marker analysis was accomplished in Molecular Biology Laboratory of School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana. Forty RAPD and SSR markers were employed to characterize and to estimate the genetic distance between 36 bitter gourd lines. Just matured leaves free from diseases, especially powdry mildew, downy mildew and viruses and developmental deformities, were collected from the research plot. Leaves collected from different plants in a line of single accession were bulked. Fresh leaves were quickly brought to the laboratory in butter paper bags where they are washed with 76% ethanol to remove traces of dirt and used fresh for DNA extraction. DNA was extracted from young healthy leaves using the modified CTAB (Cetrimide Tetradecyl Trimethyl Ammonium Bromide) method development by Saghai-Maroof *et al* (1984). The young tender and healthy leaves from five individual plants were collected separately from each of the genotypes and used for DNA isolation. DNA concentration and purity were determined by measuring the absorbance of diluted DNA solution at 260 nm and 280 nm in UV spectrophotometer. Quality of isolated DNA was checked by electrophoresis on 0.8% agarose gel and the image was visualized with an ultraviolet transilluminator.

RAPD Analysis

RAPD analysis (Williams *et al* 1990) was carried out to survey the polymorphism among the genotypes. PCR was carried out in duplicate using RAPD primers in a DNA thermal cycler (Perkin Elmer, Model 9600, USA). Each polymerase chain reaction (PCR) was carried out in a 25 µl volume containing 25 ng template DNA, 20 mM dNTPs, 1X *Taq* DNA polymerase buffer, 40ngdecanucleotide primer and 1 unit of *Taq* DNA polymerase. PCR cycling consisted of initial denaturation at 94°C for 4 minutes; followed by 45 cycles of amplification, at 64°C for 1 minute (denaturation), 37°C for 1 minute (annealing) and 72°C for 2 minutes (extension). A final extension step at 72°C for 7 minutes was followed by termination of the cycle at 4°C. The RAPD-PCR products were analyzed directly on 1.2% agarose gel in 0.5X TBE buffer. The amplified fragments were visualized and photographed under UV light using Polaroid MP4 Land Camera (Fotodyne Incorporated, New Berlin, Wisconsin, USA).

SSR Analysis

SSR analysis was carried out to investigate the genetic variation and diversity within *M. charantia*.PCR was carried out in a volume of 15µl consisting of 20-30 ng genomic DNA, 1.5µl 10X pcr buffer, 0.4µM for each primer, 1.5 Mm $MgCl_2$, 250µM each Dntp and 0.5 U Taq DNA polymerase. The amplification conditions included initial denaturation at 95°C for 5 min followed by 35 cycles of 94°C for 40 seconds, annealing at optimal temperature for 45 seconds and 72°C for 40 seconds and a final 7 min elongation step at 72°C.

Scoring of Amplified Products and Data Entry

Reproducible DNA bands, i.e., bands present in both repetitions of individual sample were scored manually.

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Both weak band with negligible intensity and smearing bands were excluded from final data analysis. Band profiles were scored in a binary mode with 1 indication the presence and 0 indicating the absence of a band. The data matrices thus obtained were entered in MS-Excel for further analysis.

Table 1. List of genotypes used in present study.

Code	Accession name	Source
1	Punjab Kareli-1	Ludhiana
2	Arka Harit	Bangalore
3	DBG-35	New Delhi
4	DBG-3	New Delhi
5	DBG-45	New Delhi
6	Punjab-14	Ludhiana
7	Pant Kareli- 2	Pantnagar
8	DBG-41	New Delhi
9	Solan Hara	Solan
10	DBG-44	New Delhi
11	Pusa Do Mausami	New Delhi
12	Pusa Visesh	New Delhi
13	Coimbatore long	Coimbatore
14	WBBG-6	West Bengal
15	DBG-40	New Delhi
16	WBBG48	West Bengal
17	WBBG-5	West Bengal
18	DBG-18	New Delhi
19	PBBG-1	Ludhiana
20	PBBG-2	Ludhiana
21	PBBG-3	Ludhiana
22	PBBG-7	Ludhiana
23	PBBG-6	Ludhiana
24	PBBG-9	Ludhiana
25	PBBG-8	Ludhiana
26	PBBG-10	Ludhiana
27	PBBG-11	Ludhiana
28	PBBG-14	Ludhiana

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Code	Accession name	Source
29	PBBG-13	Ludhiana
30	PBBG-40	Ludhiana
31	Jaunpuri long	Ludhiana
32	PBBG-20	Ludhiana
33	PBBG-31	Ludhiana
34	CO-1	Coimbatore
35	Hirkani	New Delhi
36	PBIG-56	New Delhi

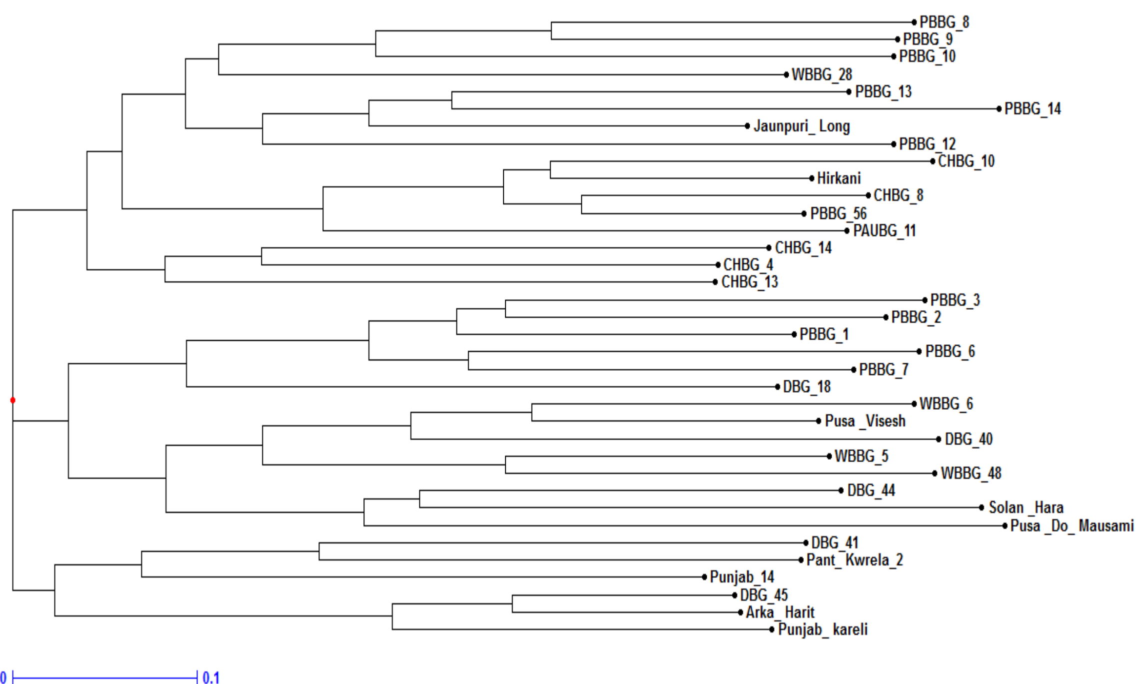

Fig. 1. Dendrogram based on molecular analysis of 36 genotypes of bitter gourd.

III. Results and Discussions

The polymorphism survey among all the genotypes was carried out using the bulked DNA sample from 5 plants in each case. A total of 40 primers were used for this purpose. Out of 36 SSR tested, three primers did not amplify, only 13 produced polymorphic bands (100%) and rest all other produced monomorphic bands. Out of 4 RAPD primers tested all amplified. The primers which produced polymorphic bands only selected to measure the diversity among 36 genotypes taken for experiment. These polymorphic primers can be regarded as reliable markers for further analysis of 36 genotypes. Polymorphic primers identified based on preliminary screening were MCMS 2, MCMS 7, MCMS 8, S 24, S 26, S 32, MCMS 43, MCMS 47, MCMS 40, MCMS 41, S 33, S 38, S 39. The size of the amplified products varied from approx. 164 bp (MCMS 10) to 300 bp (MCMS 9).

DARwin based cluster analysis (Fig. 2) showed that the grouping of 36 genotypes in three major groups. Group I had the 16 genotypes. Group I can be further divided into two subgroups. Subgroup I represents three

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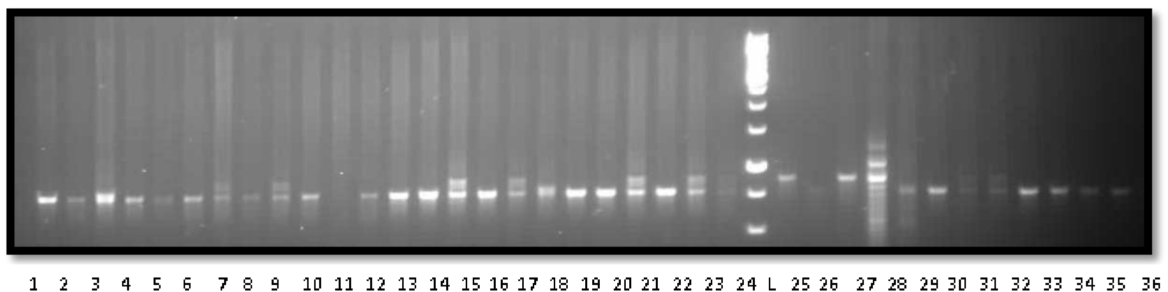
genotype i.e. PBBG-20, PBBG-31 and CO-1. All other 13 genotypes were represented in subgroup II. Very minor differences observed in sub group II. The subgroup II can further be divided into two clusters. The small cluster represented five genotypes which were DBG-3, Hirkani, PBBG-40, PBIG-56 and PBBG-11 rest of the genotypes (8) formed the large cluster. In this large cluster two lines which are closely related are PBBG-9 and PBBG-8 and PBBG-13 and PBBG-14. Group II contained 14 genotypes with 6 genotypes in subgroup I and remaining 8 genotypes in subgroup II. In subgroup I PBBG-3 and PBBG-2, PBBG-6 and PBBG-7 are more closely related. In subgroup II WBBG-6 and Pusa Visesh, WBBG-5 and WBBG-48, DBG-44 and Solan Hara are closely related. Group III comprise of 6 genotypes in which DBG-41 and Pant Kareli-2, DBG-45 and Arka Harit were more closely related.

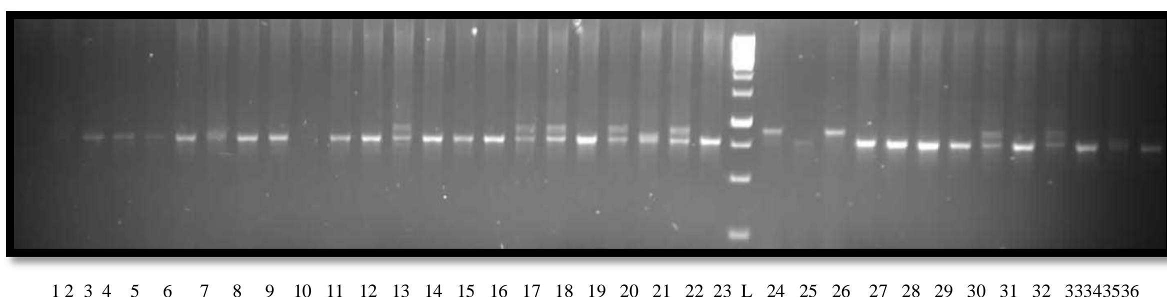
During the recent past, the development of new molecular marker systems has been successfully utilized for analysis of genetic diversity. Molecular markers are highly useful for identification of plant varieties and protection of plant breeders and farmers right. Among the molecular markers available, RAPD is simplest, efficient and economic means for cultivar identification and diversity analysis (Williams *et al* 1990). The power of RAPD for analyzing genetic diversity has been well established in a wide range of vegetable crops including cucurbits. The most reliable, efficient and economic means for diversity analysis and cultivar identification are SSR markers. The present investigation is the first comprehensive study in bitter gourd utilizing SSR markers.

The 40 primers used in the present analysis revealed polymorphism among 36 genotypes collected from different parts of India including some of the commercially released varieties by different institutes of India. The degree of polymorphism detected was relatively higher than that reported earlier in melon (Garcia Mas *et al* 2000), watermelon (Lee *et al* 1996), pumpkin (Gwanama *et al* 2000) and ashgourd (Sureja, 2003). Mo *et al* (1998) analysed the genetic diversity in melon and found that the average number of polymorphic bands per primer was 2.1. In the present study, the average number of polymorphic bands per primer was 2.1 which indicate that these identified primers are highly reliable and can further be used for evaluation of bitter gourd population.



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 L 25 26 27 28 29 30 31 32 33 34 35 36

Fig. 2. SSR Profile of the bitter gourd genotype with Primer- MCMS 40.



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 L 24 25 26 27 28 29 30 31 32 33343536

Fig 3. SSR Profile of the bitter gourd genotype with Primer- MCMS 41.

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IV. Conclusion

For molecular analysis among the 40 primers screened only 11 primers showed polymorphism. The average number of polymorphic bands per primer was 2.1. All the observations made in this study will provide valuable evidence for characterization of germplasm, breeding and bitter gourd germplasm management.

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Author's Profile

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Gurleen Sidhu, Presently I am doing my PhD in Plant Agriculture with specialization in plant breeding and genetics. I am recipient of various academic awards and scholarships during my PhD and Masters program. I got my Masters in Vegetable Science (specialization in plant breeding, genetics and biotechnology) and Bachelors in Agriculture from Punjab Agricultural University, India. During my Bachelor and Masters I was recipient of University merit scholarship and merit certificate. I was also awarded meritorious student award for securing highest OGPA during my masters. I have delivered numerous poster and oral presentations at national and international conferences. I have 10 publications until n--ow with few under review from my PhD research. Furthermore, I have been involved in a variety of volunteering experiences and extracurricular activities in addition to my academic and career-related responsibilities.

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