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Assessment of genetic diversity in advance breeding lines of Chilli (*Capsicum annuum* L.) using RAPD and Cytochrome P_{450} gene based marker system

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Abstract
Chilli (*Capsicum annuum* L.) is regarded as one of the major commercial spice crops. India is the largest producer of Chilli in the world. The present work Genetic relationship between sixteen advance breeding lines was analyzed using randomly amplified polymorphic DNA and Cytochrome P_{450} gene based markers. A total of 35 RAPD and 11 Cytochrome P_{450} gene based markers analysis generated an average of polymorphism of 37.625 % and 29.17 % respectively. Multiplex ratio (MR) was recorded highest for cytochrome P_{450} gene based markers (10.18) compared to RAPD (6.34). Based on the marker data, 16 breedling lines of Chilli were grouped into two main and two sub-clusters, without any distinct pattern. High bootstrap value obtained at major nodes indicates robustness of the dendrogram. Pearson's coefficient was determined by mantle test to establish cophenetic correlation with the value of 0.884 from both the marker system. The dendrogram and PCA plots obtained were found to be highly concordant to each other. Current research signifies that combination of these functional RAPD and non-functional cytochrome P_{450} gene based marker systems can be effectively utilized for understanding genetic variability between advance breeding lines, which can be utilize for breeding strategies and development of improved verities.

Keywords: PIC, cytochrome P450 gene based marker, RAPD, Bootstrapping, marker index

Introduction
The genus capsicum is believed to be one of the earliest domesticated plant genera and has been cultivated since 7000 year based on archaeological data (Basu and De, 2003) [3]. The genus capsicum includes 30 species in which five are under the cultivation namely, *Capsicum annuum, C. frutescens, C. chinense, C. pubescens & C. baccatum* (Bosland and Votava, 2000) [4]. Among the five cultivated species, chilli (*Capsicum annuum* L.) is the most important economical crop cultivated throughout India both in rainfed and irrigated conditions (Asati and Yadav, 2004) [1]. Chilli is indispensible spice essentially used in every Indian cuisine due to its pungency, taste, appealing colour and flavour and also used worldwide as spice, condiment, vegetables. Apart from the culinary purpose carotenoids and capsicinoids are the major medicinal components present in Chilli fruits which conntributes commercial value to the crop. In India, a total of 792.1 thousand hectares land under cultivation with the productivity of 1.5 tonnes per hectares. But, Indian productivity (1.5 t/ha) is low and further need to improve the same by developing high yielding varieties and hybrids through appropriate crop improvement programs.
Recent developments in DNA based technologies have revolutionized the utilization of molecular markers in genetics and breeding studies (Rafalski *et al.*, 1996) [25].
Assessment of genetic polymorphism is more meaningful and accurate than studying phenotypic character. Estimation of inter cultivar genetic variability and their association with trait of economic importance would facilitate identification of molecular markers associated with the traits of interest. Genetic variation among plants can be estimated through biochemical, morphological and by genomic approaches. In Capsicum several reports have been developed using morphological, cytological and biochemical markers. Barerra *et al.* (2005) characterized a total of 261 capsicum germplasm using Isozymes. But clear understanding of genomic variability can only be characterized through molecular markers. Randomly amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990) [32] is one of the

techniques which is extensively used because of its rapidity in generating data with less laboriousness compared to simple sequence repeats (SSR), inter simple sequence repeats (ISSRs), Restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphisms (AFLPs). RAPD technique is extensively used to access the genetic variation in many plants. Genetic variations among 24 accessions of Capsicum were studied by (Ayse *et al*., 2010) [2] using 2,760 RAPD primers through touch-down polymerase chain reactions. Apart from RAPD, AFLP (Guzman *et al*., 2005) [8], SSR (Patel *et al*., 2011; Julia *et al*., 2012) [22, 10] are also reported in various species of Chilli for genetic variability assessment. These techniques have both advantage and disadvantages, generating reliable information on genetic variation based on neutral regions of the genome (Karp, 2002) [13]. Cytochrome P_{450} gene based markers are novel genetic markers used for genetic diversity analysis by wider coverage of genetic bases and gives clear resolution for better understanding of genetic variability (Yamanaka *et al*., 2003) [33]. Cytochrome P_{450} based genes are present in all life forms of animals, plants, fungi, protists, bacteria, archaea, and even in viruses (Shalk *et al.,* 1999) [29]. These are the terminal oxidase enzyme in electron transfer chain called hemoproteins known to play an important role in bio synthesis of many secondary metabolite productions (Ohkawa *et al*., 1999) [18]. Thus, sequence diversity of cytochromes P_{450} genes can be successfully employed as a marker based detection for genetic variation (Panwar *et al*., 2010; Saini *et al*., 2013; Kumar *et al*., 2017) [20, 27, 14]. In the present study used the combination of non-functional RAPD and functional cytochrome P_{450} gene based markers to assess the level of genetic diversity among 16 advance breeding lines of Chilli and the outcome of the study will be a great helpful in understanding genetic variability and for crop improvement program.

Material and Methods

Collection of Plant Material

Sixteen advanced breeding lines of Chilli were collected from Horticultural Research Centre Devihosur, a specialized centre of Chilli and College of Horticultural science, Bagalkot, Karnataka, India (Table 1). Seeds were treated with thyrum @ 3g / kg of seeds and sown in portray containing soil, sand and vermicompost (2:1:1 w/v) and maintained at 26–28 °C in green house. Healthy and unfolded leaves were collected from 3 different plants of each line and stored at -80 ˚C for further use.

Exaction of genomic DNA

Genomic DNA was extracted from freshly harvested leaves by CTAB method (Sambrok and Russel 2001) [28] with minor modifications. 100 mg of leaves were finely grounded with liquid nitrogen and transferred into a 2 ml eppendorf tube containing 0.7 ml extraction buffer composed of 2 % CTAB (w/v), 0.1 M Tris chloride (pH 8.0), 0.02 M EDTA (pH 8.0), 1.4 M NaCl, 2 % PVP (w/v) and 0.25 % β-mercaptoethanol (v/v) and incubated in water bath at 60 ˚C for 30 min with regular shaking at every 5 min. To the aqueous layer equal volume of Phenol: chloroform: isoamyl alcohol (25:24:1) was added, mixed thoroughly and centrifuged at 12,000 g for 10 min at 22 ˚C. 0.7 volume chilled ethanol was added to the supernatant. The DNA was plleted by centrifuged at 12,000 X g for 20 min at 4 ˚C and the pellet was washed twice with 70 % ethanol. Ethanol was completely air dried and DNA was re-suspended in 80 µl sterile milipore water. Quantity and quality of the DNA was checked using Nano Drop (Thermo Scientific) and the samples were diluted to a concentration of 50 ng μl^{-1}.

PCR Analysis using RAPD and Cytochrome P450 gene based primers.

The lab work was carried out at Bio control laboratory, University of horticultural sciences, Bagalkot during 2015-16. Initially 50 RAPD primers and 18 Cytochrome P_{450} gene based primers were screened using 5 accessions of chilli to assess the consistency of band profiles. Out of 51, 34 RAPD (Table 1) and 11 Cytochrome P_{450} gene based primer (Table 2) producing robust amplification patterns were selected for further analysis.

PCR analysis was carried out in 0.2 ml PCR vials containing 1X PCR reaction buffer, 0.20 U *Taq* DNA polymerase, 0.2 mM dNTPs mix, 20 µM primer, 50 ng DNA template and sterile distilled water to a final volume of 20 µl. Amplification was performed with thermal cycler (Eppendorf, Germany) programmed to 40 cycles of denaturation at 94 ˚C for 1 min; primer annealing based on Tm for 2 min; primer extension at 72 °C for 2 min and final primer extension at 72 °C for 10 min (Saini *et al*., 2013) [27] for both RAPD and cytochromes P_{450} gene based primers. Annealing temperature of each primer was calculated using Oligo Analyzer 3.1 (http://www.idtdna.com/analyzer/Applications/Oligo Analyzer). The Amplified products were separated on 1.8 % agarose gel, stained with ethidium bromide (0.001%) and gel images were documented.

Table 1: List of different accessions of Chilli (*Capsicum annuum* L.) and their source of collection used in present study for genetic variability assessment.

S/No.	Code	Genotype name	Source of the breeding line Collection
	G1	DCA-136	HRS, Devihosur
	G2	DCA-192	HRS, Devihosur
	G3	DCA-199	HRS, Devihosur
	G4	DCA-223-1	HRS, Devihosur
	G5	Assam Chilli	HRS, Devihosur
	G6	Byadagi Kaddi	HRS, Devihosur
	G7	EC-28-DPS-06-07-01	COH, Bagalkot
	G8	GC-07-03	COH, Bagalkot
	G9	CH-1	COH, Bagalkot
	G10	HC-07-05	COH, Bagalkot
	G111	HC-0702	COH, Bagalkot
	G12	GPC-82	COH, Bagalkot
	G13	Pusa Jwala	COH, Bagalkot
	G14	Byadagi Dabbi	COH, Bagalkot
	G15	GC-07-02	COH, Bagalkot
	G16	HC-0714	COH, Bagalkot

HRS- Horticulture Research Station, COH- College of Horticulture

Data analysis

For data analysis, RAPD and Cytochrome P_{450} gene based primers which are well resolved and consistently reproducible banding pattern ranging from 150 to 3000 bp were selected. Amplified DNA fragments were scored manually as, present (1) or absent (0) for each analysis based on binary coding. Bands with similar migration patterns were considered as homologous and recorded cafefully. NTSYS-pc version 2.1 was used to compute pair-wise similarity matrices and similarity coefficient by means of SIMQUAL program. Dendrogram were constructed by using similarity matrix (Jaccard 1998) by the un-weighed pair-group method with arithmetical averages (UPGMA) (Rohlf, 2000). Boots strap values were calculated using Winboot software analysis system. Primer banding characteristics such as number of scoreable, number of polymorphic band, and percentage of polymorphic bands were calculated manually.

Polymorphism information content (PIC) of a band was calculated according to Anderson *et al*., (1993), $PIC = 1- \Sigma pi^2$, (pi= frequency of the i^{th} allele pattern), average heterozygosity was calculated by taking the average of each PIC value. Per cent polymorphism was calculated as, Per cent Polymorphism = p/ (m + p) and Multiplex ratio (MR) for each marker was calculated as MR = (m + p)/n. where p = is total number of polymorphic bands, m= total number of monomorphic band, p = total number of polymorphic bands and n= total number of primer combinations used (Powell *et al*., 1996) [24].

Results

RAPD Analysis

RAPD analyses were performed using 50 random primers, among these 35 RAPD markers produced polymorphic bands across the 16 genotypes. A total of 222 bands were produced from 35 markers. The sizes of amplified product were ranged from 150 to 3000bp. A representative gel profile obtained by each line by primer OPB-07 and OPB-03 is shown in the (Fig.1a and 1b). The numbers of scorable fragment produced per primer were ranged from 3 (OPJ-10; OPJ-05; OPB-07; OPD-16) to 12 (OPB-17) with an average of 6.34 bands per primer. Among 222 bands 74 bands were polymorphic with 37.625 % polymorphism (Table 2). The PIC value shows the allele diversity which is a measure of informativeness of a marker, were found to be from 0.203 (OPB-07) to 0.895 (OPB-17). The mean heterozygosity was fond to be 0.725 (average heterozygosity).

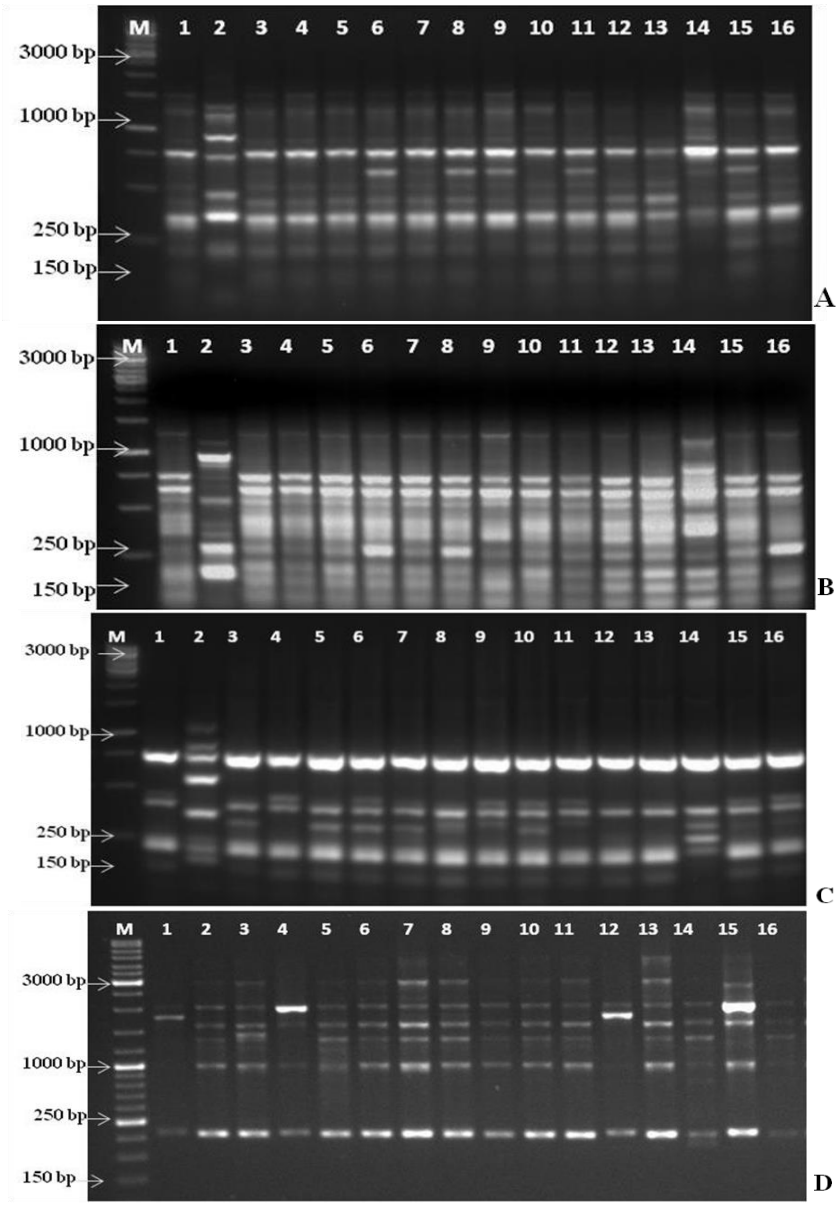

Fig 1: Banding profile of 16 advance breeding lines of Chilli (*Capsicum annuum*) with Cytochrome P_{450} gene based marker with primer combination (A) CYP2B6F, (B) CYP1A1 and RAPD Primer (C) OPA-07 (D) OPB-03 M= 1 kb ladder.

Table 2: Summary of genetic analyses obtained by using 34 RAPD primers for 16 breeding lines of Chilli (*Capsicum annuum* L.)

s/no	Primer name	Primer sequence	Tm value of primer (°C)	Polymorphic bands/total no. of bands	Per cent Polymorphism	PIC
1.	RAPD 1	CCGCCGATGG	37 °C	1/6	16.67	0.752
2.	RAPD 2	CCACACTACC	37 °C	2/6	33.33	0.772
3.	RAPD 3	CGGCCACTGT	37 °C	1/5	20.00	0.648
4.	RAPD 4	CGGCCCCGGC	37 °C	3/6	50.00	0.791
5.	RAPD 5	CGGAGAGCGA	37 °C	2/5	40.00	0.619
6.	RAPD 6	GACGGAGCAG	37 °C	1/4	25.00	0.579
7.	RAPD 7	GAAGAACCGC	37 °C	0/4	0.00	0.557
8.	RAPD 8	GACGGATCAG	37 °C	1/6	16.67	0.705
9.	RAPD 9	CGGAGAGCCC	37 °C	3/3	100.00	0.660
10.	RAPD 10	GGGTAACGCC	37 °C	2/8	25.00	0.847
11.	RAPD 11	GGACTGGAGT	37 °C	2/6	33.33	0.770
12.	RAPD 12	CGGCCCCGGT	37 °C	3/11	27.27	0.819
13.	OPJ-10	AAGCCCGAGG	37 °C	1/3	33.33	0.706
14.	AV-08	CACCGATCCA	37 °C	4/4	100.00	0.765
15.	OPJ-01	CCCGGCATAA	37 °C	8/8	100.00	0.791
16.	C-20	ACTTCGCCAC	37 °C	2/7	28.57	0.825
17.	OPB-03	CATCCCCCTG	37 °C	1/8	12.50	0.809
18.	OPA-07	CCGATATCCC	37 °C	3/9	33.33	0.862
19.	OPC-03	GGGGGTCTTT	37 °C	4/4	100.00	0.707
20.	OPB-04	GGACTGGAGT	37 °C	1/8	12.50	0.770
21.	OPB-17	GACCGCTTGT	37 °C	2/12	16.67	0.895
22.	OPJ-05	CTCCATGGGG	37 °C	1/3	33.33	0.583
23.	OPA-AC07	GTGCCCGATC	37 °C	2/8	25.00	0.820
24.	OPA-06	GGTCCCTGAC	37 °C	3/6	50.00	0.691
25.	OPA-09	GAAACGGGTG	37 °C	2/6	33.33	0.702
26.	OPA-11	CAATCGCCGT	37 °C	3/10	30.00	0.892
27.	OPA-14	CTCGTGCTGG	37 °C	1/7	14.29	0.825
28.	OPB-07	GGTGACGCAG	37 °C	1/3	33.33	0.203
29.	OPC-06	GAACGGACTC	37 °C	2/4	50.00	0.732
30.	OPC-20	ACTTCGCCAC	37 °C	2/7	28.57	0.806
31.	OPD-16	AGGGCGTAAG	37 °C	3/3	100.00	0.704
32.	OPJ-04	CCGAACACGG	37 °C	2/11	18.18	0.762
33.	OPM-16	GTAACCAGCC	37 °C	1/6	16.67	0.680
34.	OPJ-06	TCGTTCCGCA	37 °C	2/10	20.00	0.702
35.	C-16	CCACATCCAG	37 °C	2/5	40.00	0.623
36.	Average	_	_	74/222	37.625	*Hav= 0.725

Where, PIC - Polymorphic information content

Cytochrome P_{450} gene based markers analysis

Analysis of cytochromes P_{450} gene based markers was carried out using 17 cytochromes P_{450} primers. Of the 17, 11 primers produced polymorphic reproducible bands which are further selected for variability assessment. A total of 112 scoreable bands were produced in 16 breeding lines with amplicon size varying from 200-3000 bp from selected 11 set of primer combination. Of the 112 bands 32 bands were polymorphic with an average of 10.18 numbers of bands per primer. Maximum bands were observed in primer cyto04 (15) and minimum bands in CYP2C19 (4). A total of 29.17 % polymorphism observed, with an average PIC value of 0.96 (Table 3). The PIC value recorded were ranging from 0.822 (cyto04) - 0.962 (cyp2C19). A representative gel profile obtained by primer Cyp2B6F and Cyp1A1 is shown in the (Fig. 1c and 1d).

Statistical Report

Jaccard similarity coefficient reflects the genomic variability among the breeding lines, was calculated based on the Binary data (1-0) obtained by amplification of RAPD and cytochromeoP450 gene based marker. In RAPD marker (Table 4) similarity coefficient was found in between 0.212 (between G2 and G1) to 0.912 (G9 and G8) and in cytochromeo P_{450} gene based marker (Table 5) the similarity was from 0.450 (between G6 and G2) to 0.927 (between G8 and G6). The UPGMA analysis clearly displaces the genotypic relation for all the genotype, based on RAPD and Cytochrome P_{450} gene based markers data. The RAPD marker showed the clear distribution of 16 lines into two major and two minor clusters (Fig. 2) From the analysis it is clear that line G2 and G13 lines are farthest related to other breeding lines. Cluster1 consist of a single breeding line G2, whereas second cluster again subdivided in to two minor sub-clusters where the 14 lines grouped into one sub-cluster and G13 into another. The dendrogram obtained using Cytochrome P_{450} gene based markers also distributed the 16 breeding lines in to two main and two sub-clusters (Fig. 3). Here the line G2 and G5 are distinctly related to G1, G3 and G4 which are grouped into separate cluster. Bootstrap analysis was carried out from the binary data of both the marker, high bootstrap value at major nodes of the dendrogram of both the marker shows the robustness of the marker system used.

The pearson's correlation co-efficient was determined by mantles test using the matrices generated by these marker and coefficient correlation between RAPD and Cytochrome P_{450} gene based markers was 0.884. The principal component analysis of both the marker revealed that the breeding line belongs to particular cluster were grouped together in PCA plots (data shown in supplementary table 6). The comparison of both functional (cytochromeo P_{450} gene marker) and non-functional were carried out on the basis of Marker index, Average heterozygosity (Hav), Multiplex ratio (MR) and Percentage polymorphism as in the (Table 5). Highest per

cent polymorphism was observed in RAPD marker (37.62 %) compared to cytochromeo P_{450} gene based marker (29.17 %). In assessment of breeding lines, Marker index which is a measure to examine the overall usefulness of the marker from all the parameters was found highest in cytochromeo P_{450} gene based marker indicating these marker are most efficient in generating genomic diversity among the breeding lines.

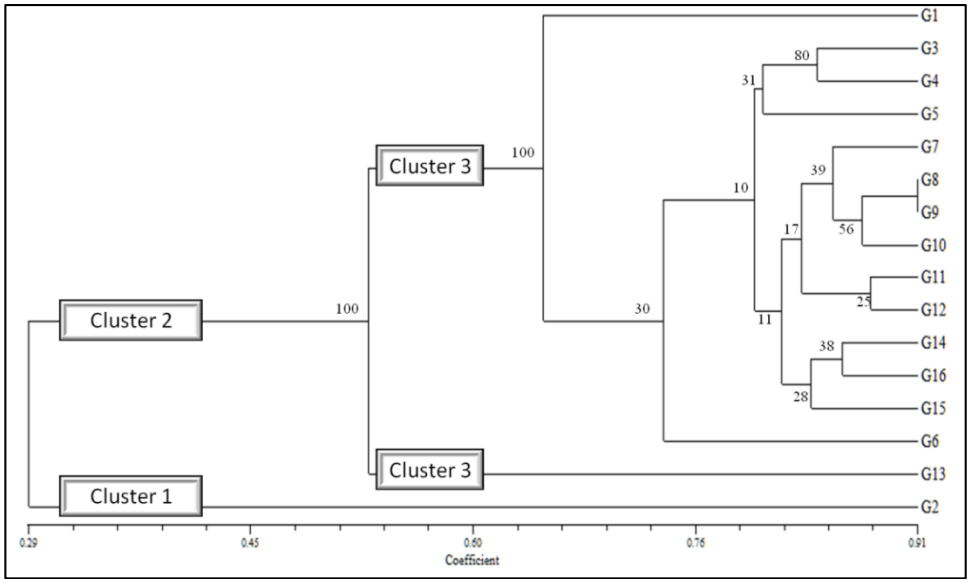

Fig 2: UPGMA Cluster analysis displaying genetic variability among advance breeding line of chilli (*Capsicum annuum*) produced by RAPD marker.

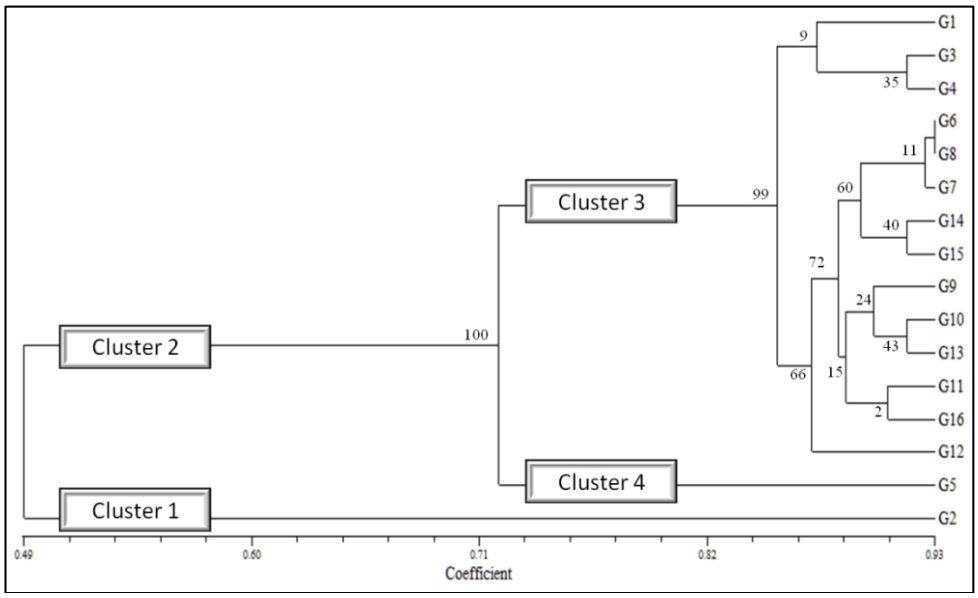

Fig 3: UPGMA Cluster analysis displaying genetic variability among advance breeding line of Chilli (*Capsicum annuum*) produced by Cytochrome P_{450} gene based marker.

Table 3: Summary of genetic analyses obtained by using cytochrome p450 for 16 breeding lines of Chilli (*Capsicum annuum* L.)

s/no	Primer name	Primer sequence	Tm value of primer (°C)	Polymorphic bands/total no. of bands	Per cent polymorphism	PIC
	CYP1A1	(F)- GCCAAGCTTTCTAACAATGC (R)- AAGGACATGCTCTGACCATT	52 °C	2/10	20.00	0.985
	CYP2B6F	(F)- GACTCTTGCTACTCCTGGTT (R)- CGAATACAGAGCTGATGAGT	52 °C	3/9	33.33	0.979
	CYP2C19	(F)- TCCTTGTGCTCTGTCTCTCA (R)- CCATCGATTCTTGGTGTTCT	52 °C	2/4	50.00	0.822
	Cyt01	(F)- GATGGTCTTCCGCGGTA (R)- CACTGGAAGGCGTGCA	52 °C	2/8	25.00	0.965
	Cyt02	(F)- CGGCTTGCTCATGGA (R)- GAGAAATAGGTGCGTGGA	52 °C	4/12	33.33	0.981
	Cyt03	(F)- GACCCAAGCAACGTCA (R)- GTGGGTTATGGCCCACA	52 °C	2/12	16.67	0.988
	Cyt04	(F)- GACGTGCCACTCTGCA	52 °C	6/15	40.00	0.990

		(R)- ACCCTAGGCTAAGGTGGA				
	Cyt05	(F)- CCACCTTGACGACCCAA (R)- TGGCCCACATATTCACCA	52 °C	4/9	44.44	0.971
	Cyt06	(F)- ACGTGCCACTCTGCAA (R)- ACCCTAGGCTAAGGTGGA	52 °C	4/13	30.77	0.971
	Cyt07	(F)- CCTGTACGACCCAAGCA (R)- TGGCCCACATATTCACCA	52 °C	3/11	27.27	0.982
	CYP1A1	(F)- GCCAAGCTTTCTAACAATGC (R)- AAGGACATGCTCTGACCATT	52 °C	0/9	0.00	0.953
	Average	_	_	32/112	29.17	*Hav= 0.96

Table 4: Jaccard similarity coefficients among 16 breeding lines of chilli (*Capsicum annuum* L.) generated from binary matrix of RAPD

Rows/col	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16
G1	1.000															
G2	0.212	1.000														
G3	0.637	0.301	1.000													
G4	0.666	0.285	0.842	1.000												
G5	0.680	0.292	0.800	0.807	1.000											
G6	0.603	0.256	0.714	0.745	0.701	1.000										
G7	0.666	0.285	0.842	0.785	0.843	0.745	1.000									
G8	0.610	0.301	0.803	0.750	0.800	0.741	0.875	1.000								
G9	0.620	0.305	0.816	0.793	0.814	0.783	0.857	0.912	1.000							
G10	0.655	0.315	0.790	0.766	0.785	0.730	0.827	0.850	0.896	1.000						
G11	0.649	0.305	0.847	0.793	0.781	0.725	0.824	0.847	0.862	0.833	1.000					
G12	0.637	0.319	0.833	0.779	0.767	0.714	0.810	0.833	0.816	0.819	0.879	1.000				
G13	0.454	0.263	0.541	0.492	0.538	0.453	0.514	0.541	0.549	0.534	0.571	0.563	1.000			
G14	0.690	0.291	0.770	0.745	0.763	0.682	0.775	0.770	0.783	0.758	0.813	0.800	0.579	1.000		
G15	0.679	0.328	0.793	0.833	0.823	0.789	0.800	0.824	0.872	0.842	0.839	0.824	0.500	0.821	1.000	
G16	0.660	0.291	0.830	0.807	0.796	0.737	0.839	0.800	0.844	0.816	0.877	0.830	0.557	0.859	0.854	1.000

Table 5: Jaccard similarity coefficients among 16 breeding lines of Chilli (*Capsicum annuum* L.) generated from binary matrix of Cytochrome P_{450} gene based marker.

Rows/col	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16
G1	1.000															
G2	0.513	1.000														
G3	0.878	0.490	1.000													
G4	0.864	0.468	0.914	1.000												
G5	0.666	0.558	0.689	0.711	1.000											
G6	0.837	0.450	0.905	0.882	0.711	1.000										
G7	0.806	0.481	0.882	0.887	0.734	0.923	1.000									
G8	0.828	0.459	0.878	0.882	0.738	0.927	0.923	1.000								
G9	0.806	0.500	0.837	0.869	0.743	0.869	0.900	0.906	1.000							
G10	0.824	0.500	0.846	0.878	0.734	0.878	0.882	0.887	0.909	1.000						
G11	0.833	0.481	0.864	0.869	0.761	0.878	0.882	0.869	0.900	0.909	1.000					
G12	0.779	0.545	0.828	0.869	0.734	0.869	0.873	0.860	0.864	0.891	0.891	1.000				
G13	0.819	0.513	0.833	0.864	0.711	0.864	0.869	0.864	0.887	0.914	0.887	0.869	1.000			
G14	0.833	0.454	0.873	0.887	0.716	0.905	0.891	0.905	0.891	0.900	0.900	0.864	0.914	1.000		
G15	0.801	0.477	0.860	0.864	0.711	0.891	0.869	0.864	0.860	0.869	0.878	0.842	0.882	0.914	1.000	
G16	0.846	0.486	0.878	0.864	0.702	0.882	0.869	0.855	0.860	0.878	0.905	0.860	0.873	0.896	0.891	1.000

Table 6: Comparative summary of RAPD and Cytochrome P_{450} based marker analysis.

Sl. No	Molecular Marker	RAPD Marker	Cytochrome P_{450} gene based markers
1	Number of breeding lines	16	16
2	Total No. of bands	222	112
3	Number of Polymorphic bands	74	32
4	Percent polymorphism	37.625	29.17
5	Multiplex Ratio	6.34	10.18
6	Average Heterozygosity	0.725	0.97
7	Marker Index	4.59	9.77

Discussion

Chilli is an indispensable spice crop used as an important ingredient in a wide variety of cuisines in all part of the world. India is the largest producer of Chilli in the world and constitute around 17, 3000 ha of land under cultivation (National horticultural Board 2015) [16]. Chilli genotype constitutes a rich source of biodiversity, so understanding genetic variability will play a pivotal role for identification, conservation, utilization and cultivar development. There are several approaches *viz*., phenotypic, biochemical and genotypic markers are available for genetic variability assessment. Traditional methods based on phenotypic trait are

time consuming, laborious and difficult to discriminate individual species based on morphological data (Pickersgill *et al*., 1988) [23], due to interference of various environmental factors. In comparison with the traditional methods, gene based molecular marker provides the consistent and authenticated data which can be successfully employed for genetic variability assessment.

In Chilli, various marker system have been developed to understand the genetic variability among different species *viz*., morphological (Julia *et al*., 2012) [10], RAPD (Kang *et al*., 2001) [12], AFLP (Lee *et al*., 2004) [15], SSR (Paran *et al*., 1998) [21] and SNP (Jung *et al*., 2010) [11]. However very scanty information available on genetic variability assessment of advanced breeding lines. The present research focussed on diversity analysis of 16 advance breeding lines of chilli using combination of functional (Cytochrome P_{450} gene based) and of non-functional (RAPD based) marker.

RAPD markers have advantageous over other morphological markers as they produce vast number of bands independent of environmental effects. Previously Eric *et al*. (2005) [7] studied the genetic variability using RAPD in Chilli land races from northern new maxico and Ays *et al*. (2010) [2], reported genetic variability using 2,760 RAPD markers among 24 accessions belonging to 11 species of Capsicum. In our study, out of 50 RAPD primer tested 34 primers produced reproducible bands across 16 advance breeding lines of Chilli. Out of total 222 bands 74 (37.62%) were polymorphic in nature. Cluster analysis constructed based on binary data according to UPGMA method shows that G1 (DCA-136) is farthest related to G2 (DCA-192), indicating breeding lines have not distributed according to their geographical locations. Julia *et al*., (2012) [10], reported the similar results among different Chilli landraces from northern part of India. Similar results were also observed in other plants e.g Olea europaea (Bronzini de Caraffa *et al*., 2002) [5] and Andrographis paniculata (Padmesh *et al*., 1999) [19]. Contrasting results have also been reported where distribution of genotypes taken place based on their geographical locations (Panwar *et al*., 2010; Das *et al*., 2007) [20, 6]. The cluster or dendrogram obtained by marker data was bootstrapped using WINBOOT program with 2,000 replications (Hedges, 1992) [9] wherein high value obtained at major nodes suggest the robustness of the dendrogram.

The RAPD markers are the arbitrary dominant markers and generally record the genetic diversity in neutral regions of the genome and fail to distinguish diversity based on entire genome. Therefore, higher resolution marker are needed for functional genomic assessment in multi gene family particularly in advance breeding lines where there is peculiar need of crucial consideration of variability assessment (Kumar *et al*., 2017; Karp, 2002) [14, 27]. Cytochrome P_{450} gene based markers are mono-oxygenases gene family which play important role in biosynthesis of many secondary metabolites and in oxidative detoxification (Ohkawa *et al*., 1998) [17] in support many Cytochrome P_{450} gene families have found in various plant species (Yamanaka, 2003) [33]. In present research 11 Cytochrome P_{450} gene based markers produced 32 polymorphic bands among 112 bands with an average of 29.17 % polymorphism. Low percent polymorphism was produced by Cytochrome P_{450} gene based marker compared to RAPD, similar observation also recorded in genetic diversity assessment of commercially grown *Moringa oleifera* and in finger millet accessions (Saini *et al*., 2013; Panwar *et al*., 2010) [27, 20]. Cluster analysis based on UPGMA 16 lines distributed into two main clusters and two sub-cluster. Here line G2 is distinctly related to G1 similar as RAPD marker system with the high bootstrap value indicating robustness of the dendrogram. The comparison PIC value (a parameter for assessing impact of marker) of both the marker indicates that RAPD primer was from 0.203 to 0.895 in primer OPV-17 and OPV-07 with the mean of 0.725. PIC value of Cytochrome P_{450} gene based markers ranged from 0.822 (primer CYP2C19) to 0.990 (primer Cyt04) with an average heterozygosity of 0.97.

The pearson's correlation co-efficient was determined by mantles test and high similarity co-efficient was recorded (R^2 = 0.884) between both the marker indicting similar fit of similarity matrix. These results indicate that close relationship within breeding lines selected for severity assessment. Similar observations were made in thirty genotypes of finger millet (Das *et al*., 2007) [6]. The comparative analysis of RAPD and Cytochrome P_{450} gene based markers revels that Cytochrome P_{450} gene based markers gene are to be the most efficient marker for assessment of genetic diversity of advance breedling lines in Chilli as it generated high marker index (9.77), average heterogeneity (0.97) and multiplex ratio (10.181) compared to RAPD. These markers also proved to be most efficient in genetic evaluation of most of the species like as advance breeding lines of *Moringa oleifera* (Kumar *et al*., 2017) [27], and in rice species (Tanaka *et al*., 2001; Yamanaka *et al*., 2003) [30, 33].

From the above findings it can be used for authentication of genetic variability among the advance breeding lines of Chilli and the combination of functional and non-functional marker system provide the useful platform for recognition of stable high yielding lines for situ conservation cultivar development.

Conclusion

In assessment of 16 advance breeding lines of Chilli using RAPD and Cytochrome P_{450} gene based markers, both the markers revealed high level of genetic variability within the breeding lines. Thus detection of high level polymorphism, will definitely plays an important role in crop breeding strategies, which can be useful for selection of parental line with a goal of high yield, as Chilli is one the major spice and have huge commercial value. These variations, among the cultivars can be explored for crop breeding strategies for production of Nutraceutically rich, high yielding variety with better adaptability to vast climatic condition and resistant to various biotic stresses.

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Compliance with ethical standards

Conflict of interest the authors declare that no conflict of interest exists.

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