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RESEARCH ARTICLE

Assessment of Molecular Diversity and Phylogenetic Analysis Among Chilli Genotypes Using Start Codon Targeted (SCoT) Markers

Hanuman Ram[1], DK Samadia[1], Chet Ram[1]* and AK Verma[1]

Abstract

Assessment of genetic diversity is pivotal to identify genotypes for the crop improvement programme in chilli. Therefore, the present study was carried out to identify and characterise genetic variation among 45 chilli genotypes using Start Codon Targeted Polymorphism (SCoT) markers. Out of 18 primers, ten markers revealed significant polymorphism among the chilli accessions studied, with amplification ranging from 280 bp to 2.0 kb. A total of 81 alleles were obtained, ranging from 5 to 10 alleles, with an average of 8.1 alleles per primer. Out of 81 alleles, sixty-two (76.54%) were found polymorphic in nature. The genetic similarity among the analysed genotypes ranged from 24.2 to 100%. The UPGMA dendrogram was constructed to establish the genetic relationship among the chilli genotypes using Jaccard's coefficient. Cluster analyses grouped 45 genotypes into two major groups, which was also supported by principal coordinate analysis (PCoA). Analysis of molecular variance (AMOVA) showed a significant estimated value at a degree of 999 permutations. The percentage of variability was higher among populations (55%) than within populations (45%). The mean Nei's gene diversity (h) was 0.2233 and the mean Shannon's Information Index (i) was 0.3470, with the highest of both given by the SCoT-7 marker. The SCoT marker-based distinguishable genetic variation in the chilli genotypes may serve as a potential aid in chilli improvement programme. It demonstrates the utility of SCoT markers for estimating molecular diversity and genotype identification in chilli. This study also suggests the potential of SCoT markers in further association studies in chilli.

Keywords: Chilli, Diversity, PCoA, Polymorphism, SCoT markers

[1]ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan, 334006, India.

***Author for correspondence:**
chet.ram2@icar.org.in

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Introduction

Chilli, scientifically categorized under the genus *Capsicum*, is one of the important members of the Solanaceae family. Native to Central and South America, chilli was domesticated more than 6,000 years ago and is now used extensively in industry, medicine, and cooking all over the world (Bosland and Votava, 2012). Among *Capsicum* spp., *Capsicum annuum*, *C. chinense*, *C. frutescens*, *C. baccatum*, and *C. pubescens* are the main domesticated species (Pickersgill, 2007). Chillies are also nutritionally dense, containing high levels of vitamin C, minerals, amino acids, provitamin A (β-carotene), flavonoids, and phytochemicals, making them useful for pharmacological and dietary applications (Materska and Perucka, 2005; Olatunji and Afolayan, 2018; Palma-Orozco *et al.*, 2021). Capsaicinoids have been studied for their involvement in pain relief, metabolism stimulation, and cardiovascular protection in addition to their antioxidant and antimicrobial activities (Reyes-Escogido *et al.*, 2011). Due to their agronomic

importance, biological diversity, and therapeutic potential, chilli continues to be a focal point of research in plant science, nutrition, and pharmacology. Chillies are still focused on by plant science, pharmacology, and nutritional studies due to their genetic diversity, therapeutic potential, and agronomic importance.

Plant adaptation, resilience, and evolution are significantly influenced by genetic diversity. Genetic diversity at the molecular level includes differences across and within plant populations in terms of DNA sequences, gene architectures, allelic frequencies, and genome organization (Frankham *et al*., 2002). Phenotypic traits diversity, including disease resistance, drought tolerance, yield, and quality are based on these molecular differences and is essential for crop improvement and breeding. In addition to managing genetic resources and identifying unique genotypes, an understanding of molecular genetic diversity is necessary for mapping genes associated with desired agronomic qualities using marker-assisted selection (MAS) and association mapping (Gupta *et al.*, 2001).

The researchers have characterized several vegetable germplasm, including chilli, using morphological markers (Rahevar *et al.*, 2021; Samadia *et al.*, 2024). However, the genetic diversity inferred by morphological markers is not precise due to the influence of environmental factors. Thus, molecular markers can provide accurate diversity at the molecular level. Maintaining and leveraging genetic variability at the molecular level is pivotal for sustainable agriculture and food security in the face of habitat loss and climate change. The evaluation of genetic diversity in plants has been completely transfigured by the advancement of molecular markers. Numerous methods have been executed to measure and examine genetic variability, including RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeats), and SNP (Single Nucleotide Polymorphisms) (Powell *et al.*, 1996; Semagn *et al.*, 2006). In continuation, the short, conserved areas that surround the ATG translation initiation codon in plant genes serve as the basis for the new class of gene-targeted molecular markers known as Start Codon Targeted (SCoT) markers (Collard and Mackill, 2009). The functional nature of SCoT markers, which target conserved gene areas, makes them more trustworthy for identifying functional genetic variation related to the trait of interest than other anonymous markers like RAPD or AFLP. They are cost-effective and adaptable to a variety of plant species; they are useful in genetic diversity analysis, DNA fingerprinting, population structure assessment, phylogenetic studies, and QTL mapping; they are highly reproducible because longer primers and higher annealing temperatures are needed; and they don't require prior sequence information; able to identify polymorphisms in coding areas, which are frequently linked to adaptability and expressed traits. These markers' broad applicability in plant breeding, conservation genetics, and cultivar identification has been demonstrated by their successful use in a range of crops, including rice (*Oryza sativa*), wheat (*Triticum aestivum*), grapes (*Vitis vinifera*), and potato (*Solanum* spp.) (Xiong *et al.*, 2011; Gorji *et al.*, 2011). However, the utilization of SCoT markers for assessing genetic diversity is limited in chilli (Tsaballa *et al.*, 2015; Gupta *et al.*, 2019; Igwe *et al.*, 2019). The existing research gap limits the utility of gene-targeted molecular diversity for enhancing chilli breeding programmes. Therefore, the current investigation was formulated with the aims to (1) harness the SCoT marker-based genetic potential in chilli, (2) identify the unique genotype and (3) phylogenetic relationship among the chilli genotypes. Thus, the study was performed to assess molecular diversity and phylogenetic relationship among 45 chilli genotypes by employing 10 SCoT markers. The study highlights the promising potential of SCoT markers for advancing chilli crop improvement programmes.

Materials and Methods

Plant materials

The experimental material for the present investigation comprised 45 chilli genotypes, including four adapted varieties (JCA-283, Pusa Jwala, Pusa Sadabahar and RCH-1) (Table 1). The seeds of each genotype were sown

Table 1: List of genotypes used for molecular diversity and phylogenetic analysis

S. No.	Accession/ collector number	IC number	Source	S. No.	Accession/ collector number	IC number	Source
1.	AHM-05	IC-347001	ICAR-CIAH, Bikaner	6.	AHM-93	IC-347089	ICAR-CIAH, Bikaner
2.	AHM-31	IC-347027	ICAR-CIAH, Bikaner	7.	AHM-115	IC-347111	ICAR-CIAH, Bikaner
3.	AHM-35	IC-347031	ICAR-CIAH, Bikaner	8.	Chilli thin-1	--	ICAR-CIAH, Bikaner
4.	AHM-42	IC-347038	ICAR-CIAH, Bikaner	9.	Chilli thin-2	--	ICAR-CIAH, Bikaner
5.	AHM-55	IC-347051	ICAR-CIAH, Bikaner	10.	Chilli thick-1	--	ICAR-CIAH, Bikaner

S. No.	Accession/ collector number	IC number	Source	S. No.	Accession/ collector number	IC number	Source
11.	Chilli-dwarf	--	ICAR-CIAH, Bikaner	29.	AHM-192	IC-446364	ICAR-CIAH, Bikaner
12.	KSB-9	IC-261862	ICAR-CIAH, Bikaner	30.	AHM-193	IC-446365	ICAR-CIAH, Bikaner
13.	AHM-154	IC-446326	ICAR-CIAH, Bikaner	31.	AHM-194	IC-446366	ICAR-CIAH, Bikaner
14.	AHM-162	IC-446334	ICAR-CIAH, Bikaner	32.	AHM-197	IC-446369	ICAR-CIAH, Bikaner
15.	AHM-164	IC-446336	ICAR-CIAH, Bikaner	33.	AHM-198	IC-446370	ICAR-CIAH, Bikaner
16.	AHM-165	IC-446337	ICAR-CIAH, Bikaner	34.	AHMHR-01	--	ICAR-CIAH, Bikaner
17.	AHM-167	IC-446338	ICAR-CIAH, Bikaner	35.	MC-2	--	ICAR-CIAH, Bikaner
18.	AHM-172	IC-446344	ICAR-CIAH, Bikaner	36.	MC-1	--	ICAR-CIAH, Bikaner
19.	AHM-175	IC-446347	ICAR-CIAH, Bikaner	37.	JCA-283	--	JNKVV, Jabalpur
20.	AHM-177	IC-446349	ICAR-CIAH, Bikaner	38.	Pusa Jwala	--	ICAR-IARI, New Delhi
21.	AHM-179	IC-446351	ICAR-CIAH, Bikaner	39.	Pusa Sadabahar	--	ICAR-IARI, New Delhi
22.	AHM-180	IC-446352	ICAR-CIAH, Bikaner	40.	RCH-1	--	AU, Jodhpur
23.	AHM-181	IC-446353	ICAR-CIAH, Bikaner	41.	AHMHR-02	--	ICAR-CIAH, Bikaner
24.	AHM-182	IC-446354	ICAR-CIAH, Bikaner	42.	AHMHR-03	--	ICAR-CIAH, Bikaner
25.	AHM-183	IC-446355	ICAR-CIAH, Bikaner	43.	AHMHR-04	--	ICAR-CIAH, Bikaner
26.	AHM-184	IC-446356	ICAR-CIAH, Bikaner	44.	AHMHR-05	--	ICAR-CIAH, Bikaner
27.	AHM-189	IC-446361	ICAR-CIAH, Bikaner	45.	AHMHR-06	--	ICAR-CIAH, Bikaner
28.	AHM-191	IC-446363	ICAR-CIAH, Bikaner				

in a protective vegetable nursery bed (Ram *et al.*, 2024) at the vegetable research field of ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan, India (28.1115° N and 73.3497° E). The seedlings were watered thrice a week with a drip irrigation system. The healthy and disease-free leaf samples were collected from nursery-staged seedlings (28-days-old). The leaf samples were wrapped in the aluminium foil and immediately frozen in liquid nitrogen (-196°C) and further stored at a deep freezer (-80°C) for DNA isolation.

DNA isolation and SCoT marker profiling

Molecular work was undertaken at the Molecular unit, Biotechnology laboratory, ICAR-CIAH, Bikaner. The genomic DNA was isolated from 100 mg of fresh young leaf tissue following the CTAB method in the presence of liquid nitrogen with some minor modifications (Murray and Thompson, 1980). DNA was precipitated with chilled iso-propanol, and subsequently, the DNA pellet was rinsed with 70% ethanol for 10-15 min. to remove excess amount of CTAB and other salt precipitates. The pellet was dried at room temperature for up to 1 h and dissolved in TE buffer (*p*H=8). The purified DNA was quantified electrophoretically on 0.8% agarose gel. The genomic DNA was diluted to 10 ng/µl as a working stock for PCR analysis. PCR reaction was carried out using a Thermal Cycler machine (Bio-Rad Laboratories Inc., USA) in a 15 µl reaction volume, composed of 2.0 µl of 10 ng genomic DNA, 7.5 µl of 2X PCR master mixture (Takara Bio Inc., Japan), 0.5 µl $Mgcl_2$, 2.0 µl SCoT primer and 3.0 µl nuclease-free water (Himedia, India). PCR tubes containing the reaction components were gently tapped and given a brief spin to allow proper settling of the reaction mixture. PCR amplification was performed with initial denaturation at 94°C for 3 min. followed by 40 cycles of denaturation at 94°C for 30 s, annealing step at 45°C for 30 s, primer extension at 72°C for 2 min. and a final extension at 72°C for 10 min.

Gel electrophoresis and allele scoring

The amplicons were resolved electrophoretically on 1.5% agarose gel prepared in 1XTBE buffer. The DNA fragments (SCoT alleles) were visualized by staining with ethidium bromide (10 mg/ml) and finally documented using a gel documentation system (Syngene, USA). The amplicons were scored as alleles for each of the SCoT marker loci. The alleles were scored manually as a binary matrix (0 and 1, where 0 and 1 are considered as absence and presence of the alleles, respectively). The allele sizes (base pairs) were determined by comparing with a 100 bp standard DNA ladder (Bio-Helix, Taiwan). The allele data (binary matrix) from all the markers were used for statistical analysis.

Clustering and population structure analysis

The binary matrix data were further used for clustering analysis among the genotypes. The clustering was inferred using Jaccard's coefficient, and a tree was obtained using the UPGMA model in NTSys 2.0 software (Rohlf, 2000). To determine the number of population structures present among the 45 chilli genotypes, the

binary data were analysed in STRUCTURE 2.0 software with the admixture model (Pritchard *et al.*, 2000).

Statistical analysis

Total number of alleles, major allele frequency, gene diversity, heterozygosity and polymorphic information content (PIC) were estimated using PowerMarker V3.25 (Liu and Muse, 2005). Any allele appearing in only one genotype was considered aunique allele, whereas an allele with a frequency of <0.05 was considered arare allele. Genetic dissimilarity was calculated for pairwise comparison of genotypes using Jaccard's coefficient with 1000 bootstraps. Principal coordinate analysis (PCoA) was calculated to supplement the clustering pattern (Gower, 1966). SCoT allelic composition for each genotype at every marker locus was determined by counting the number of alleles per locus and the allele frequencies and polymorphism information content (PIC) were determined according to the formula given by Botstein *et al.*, (1980).

$$PIC = \left(1 - \sum_{i=1} \hat{p}_i^2\right) \frac{2n}{2n-1} - \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2\hat{p}_i^2 \hat{p}_j^2$$

Where *pi* was the estimated allele frequencies of *k* alleles (*i*=1 to *k*) and *n* was the number of individuals. Analysis of Molecular Variance (AMOVA) was carried out using GenAlEx 6.5 (Peakall and Smouse, 2012). In addition, genetic indices such as observed number of alleles (na), number of effective alleles (ne), Shannon's information index (i), Nei's observed heterozygosity (h) and expected heterozygosity (*He*) were also calculated using GenAlEx v6.503 (Peakall and Smouse, 2012).

Results and Discussion

SCoT allele characteristic and polymorphism

To assess the SCoT marker-based polymorphism among the chilli genotypes, eighteen SCoT markers were profiled on the genomic DNA of chilli genotypes. Consequently, ten SCoT markers were amplified polymorphic alleles among the genotypes (Fig. 1) and used for the genetic diversity assessment among chilli genotypes. Eight SCoT markers were excluded from the analysis due to their monomorphic nature of amplification. A total of 81 alleles were obtained, ranging from 5 to 10 alleles, with an average of 8.1 alleles per locus. Out of 81 alleles, 62 (76.54%) were found polymorphic in nature. The allele size ranged from 280 bp to 2.0 kb, thereby revealing a significant level of genetic diversity among the chilli genotypes. Similarly, the genetic criteria pertain to efficiency of the markers, such as percentage of polymorphism, PIC, *He* and resolution power, which also showed significance values among the chilli genotypes. The moderate level of PIC value ranged from 0.25 to 0.58, with a mean value of 0.40 generated by the SCoT markers. Likewise, the *He* value ranged from 0.30 to 0.66 with a mean of 0.46. Additionally, SCoT markers produced resolution power ranging from 1.82 to 4.58 among chilli genotypes. On the contrary, the four SCoT markers, namely SCoT2, SCoT3, SCoT10 and SCoT11, were unable to produce resolutions among the alleles. Statistics of the polymorphism are presented in Table 2. The SCoT

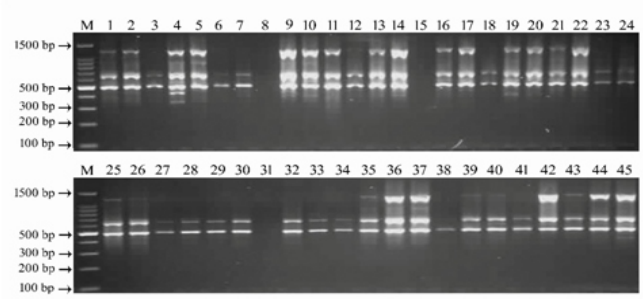

Fig. 1: Representative gel image of SCoT7 amplification on genomic DNA of chilli genotypes.
The M and numerical 1-45 indicate the 100 bp standard DNA ladder and chilli genotypes, respectively.

Table 2: Polymorphism and marker efficiency of analysed SCoT markers in chilli

Marker	Sequence (5' – 3')	Amplicon size (bp)	No. of alleles	%Polymorphism	He	PIC value	Rp
SCoT2	CAACAATGGCTACCACCC	300-1100	10	70.00	0.49	0.44	--
SCoT3	CAACAATGGCTACCACCG	500-1600	10	90.00	0.62	0.54	--
SCoT4	CAACAATGGCTACCACCT	450-1000	5	60.00	0.30	0.25	1.82
SCoT5	CAACAATGGCTACCACGA	500-1000	6	83.33	0.49	0.37	2.76
SCoT6	CAACAATGGCTACCACGC	400-1000	8	62.50	0.47	0.36	3.64
SCoT7	CAACAATGGCTACCACGG	380-2000	10	90.00	0.42	0.33	3.91
SCoT8	CAACAATGGCTACCACGT	300-1350	9	88.89	0.47	0.36	4.58
SCoT9	CAACAATGGCTACCAGCA	280-870	7	85.7	10.44	0.34	2.53
SCoT10	CAACAATGGCTACCAGCC	550-1200	10	0.00	0.66	0.58	--
SCoT11	AAGCAATGGCTACCACCA	400-750	7	71.43	0.55	0.46	--
Mean	---	---	8.1	60.00	0.49	0.40	2.41

markers, as new gene-targeted marker systems, have been substantially utilised for characterizing germplasm of numerous crop plants, including solanaceous crops (Shahlaei *et al.*, 2014; Gupta *et al.*, 2019; Kulyan *et al.*, 2023; Altaf *et al.*, 2025). The SCoT marker has been considered as an effective and reproducible marker and resulted in high polymorphism correlated with traits of biological interest as compared to other random marker systems (Collard and Mackill, 2009; Shahlaei *et al.*, 2014). The SCoT marker analysed in the present study generated a substantial level of polymorphism among the chilli genotypes, indicating a wide level of genomic divergence among the accessions. Out of eighteen SCoT primers screened, ten primers were found polymorphic, while eight primers were excluded from the analysis due to their monomorphic allele pattern. The SCoT alleles are in accordance with the inherent nature of gene-targeted SCoT marker systems, where amplification efficiency varies according to primer–genome specificity (Collard and Mackill, 2009). The total of 81 alleles obtained with an average of 8.1 alleles per locus and 76.54% polymorphism indicated a higher resolution capacity of SCoT markers in chilli, demonstrating their power in revealing genomic variability in chilli in the present study. Similar high allele richness and polymorphism using SCoT markers have been reported earlier in *Capsicum* spp. (Gupta *et al.*, 2019; Igwe *et al.*, 2019; Abou-Sreea *et al.*, 2021). The moderate PIC values (0.25–0.58) and *He* values (0.30–0.66) obtained in this experiment further confirm the efficiency and informativeness of SCoT markers as powerful molecular tools for genetic diversity assessment among chilli genotypes. The findings of the present investigation were consistent with previous findings in *Capsicum* and other Solanaceae crops (Feng *et al.*, 2018; Gupta *et al.*, 2019; Igwe *et al.*, 2019; Shilpa *et al.*, 2021). The resolution power (Rp), ranging from 1.82 to 4.58, additionally indicated that these primers possess strong discriminatory potential in differentiating chilli genotypes at the molecular level. However, some markers (SCoT2, SCoT3, SCoT10 and SCoT11) have not produced allelic resolutions, which might be due to the low level of polymorphism in the present study. The similar discriminatory potential of SCoT markers in terms of resolution power has been observed in various crops (Bhawna *et al.*, 2017; Igwe *et al.*, 2017; Rai, 2023).

Genetic diversity analysis among chilli genotypes

Based on the genetic diversity indices such as observed number of alleles (na), number of effective alleles (ne), Shannon's information index (i) and Nei's observed heterozygosity (h), all the chilli genotypes showed a significant level of genetic diversity (Table 3). The observed and effective number of alleles generated by 10 SCoT markers in 45 chilli genotypes ranged from 1.40 (SCoT4) to 2.00 (SCoT5) and 1.23 (SCoT4) to 1.52 (SCoT8), with meansof 1.75 and 1.36, respectively. The mean of Nei's observed heterozygosity was found to be 0.22, with ranging from 0.15 (SCoT4) to 0.30 (SCoT8). Similarly, Shannon's information index ranged from 0.22 (SCoT4) to 0.46 (SCoT8) with a mean of 0.34. The genetic diversity analysis identified the SCoT8 as a more informative marker to characterise45 chilli genotypes. Whereas the SCoT4 markers have generated the least genetic diversity value in all the parameters. The number of alleles (na =1.40 - 2.00; mean 1.75) and number of effective alleles (ne = 1.23 – 1.52; mean 1.36) align with earlier reports in *Capsicum* and tomato, where SCoT markers generally produce moderate to high allelic richness (Shahlaei *et al.*, 2014; Gupta *et al.*,

Table 3: SCoT markers based on allelic diversity in 45 chilli genotypes

Marker	*na*	*ne*	*h*	*i*
SCoT2	1.80	1.25	0.18	0.30
SCoT3	1.90	1.26	0.20	0.34
SCoT4	1.40	1.23	0.15	0.22
SCoT5	2.00	1.29	0.21	0.36
SCoT6	1.63	1.34	0.21	0.31
SCoT7	1.80	1.38	0.23	0.36
SCoT8	1.89	1.52	0.30	0.46
SCoT9	1.71	1.44	0.23	0.34
SCoT10	1.67	1.42	0.25	0.37
SCoT11	1.71	1.44	0.25	0.37
Mean	1.75	1.36	0.22	0.34

2019). Shannon's information index (i), ranging from 0.22 to 0.46 (mean 0.34), and Nei's heterozygosity (h = 0.15–0.30; mean 0.22), further confirm the presence of appreciable intra-specific variability. Such diversity estimates are comparable to those documented in chilli and other vegetable crops using SCoT and related co-dominant markers like SSRs (Gupta *et al.*, 2019; Ram *et al.*, 2021).

Structure-based diversity analysis

To determine the genetic populations present in 45 chilli genotypes, the binary matrix data were analysed in the STRUCTURE software. The delta K value revealed three population structures present in 45 chilli genotypes (Fig. 2). Population structure I consisted of21 chilli genotypes, out of which 04 genotypes were found

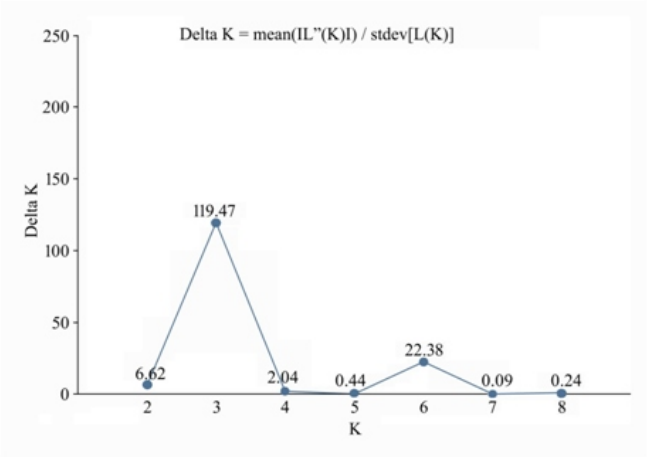

Fig. 2: Eigen value (Delta K) indicating the number of populations among 45 chilli genotypes.

to be admixture type. Similarly, population cluster II contained 12 genotypes with 3 admixture types. The population structure III consisted of08 highly porous, 3 admixture type and one genotype as a hybrid type (Fig. 3). The low level of admixture among the genotypes might be due to self-pollination nature of the crop. Population structure analysis identified three distinct genetic populations among the 45 chilli genotypes studied, supported by delta K analysis, which agrees with multiple population structure studies in chilli revealing 2–4 major genetic groups due to narrow reproductive behaviour and region-specific domestication (Taranto *et al.*, 2016). The comparatively

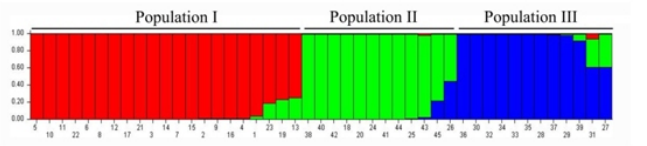

Fig. 3: Population structure analysis among 45 chilli genotypes.

lower proportion of admixture genotypes recorded might be attributed to the often-cross-pollinated nature of *Capsicum annuum*, thereby restricting intensive gene flow, as also suggested earlier by Pérez-Martínez *et al.,* (2022).

Clustering study

The UPGMA cluster analysis grouped 45 genotypes into three major clusters, some of them having sub-clusters (Fig. 4). Cluster one emerged as the largest cluster,

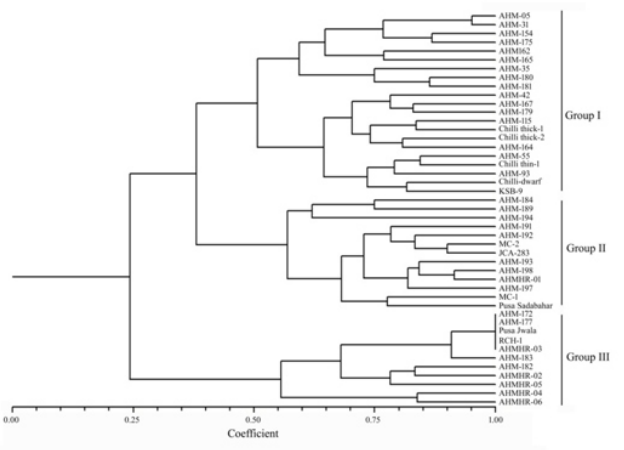

Fig. 4: UPGMA clustering analysis among 45 chilli genotypes.

possessing twenty-one genotypes with a 0.48 to 0.90 similarity index. Similarly, cluster 2 was found second largest cluster, having thirteen genotypes, including two varieties (JCA-283 and Pusa Sadabahar) with a 0.58 to 0.87 similarity index. Cluster 3 consisted ofeleven chilli genotypes, including two varieties (Pusa Jwala and RCH-1). In cluster 3, five genotypes AHM-172, AHM-173, RCH-1, Pusa Jwala and AHMHR-3 were observed closest to each other in the phylogeny analysis, suggested the 100% similar based on the genetic level. The UPGMA-based clustering of genotypes also validated three primary genetic groups, which were further confirmed and supported by the STRUCTURE results. The clustering of commercial varieties such as Pusa Jwala, RCH-1, Pusa Sadabahar and their close grouping with specific accessions suggests shared allelic origin and similar genetic background, as also observed by Nagy *et al.,* (2007) and Dhaliwal *et al.,* (2014) in phylogenetic studies of chilli cultivars.

Molecular variability and coordination among chilli genotypes

An analysis of molecular variance (AMOVA) study was performed to analyse the distribution of genetic variations among or within populations of 45 chilli genotypes. Consequently, the analysis showed a

Table 4: AMOVA showing genetic variation among and within populations of 45 chilli genotypes

Source	df	SS	MS	Est. Var.	%
Among Pops	2	287.123	143.561	9.443	55%
Within Pops	4	2327.4	337.796	7.796	45%
Total	4	4614.556		17.239	100%

significant estimated value at a degree of 1000 permutations (Table 4). The results revealed that most of the variation (55%) was found among the populations rather than within populations (45%). Principal coordinate analysis (PCoA) was further performed to assess the cluster coordinates according to the genetic variability among 45 chilli genotypes. The findings of coordinate analysis revealed four clusters among 45 chilli genotypes with distribution in four quadrangles; however, the distribution of the genotypes across the quadrangles was not uniform. The maximum number of genotypes (21 genotypes) was presented by quadrangle 1, followed by quadrangle 4 (12 genotypes). Whereas, the quadrangle 2 presented the minimum number of genotypes clustered (4 genotypes). The coordinates 1 and 2 explained per centvariation of 54.56% and 19.94% with a cumulative per centvariation of 81.72% (Fig. 5). AMOVA indicated that maximum molecular variation resided among the populations rather than within populations, which clearly supports a structured genetic architecture in chilli. Similar trend of higher inter-population variance has been documented in chilli across different geographic origin-based studies (Yumnam *et al.*, 2012; Abhinaya *et al.*, 2016). The PCoA further supported cluster-based grouping of chilli genotypes, where differential spatial distribution of genotypes across quadrants indicated heterogeneous genetic divergence among the chilli genotypes studied. Thus, the combined marker efficiency statistics, clustering pattern, population structure, and AMOVA outputs confirmed that SCoT markers exhibit high potential for precise discrimination, population stratification and genetic divergence in chilli genotypes.

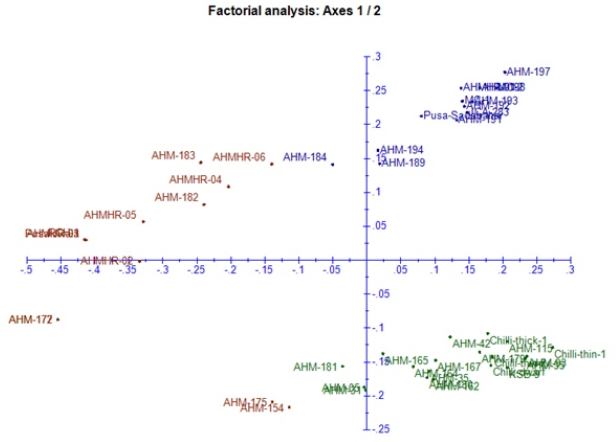

Fig.5: Principal coordinate analysis (PCoA) of 45 chilli genotypes.

In a nutshell, the findings of this study revealed a substantial level of allelic variation and polymorphism in chilli genotypes. Out of 81, ten SCoT markers were found informative to generate 81 alleles with 60.00% mean polymorphism. The moderate level of PIC value, ranging from 0.25 to 0.58, reflecting informativeness of SCoT markers analysed in this study. Three clusters corroborated with three population structures also infer the utility of the SCoT markers to identify the genotypes according to their phylogenetic proximity. Similarly, the AMOVA showed significant genetic variation among and within populations of 45 chilli genotypes. Overall, SCoT markers in this study were highly informative, revealing significant genetic diversity among chilli genotypes, which provided sufficient genetic distance to identify promising contrasting parents for future heterosis breeding, trait pyramiding and molecular breeding program development.

Conclusion

The present study confirmed that SCoT markers are highly efficient and reliable for assessing molecular polymorphism and genetic diversity in chilli. The high mean of per cent polymorphism (60%), moderate mean PIC values (0.40), superior resolution power (mean 2.41) and clear genetic clustering obtained in this study collectively indicated that SCoT markers have strong discriminatory strength for genome differentiation in *Capsicum annuum*. The STRUCTURE, UPGMA and PCoA-based grouping consistently classified the germplasm into three genetically distinct populations, which suggests the existence of structured genetic divergence within the evaluated collection. AMOVA further validated this pattern by demonstrating that the majority of genetic variance in chilli lies among populations (55%) rather than within populations (45%), signifying clear differentiation and limited gene flow among the genotype groups.

From a breeding perspective, identification of genetically distant clusters and diverse genotypes is

extremely valuable for parental selection in hybrid, heterosis and trait-specific breeding programmes. The genotypes belonging to genetically distant clusters can be strategically used to maximise heterotic response, broaden allelic spectrum, enhance recombination potential and pyramid desirable traits effectively in next-generation breeding. Therefore, the diversity detected through SCoT markers analysis in this study provides a valuable molecular basis and genetic resource framework for designing future chilli improvement, hybrid development and molecular breeding strategies.

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