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Insights into the genetic diversity of chilli pepper (Capsicum annuum L.) using morphological traits and microsatellite markers

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Abstract

Gene pool diversity is being reshaped due to preference of stakeholders for varieties with specific traits, along with government policies that place greater responsibility on breeders to preserve and enhance genetic diversity which is an essential component of crop improvement programs. Keeping this in view, the present investigation was undertaken to assess morphological and molecular diversity using nine morphological descriptors, 35 quantitative traits, and 60 simple sequence repeat (SSR) markers across a collection of 32 chilli pepper genotypes. Seven morphological descriptors were discovered to be polymorphic, with the intensity of anthocyanin color of nodes having the greatest Shannon diversity index (0.95). The studied genotypes were divided into seven groups by Mahalanobis D^2 with the maximum inter-cluster distance (48.98) between clusters V and VI and the highest intra-cluster distance by cluster V (30.33). The marketable red ripe fruits per plant made the greatest contribution to genetic divergence (23.99%). Molecular markers revealed wide diversity among genotypes with average polymorphism of 0.426 and the polymorphic information content (PIC) varied from 0.154 to 0.847 with primer BM 61910 having maximum PIC representing their efficacy in detecting genetic diversity. Based on SSR data using the UPGMA approach and structural analysis, 32 genotypes were categorized into two major clusters, resulting in three distinct population groupings. In addition, based on their genetic similarities and geographic origin the neighbor-joining tree inferred three groupings. This study provides essential insights into morphological and molecular markers, highlighting their significance in assessing genetic diversity and their potential application in chilli breeding and genetic improvement.

Introduction

Chilli (*Capsicum annuum* L.; 2n = 2x = 24) is a valuable cash crop farmed across the world for a variety of applications including fresh/cooked vegetables, pickles, dried spice or condiment, herbs and processing industry[1]. Chilli, often known as hot pepper, is a member of the *Capsicum* genus in the Solanaceae family that is native to Central and South America[2] and has genome size of 3.48 gigabytes. Chilli peppers contain a variety of bioactive components, such as ascorbic acid and capsaicin, which have significant pharmacological or physiological effects[3]. It prized for its pungency, color, oleoresin, and aroma on the international market[4]. From imparting pungency to global cuisines to driving the livelihoods of millions of farmers, *Capsicum annuum* embodies both cultural significance and economic value.

Chilli regarded as facultative cross-pollinating species in terms of breeding, and out-crossing to the level of 90% has been documented[5]. India, being the world's largest chilli producer, has enormous potential to enhance output in order to encourage exports while also fulfilling domestic needs. Efforts are required to improve the prevailing productivity of chilli as fresh crop or spice and other industrial purposes through focussed breeding program[6]. Despite continual efforts at many levels, chilli productivity has remained stagnant. This might be due to a variety of factors including the lack of appropriate cultivars/hybrids, biotic and abiotic stressors, cultivar genetic drift, and the emergence of novel pathogenic races[7]. In an era of climate uncertainty and escalating food demands, understanding the genetic blueprint of crop plants has become more critical than ever.

As a result, there is a necessity to generate high yielding cultivars or hybrids loaded with superior quality traits by genetically reconstructing chilli germplasm in order to increase production. Intensive breeding programs focused on maximizing yield and crop uniformity, driven by market demands have contributed significantly to the reduction of genetic diversity and accelerated genetic erosion i.e. loss of useful gene(s) in the germplasm[8]. Genetic diversity plays a crucial role in crop improvement programs, providing essential genetic material needed to develop superior varieties with enhanced resilience, improved nutritional value, higher productivity and better adaptability to diverse environmental conditions, thereby supporting overall food security[9]. The conservation and viable utilization of plant genetic resources are keys to continuous improvement of *Capsicum*. Breeding programs for *Capsicum* improvement would be facilitated by the availability and complete characterization of genetically diverse genotypes.

The remarkable phenotypic diversity observed in chilli reflects an equally intricate underlying genetic framework that remains only partially deciphered. Comprehensive assessment of this diversity, integrating morphological traits with molecular markers is pivotal for unlocking its full breeding potential. Various approaches such as allele mining and TILLING (Targeted Induced Local Lesions in Genomes) are available for evaluating genetic diversity among different genotypes. However, these techniques are

often costly and labor-intensive. As a more efficient alternative, molecular markers offer a practical and effective means to analyze genetic diversity in chilli. A variety of molecular marker system, include amplified fragment length polymorphisms (AFLPs)[10], restriction fragment length polymorphism (RFLPs)[11, 12], random amplified polymorphic DNA (RAPDs)[13, 14], simple sequence repeat (SSRs) also known as microsatellite marker[15, 16], insertion/ deletion polymorphisms (InDels)[17, 18, 19] and single nucleotide polymorphism (SNPs)[4, 20], have been adopted in *Capsicum* molecular genetics research over the past few decades. Among the molecular markers, simple sequence repeat has become one of the most absorbing markers for plant genetics and breeding[21]. Utilization of SSRs is an ideal approach for assessing genetic diversity among a large number of *C. annuum* accessions in a short period of time and at a low cost because they are highly reproducible, co-dominant, multi-allelic nature, distribution throughout the genome, co-dominant nature of inheritance, and easy detection by PCR, making them most suitable for whole genome characterization[22]. The polymorphism apparent between the genotypes by molecular markers is independent of environmental components. For efficient utilization of *Capsicum* genotypes, sufficient understanding of the genetic diversity among the genotypes' is essential. Therefore, in the present study, we performed population structure analysis among 32 chilli pepper genotypes through applying 60 SSR markers and various horticultural markers. The information generated from this work allowed as to determine the structure of genetic diversity within the chilli pepper genotypes, and its utilization in breeding program of peppers.

Materials and methods

Plant material and experiment layout

The present investigation was conducted at the Research Farm, Department of Vegetable Science and Floriculture, Chaudhary Sarwan Kumar Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India. A total of 32 genotypes of *C. annuum* belonging to different ecological locations of India were included for the morpho-molecular diversity analysis. The selfed seed of each genotype were grown in three replications during the months of May to October of the year 2019-20 adopting a plant and row spacing of 45 × 45 cm. Ten plants of a genotype were planted in each replication. Table 1 lists the specifics of the experimental materials.

Table 1
List of chilli pepper genotypes involved in present research along with their source

Sr. No.	Genotypes	Pedigree	Source	Sr. No.	Genotypes	Pedigree	Source
1	DPCH 6 – 2	Pusa Sadabahar × Surajmukhi	CSKHPAU[a]	17	DPCH 39 – 11	LCA-436 × Pant C-1	CSKHPAU
2	DPCH 9Y	Chilli Sonal × Surajmukhi	CSKHPAU	18	DPCH 39 – 12	LCA-436 × Pant C-1	CSKHPAU
3	DPCH 10PP	Chilli Sonal × Surajmukhi	CSKHPAU	19	DPCH 40 – 2	LCA-436 × Pant C-1	CSKHPAU
4	DPCH 13 – 1	Chilli Sonal × Surajmukhi	CSKHPAU	20	DPCH 104-1	Selection from local Unknown material	CSKHPAU
5	DPCH 21	Arka Lohit × Surajmukhi	CSKHPAU	21	DPCH 501	Selection from local Unknown material	CSKHPAU
6	DPCH 22C	Arka Lohit × Surajmukhi	CSKHPAU	22	DPCH 502	Selection from local Unknown material	CSKHPAU
7	DPCH 28 – 12	Pusa Jwala × Surajmukhi	CSKHPAU	23	DPCH-US-1	Selection from local Unknown material	CSKHPAU
8	DPCH 28 – 13	Pusa Jwala × Surajmukhi	CSKHPAU	24	DPCH-US-2	Selection from local Unknown material	CSKHPAU
9	DPCH 29 – 11	Pusa Jwala × Surajmukhi	CSKHPAU	25	2019/CHIVAR-1	Not known	IIVR[b]
10	DPCH 29 – 12	Pusa Jwala × Surajmukhi	CSKHPAU	26	2019/CHIVAR-5	Not known	IIVR
11	DPCH 32 – 11	Pusa Jwala × Surajmukhi	CSKHPAU	27	2019/CHIVAR-6	Not known	IIVR
12	DPCH 32 – 21	Pusa Jwala × Surajmukhi	CSKHPAU	28	2019/CHIVAR-7	Not known	IIVR
13	DPCH 33 – 2	Pusa Jwala × Anugraha	CSKHPAU	29	2019/CHIVAR-9	Not known	IIVR
14	DPCH 38–121	LCA-436 × Pant C-1	CSKHPAU	30	Him Palam Mirch-1	Pusa Jwala × Surajmukhi	CSKHPAU
15	DPCH 38–122	LCA-436 × Pant C-1	CSKHPAU	31	Him Palam Mirch-2	LCA-436 × Pant C-1	CSKHPAU
16	DPCH 38 – 22	LCA-436 × Pant C-1	CSKHPAU	32	Surajmukhi	Selection	CSKHPAU

[a] CSKHPKV = Chaudhary Sarwan Kumar Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India; [b] IIVR = Indian Institute of Vegetable Research, Varanasi, India

Phenotyping

All the genotypes were phenotyped for nine distinctiveness, uniformity and stability (DUS) descriptors proposed by the Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) for chilli (hot pepper), bell (sweet) pepper and paprika (*C. annuum* L.), New Delhi, India, and 35 other morphological and quality traits. The other morphological traits viz., days to 50% flowering, days to first harvest, leaf width (cm), leaf length (cm), leaf petiole (cm), average dry fruit weight (g), dry fruit yield per plant (g) and number of seeds per fruit plus the traits measured from both fresh marketable crop/green and red ripe (spice) crop/red namely, primary branches per plant, secondary branches per plant, plant height (cm), fruit length (cm), fruit girth (cm), pedicel length (cm), average fruit weight (g), marketable fruits per plant, total fruits per plant, percent marketable fruits per plant, fruit yield per plant

and harvest duration, and three quality traits such as ascorbic acid content (mg/100g), capsaicin content (%) and oleoresin content (ASTA units) were estimated.

Molecular genotyping

PCR amplification and band profiling

Total genomic DNA was isolated from young leaves of 45 days old transplanted seedlings using CTAB method[23] with slight modifications. DNA thus obtained was quantified and diluted by using nuclease free water. Concentration and purity of DNA samples were assessed using both nanodrop spectrophotometer, while integrity of DNA was analyzed using 1% agarose gel and stored at -20˚C. Amplification reactions were performed in a total volume of 10 µl, containing 1 µl genomic DNA, 0.6 µl of forward primer, 0.6 µl of reverse primer, 3.5 µl of master mix and 4.3 µl of ddH_2O. PCR tubes containing reaction mixture were thoroughly mixed by using spinner and subjected to the gradient thermocycler with the following program: initial denaturation step at 94˚C for 3 minutes, then 35 cycles of denaturation at 94˚C for 1/2 minute, annealing temperature varies from 55 to 64 ˚C and extension at 72 ˚C for 1 minutes and, final extension was performed at 72 ˚C for 5 minutes. The PCR products were then stored at 4 ˚C for ∞. The amplified PCR products were verified by 3% agarose gel electrophoresis in 1X TAE buffer, with 100bp DNA ladder to estimate band size and electrophoresis were carried out at 80 voltage and current 120 for 2–3 hours and viewed under gel documentation unit. A total of 60 chilli SSR primer pairs (Table S1) were screened for polymorphism and fifteen primers were showed polymorphism which were scored for further analysis and rest of the primers were excluded from the study.

Statistical analysis

DUS data analysis

Binary data of nine DUS descriptors was subjected to construct the dendrogram using NTSYS-pc (version 2.02). The diversity index (DI) was calculated for each descriptor according to Sharma and Das[24]. $\mathrm{H} = -\sum_{i=1}^{R} Pi \ln Pi$

Morphological diversity analysis

Phenotypic data of 35 characters (other than DUS descriptors) was subjected to Mahalanobis D^2 statistics[25] followed by grouping into different clusters by Toucher's method as suggested by Rao[26]. For conducting D^2 analysis, the computer program WINDOSTAT 8.0 developed by Indostat Services was used. Mahalanobis D^2 analysis between two genotypes estimated on the basis of the 'p' characters is given by the equation: $D^2 = \sum_{i=1}^{p} \sum_{j=1}^{p} wij(Xi1 - Xi2)(Xj1 - Xj2)$, Where, wij = variance-covariance matrix, w^{ij} = reciprocal of (wij), (i j = 1,2……, p), X_{i1} = sample mean for i^{th} character for first sample, X_{i2} = sample mean for i^{th} character for second sample. In addition, the grouping pattern of the 32 chilli genotypes was estimated by principal component analysis (PCA) in software XLSTAT using EIGEN procedure on the basis of correlation coefficient between two genotypes.

Molecular analysis

For the estimation of genetic diversity of chilli genotypes, molecular data of each polymorphic marker was converted into binary format as 1 for presence and 0 for absence at each locus. Similarity matrix were generated using Jaccard's coefficient, $J_{ij} = C_{ij}/(n_i + n_j - c_{ij})$, where '$C_{ij}$' is the number of positive matches between two genotypes, while n_i and n_j is the total number of band in genotype i and j, respectively, in SIMQUAL program of NTSYS–PC package (version 2.02)[27], whereas Genetic distances (GD) were calculated as GD = 1 – $[C_{ij}/(n_i+n_j- C_{ij})]$ and dendrogram was constructed using the UPGMA algorithm in SAHN program of NTSYS – PC package (version 2.02).

The Polymorphic information content (PIC) were calculated as follows: $PIC = 1 - \sum_{i=1}^{k} P_i^2$, where *k* is the total number of alleles and p_i is the frequency of the i^{th} allele in the set of genotypes investigated[28]. Genetic structure of the 32 chilli genotypes

was determined by following Bayesian model-based program STRUCTURE (v.2.3.3)[29]. The analysis was done with k ranging from 1 to 10 using an admixture model with 10,000 burning periods and 10,000 replicates. The final peak of plotting LnPD values were determines using online web-based Structure Harvester program. Analysis of molecular variance (AMOVA) and the patterns of genetic relationship contained in the matrix were visualized by Principal Coordinates Analysis (PCoA) in GenALEx 6.5[30].

Results

Agro-morphological diversity and characterization

All 32 chilli pepper cultivars had consistent ratings for nine morphological descriptors. Except for leaf shape and leaf pigmentation, which were determined to be monomorphic and were omitted from the research (Fig. 1). The Shannon diversity index for nine variables varied from 0.32 (leaf pigmentation and leaf shape) to 0.95 (intensity of anthocyanin color of nodes) (Table 2). The similarity coefficients varied from 0.53 to 1.0. At a genetic similarity of 0.81, the cluster analysis divided all of the genotypes into seven groups (Fig. 2).

Table 2
Characterization of 32 diverse chilli genotypes based on DUS as per PPV & FRA

Trait	Class or scale of descriptor	Frequency	Relative frequency (%)	Diversity index (DI)
Plant growth habit	Upright	16	50	0.82
	Semi-spreading	14	44	
	Spreading	2	6	
Leaf: Intensity of green color	Light	1	3	0.48
	Medium	1	3	
	Dark	30	94	
Leaf shape	Lanceolate	32	100	0.32
	Ovate	-	-	
	Broad elliptic	-	-	
Leaf pigmentation	Absent	32	100	0.32
	Present	-	-	
Intensity of anthocyanin coloration of nodes	Weak	11	34	0.95
	Medium	11	34	
	Strong	10	32	
Intensity of fruit color at first harvest	Light	11	34	0.94
	Medium	12	38	
	Dark	9	28	
Fruit color at maturity	Orange	1	3	0.61
	Orange-red	4	13	
	Red	27	84	
Fruit shape	Moderately triangular	6	18	0.91
	Narrowly triangular	14	44	
	Horn shaped	12	38	
Fruit shape at pedicel attachment	Acute	19	59	0.70
	Round	13	41	
Mean				0.67
Maximum				0.95
Minimum				0.32
Range				0.63

Mahalanobis D^2 diversity analysis

The multivariate analysis (D^2) illustrating genetic divergence, arranged 32 genotypes into seven clusters, cluster I was the largest with 13 genotypes followed by cluster V with six genotypes, clusters II and III each had five genotypes and clusters IV, VI and VII were mono-genotypic following Tocher's procedure depicted through dendrograms (Fig. 2 and Table 3). The intra and inter cluster D^2 values presented in Table 4 revealed that cluster IV, VI and VII showed minimum intra-cluster D^2 value (0), whereas, maximum

intra-cluster D^2 value (30.33) was shown by cluster V followed by cluster III (27.71), cluster I (22.83) and cluster II (20.61). Moreover, the minimum inter-cluster D^2 value was observed between the cluster I and IV (27.47) followed by cluster IV and V (28.32), although, the maximum inter-cluster D^2 value was recorded between the clusters V and VI (48.98), followed by cluster IV and VI (47.79), cluster II and III (42.59) and cluster V and VII (42.50).

Table 3

Distribution of chilli pepper genotypes in different clusters on the basis of Mahalanobis D^2-analysis

Clusters	Number of genotypes	Genotypes included
I	13	DPCH-38-121', 'DPCH-38-122', 'DPCH-501', 'DPCH-32-21', 'DPCH-38-22', 'Him Palam Mirch-2', 'DPCH-39-12', 'DPCH-502', 'DPCH-13-1', 'DPCH-28-13', 'DPCH-104-1', '2019/CHIVAR-6' and 'DPCH-33-2'
II	5	'DPCH-10PP', 'DPCH-28-12', 'DPCH-9Y', 'DPCH-21' and '2019/CHIVAR-1'
III	5	'DPCH-6-2', 'DPCH-22C', '2019/CHIVAR-7', 'DPCH-39-11' and 'DPCH-29-11'
IV	1	'DPCH-29-12'
V	6	'DPCH-US-1', 'Surajmukhi', 'DPCH-32-11', 'Him Palam Mirch-1', 'DPCH-US-2' and 'DPCH-40-2'
VI	1	'2019/CHIVAR-9'
VII	1	'2019/CHIVAR-5'

Table 4

Average intra and inter-cluster values of D^2 and $\sqrt{D^2}$ among clusters

Clusters	I	II	III	IV	V	VI	VII
I	**22.83**	34.90	30.34	27.47	33.68	38.49	33.02
	(4.77)	(6.31)	(5.50)	(5.24)	(5.80)	(6.20)	(5.74)
II		**20.61**	42.59	35.12	40.34	37.63	40.12
		(4.53)	(6.52)	(5.92)	(6.35)	(6.13)	(6.33)
III			**27.71**	34.63	39.58	36.04	38.79
			(5.26)	(5.88)	(6.29)	(6.00)	(6.22)
IV				**0.00**	28.32	47.79	39.48
				(0.00)	(5.32)	(6.89)	(6.28)
V					**30.33**	48.98	42.50
					(5.50)	(6.99)	(6.51)
VI						**0.00**	34.28
						(0.00)	5.85
VII							**0.00**
							(0.00)

Bold values are intra-cluster distance, Data in parenthesis are $\sqrt{D^2}$ value

Cluster mean analysis

Cluster IV and V was observed to be the most important with maximum cluster means for most of the valuable traits. Cluster IV showed maximum means for plant height (green and red chilli), marketable green and red ripe fruit yield per plant, harvest duration (green), red fruit girth, average dry fruit weight, dry fruit yield per plant, number of seeds per fruit, capsaicin content

whereas, minimum means were recorded for pedicel length (green and red) (Table 5). Similarly, cluster V showed maximum means for secondary branches per plant (green and red), marketable green fruits per plant, total green fruits per plant, per cent marketable green fruits per plant, marketable red ripe fruits per plant, total red ripe fruits per plant, per cent marketable red ripe fruits per plant, ascorbic acid and oleoresin content. On the other hand, cluster VII revealed maximum means for fruit length (green and red), leaf petiole (green), fruit girth (green), pedicel length (green), average green fruit weight, primary branches per plant (red), average red ripe fruit weight and desirable means for early flowering and fruit harvesting. In addition, cluster III had maximum means for leaf width and leaf length, cluster II for harvest duration (red) and cluster VI for primary branches per plant (green) and pedicel length (red). However, cluster VI exhibited higher means for the traits namely, days to flowering and first harvest indicating genotypes with late maturity. The relative percent contribution of studied characters for genetic divergence (Table 6) showed that marketable red ripe fruits per plant contributed maximum (23.99%) towards genetic divergence followed by leaf length (16.33%), leaf petiole (13.10%), percent marketable red ripe fruits per plant (10.48%) and average dry fruit weight (5.85%).

Table 5
Cluster means for different traits of chilli pepper genotypes distributed in seven clusters

Traits	I	II	III	IV	V	VI	VII	Mean	Maximum	Minimum
Fresh marketable crop										
Days to flowering	44.10	40.13	44.07	43.00	41.83	46.67**	35.00*	30.45	46.67	35.00
Days to first harvest	55.26	52.73	56.13	54.33	53.33	57.67**	44.33*	38.83	57.67	44.33
Leaf width (cm)	3.68	3.08*	4.47**	3.64	4.04	4.27	3.29	2.70	4.47	3.08
Leaf length (cm)	8.63	7.43*	10.31**	8.30	8.98	9.84	7.75	6.21	10.31	7.43
Leaf petiole (cm)	4.89	3.93*	5.30	4.60	4.72	5.43	6.48**	3.56	3.93	6.48
Primary branches per plant	2.97	2.61	2.82	2.33*	2.86	3.58**	3.17	2.06	3.58	2.33
Secondary branches per plant	12.09	11.19	11.54	9.00*	13.09**	10.05	9.00*	6.41	13.09	9.00
Plant height (cm)	61.85	62.42	60.32	91.19**	56.82	68.91	50.97*	44.33	91.19	50.97
Fruit length (cm)	9.28	8.03	7.64	6.21*	7.37	9.51	9.88**	5.98	9.88	6.21
Pedicel length (cm)	3.52	3.25	3.45	2.63*	3.35	3.51	3.70**	2.44	3.70	2.63
Fruit girth (cm)	3.29	3.12	3.17	3.47	3.00*	3.20	3.82**	2.32	3.82	3.00
Average green fruit weight (g)	4.94	4.11	3.94	4.47	3.37*	4.42	7.77**	3.13	7.77	3.37
Marketable green fruits per plant	90.80	72.97	84.98	128.76	145.92**	40.81*	50.52	61.15	145.92	40.81
Total green fruits per plant	100.74	93.18	92.93	137.92	154.00**	58.03*	62.60	69.62	154.00	58.03
Per cent marketable green fruits per plant	90.01	77.49	90.59	93.34	94.69**	70.36*	80.66	61.73	94.69	70.36
Marketable green fruit yield per plant (g)	443.20	296.39	332.16	574.50**	490.57	180.42*	391.67	279.14	574.50	180.42
Harvest duration	59.38	59.20	58.73	59.67**	57.89	56.33*	57.33	41.79	59.67	56.33

Table 5
continued

Red ripe (spice) crop										
Primary branches per plant	2.96	2.53	2.70	2.33*	2.86	3.05	3.47**	2.01	3.47	2.33
Secondary branches per plant	12.11	10.63	11.24	8.83	12.32**	8.75*	10.42	7.60	12.32	8.75
Plant height (cm)	58.49	56.62	58.14	83.11**	53.96	65.92	50.04*	41.88	83.11	50.04
Fruit length (cm)	9.23	7.99	7.53	6.38*	7.36	9.72	9.75**	5.98	9.75	6.38
Pedicel length (cm)	3.70	3.27	3.73	2.71*	3.60	4.30**	3.44	2.53	4.30	2.71
Fruit girth (cm)	3.60	3.54	3.52	4.15**	3.34*	3.88	4.07	2.66	4.15	3.34
Average red ripe fruit weight (g)	4.74	3.86	3.59	4.82	3.02*	4.93	7.57**	3.13	7.57	3.02
Marketable red ripe fruits per plant	72.22	40.20	60.74	77.57	109.73**	29.80*	42.67	41.91	109.73	29.80
Total red ripe fruits per plant	86.63	76.69	77.48	91.96	123.10**	53.89*	71.00	57.68	123.10	53.89
Per cent marketable red ripe fruits per plant	82.97	51.01*	78.72	84.35	89.15**	55.36	60.12	51.65	89.15	51.01
Red ripe fruit yield per plant (g)	333.99	144.11*	211.74	373.83**	330.60	147.00	322.50	192.26	373.83	144.11
Harvest durations	46.13	46.27**	45.07	44.67	45.50	40.33*	40.33*	25.91	46.27	40.33
Average dry fruit weight (g)	0.81	0.73	0.68	0.93**	0.64	0.62	0.55*	0.50	0.93	0.55
Dry fruit yield per plant	58.11	27.45	41.42	71.85**	71.21	18.39*	23.49	31.67	71.85	18.39
Number of seeds per fruit	52.62	51.13	48.60*	57.00**	50.00	51.33	52.67	36.82	57.00	48.60
Ascorbic acid (mg/100g)	56.99	52.60	44.24	55.34	64.50**	34.99*	47.77	36.71	64.50	34.99
Capsaicin content (%)	0.21	0.20	0.21	0.25**	0.23	0.19	0.17*	0.15	0.25	0.17
Oleoresin content (ASTA Units)	51.15	45.62	45.49	36.24*	52.82**	36.27	39.52	31.15	52.82	36.24
*Minimum; **Maximum										

Table 6
Relative contribution (%) of individual trait to the genetic divergence among chilli pepper genotype

Source	Contribution %	Times ranked 1st
Fresh marketable crop		
Days to flowering	0.00%	0
Days to first harvest	0.00%	0
Leaf width (cm)	0.00%	0
Leaf length (cm)	16.33%	81
Leaf petiole (cm)	13.10%	65
Primary branches per plant	0.00%	0
Secondary branches per plant	0.00%	0
Plant height (cm)	0.20%	1
Fruit length (cm)	3.63%	18
Pedicel length (cm)	0.60%	3
Fruit girth (cm)	0.00%	0
Average green fruit weight (g)	0.20%	1
Marketable green fruits per plant	4.64%	23
Total green fruits per plant	0.20%	1
Per cent marketable green fruits per plant	0.60%	3
Marketable green fruit yield per plant (g)	2.02%	10
Harvest duration	0.00%	0
Red ripe (spice) crop		
Primary branches per plant	0.00%	0
Secondary branches per plant	0.20%	1
Plant height (cm)	0.00%	0
Fruit length (cm)	4.44%	22
Pedicel length (cm)	0.00%	0
Fruit girth (cm)	1.81%	9
Average red ripe fruit weight (g)	0.20%	1
Marketable red ripe fruits per plant	23.99%	119
Total red ripe fruits per plant	0.60%	3
Per cent marketable red ripe fruits per plant	10.48%	52
Red ripe fruit yield per plant (g)	3.43%	17
Harvest durations	0.20%	1
Average dry fruit weight (g)	5.85%	29
Dry fruit yield per plant	1.61%	8

Source	Contribution %	Times ranked 1st
Number of seeds per fruit	0.60%	3
Ascorbic acid (mg/100g)	0.60%	3
Capsaicin content (%)	0.60%	3
Oleoresin content (ASTA Units)	3.83%	19

Principal component analysis (PCA)

The principal component analysis revealed that nine principal components PC1, PC2, PC3, PC4, PC5, PC6, PC7, PC8 and PC9 with eigenvalues 8.19, 5.648, 4.809, 3.332, 2.432, 1.796, 1.483, 1.157 and 1.045, respectively, have accounted for 85.41% of total cumulative variability among genotypes (Table 7). The first three principal components mainly accounted for about 53.27% total variation. The first principal component (PC1) was the most important and explained 23.40% of the total variance which was mainly contributed by marketable green and red fruits per plant. PC2 accounted for 16.13% variation and was attributed mainly due to marketable green and red fruit yield per plant. PC3 contributed 13.74% of the total variance maximum through leaf width and leaf length. PC4 contributed 9.52%, PC5 contributed 6.94% of variation, PC6 accounted for 5.13% variation, PC7 contributed 4.23% of total variance, PC8 contributed 3.30% of total variance and PC9 contributed 2.98% of variation. In all the principal components, capsaicin content resulted in highest positive value followed by oleoresin content, leaf petiole, plant height (green and red), harvest duration (green and red), secondary branches per plant, fruit girth (green and red), marketable fruits per plant (green and red), leaf length and leaf width.

Table 7
Principal component analysis (PCA) for different traits in 32 chilli pepper genotypes

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalue	8.190	5.648	4.809	3.332	2.432	1.796	1.483	1.157	1.045
Variability (%)	23.400	16.136	13.741	9.521	6.948	5.132	4.239	3.306	2.985
Cumulative %	23.400	39.537	53.278	62.799	69.747	74.879	79.118	82.424	85.409
Fresh marketable crop									
Days to 50% flowering	0.057	0.136	0.309	0.245	-0.018	-0.104	-0.217	-0.014	0.085
Days to first harvest	0.064	0.067	0.308	0.245	-0.031	-0.187	-0.222	-0.100	0.011
Leaf width	0.141	0.041	0.334	0.070	-0.139	0.094	0.091	-0.080	0.136
Leaf length	0.103	0.057	0.323	0.177	-0.169	0.006	0.106	-0.074	-0.025
Leaf petiole	-0.022	0.079	0.204	-0.120	-0.034	0.064	0.505	-0.273	0.263
Primary branches per plant	-0.033	0.129	0.250	-0.095	0.182	0.314	-0.110	0.134	-0.138
Secondary branches per plant	0.126	0.074	0.146	-0.039	0.416	-0.130	0.146	-0.260	-0.235
Plant height	0.000	0.127	-0.056	0.484	0.026	-0.105	0.099	0.100	0.009
Fruit length	-0.176	0.274	0.013	-0.131	0.036	-0.292	-0.007	0.079	0.028
Pedicel length	-0.119	0.213	0.123	-0.244	-0.029	0.080	-0.245	-0.047	0.005
Fruit girth	-0.151	0.220	-0.101	0.028	-0.117	0.366	-0.016	-0.186	-0.117
Average green fruit weight	-0.226	0.258	-0.065	-0.073	0.033	0.121	0.206	0.093	-0.059
Marketable green fruits per plant	0.310	0.057	-0.120	0.055	-0.011	0.113	-0.049	-0.013	-0.085
Total green fruits per plant	0.298	0.030	-0.155	0.058	-0.005	0.086	-0.082	0.000	-0.100
Percent marketable green fruits per plant	0.283	0.156	0.036	0.026	0.042	0.186	0.120	-0.074	-0.009
Harvest durations	0.018	0.019	0.009	0.115	0.470	0.009	0.001	0.275	0.368
Marketable green fruit yield per plant	0.147	0.291	-0.188	0.040	0.042	0.182	0.021	0.017	-0.121

Table 7
continued

Red ripe (spice) crop									
Primary branches per plant	-0.010	0.115	0.251	-0.185	0.125	0.156	-0.061	0.375	0.056
Secondary branches per plant	0.127	0.125	0.187	-0.074	0.355	-0.099	0.211	-0.166	-0.255
Plant height	-0.002	0.167	0.006	0.454	-0.093	-0.097	0.115	0.069	0.082
Fruit length	-0.179	0.267	0.013	-0.135	-0.001	-0.297	-0.007	0.097	0.019
Pedicel length	-0.057	0.149	0.281	-0.208	-0.047	0.005	-0.280	-0.149	0.119
Fruit girth	-0.158	0.200	-0.094	0.171	-0.014	0.368	0.020	-0.126	-0.160
Average red ripe fruit weight	-0.218	0.248	-0.053	-0.001	-0.035	-0.007	0.255	0.216	0.001
Marketable red fruits per plant	0.315	0.066	-0.025	-0.158	-0.136	-0.024	-0.001	0.046	0.022
Total red ripe fruits per plant	0.285	-0.017	-0.080	-0.203	-0.131	-0.055	-0.034	0.044	-0.012
Per cent marketable red ripe fruits per plant	0.272	0.184	0.072	-0.041	-0.089	0.028	0.032	0.012	0.112
Average dry fruit weight	-0.073	0.246	-0.193	0.124	0.062	-0.169	-0.018	-0.145	0.006
Dry fruit yield per plant	0.256	0.197	-0.106	-0.087	-0.155	-0.129	0.010	-0.041	0.007
Number of seeds per fruit	-0.081	0.267	-0.027	0.066	-0.192	-0.068	-0.367	-0.045	-0.111
Red ripe fruit yield per plant	0.159	0.286	-0.075	-0.137	-0.176	-0.095	0.142	0.196	0.062
Harvest duration	0.043	0.090	-0.173	0.026	0.385	-0.057	-0.258	-0.264	-0.079
Ascorbic acid	0.141	0.147	-0.217	-0.127	0.129	-0.237	-0.008	-0.020	0.199
Capsaicin content	0.096	0.081	-0.121	0.076	0.185	0.310	-0.172	0.015	0.559
Oleoresin content	0.162	-0.020	0.113	0.082	0.096	0.012	-0.018	0.521	-0.377

Diversity analysis at molecular level

Genetic diversity and population structure analysis

SSR primers were used for molecular marker analysis among the 32 chilli pepper genotypes. A total of 60 SSRs chilli primers were screened for PCR amplification and 15 primers showed polymorphism (Fig. 4). SSR produced amplification products in size range of 100–300 bp, makers detected a total of 41 alleles with an average of 2.73 alleles per locus (Table 8). The polymorphic information content (PIC) was ranged from 0.154 to 0.847 with an average value of 0.426. Among the 15 polymorphic SSR markers, BM 61910 was the most informative with high PIC value of 0.897 followed by HPMSE 004 (0.680) pointing towards good polymorphism level among the genotypes. The genotypes were grouped into two major clusters A and B using UPGMA algorithm (Fig. 5). Cluster A comprised of 31 genotypes whereas one genotype was placed in cluster B. Cluster A further divided into four sub-clusters A_1, A_2, A_3 and A_4 (Table 9 and Fig. 5) comprising of 7, 20, 2 and 2, respectively, sub-cluster A_2 comprised of 20 genotypes, whereas, sub-cluster A_3 and A_4 comprised of two genotypes. Cluster B comprised of only 'DPCH-502' genotype. The genetic similarity coefficient among genotypes ranged from 0.45 to 1.00 with an average of 0.72. The genetic identity was further confirmed by subjecting the marker data to construct the neighbor-joining tree (Fig. 6) using the DARwin software version 5.0.158[31]. The cluster tree generated with the described SSR markers recognized three major groups (Table 10, Fig. 6). Cluster I comprised of five genotypes whereas, cluster II comprised of 15 genotypes which divided into two sub-clusters namely, cluster IIA and cluster IIB and cluster III comprised of 12 genotypes.

Table 8
List of polymorphic SSR primers, their product size, alleles amplified and polymorphism information content (PIC) among 32 chilli pepper genotypes

Sr. No.	Primer	Primer's sequence (Forward and Reverse)	Number of fragments	Fragments size (bp)	Polymorphic information content (PIC)
1	BM 61910	ATTGTGATAGCAACCCCTGG CACAGATGAGGGCACAAATG	3	175–225	0.897
2	CAMS 885	AACGAAAAACAAACCCAATCA TTGAAATTGCTGAAACTCTGAA	3	100–175	0.553
3	CAMS 340	TTTATGCCCATTCACAAAATAA GGACGAATTCACCGAGTGC	3	150–200	0.507
4	CAMS 072	CCCGCGAAATCAAGGTAAT AAAGCTATTGCTACTGGGTTCG	3	150–275	0.556
5	HPMS E 004	TGGGAAGAGAAATTGTGAAAGCA CAATGCCAACAATGGCATCCTA	4	150–175	0.680
6	HPMS E 046	TCCTCAAGATTGTTGTCATCATCA AAAGGCATTTTCATTTTCGACTTT	3	175–200	0.347
7	CAMS 070	CCCTGAACTTGTCCTCCAAA GGGTATGGGGTGTAGGTGTG	3	100–300	0.401
8	HPMS E 075	GCGGCTCAGCAGAAAGAGAGAG TGCCACAGCTGGAGAACGTAAA	3	175–200	0.405
9	HPMS E 013	GCGCCAAGTGAGTTGAATTGAT CACCAATCCGCTTGCTGTTGTA	2	100–175	0.154
10	GPMS 169	TCGAACAAATGGGTCATGTG GATGAGGGTCCTGTGCTACC	2	150–200	0.298
11	HPMS E 005	TGCCTCAGTTTCCCAACCCT ACCAACACCGTAACGCACCC	2	150–175	0.336
12	CAMS 460	CCTTTCACTTCAGCCCACAT ACCATCCGCTAAGACGAGAA	2	150–200	0.352
13	CAMS 156	CCCTATGCTTTCACAACTCCT GACGTGGTTATGACGATAGGC	3	100–275	0.318
14	CAMS 051	ACCCAGTTCCCTTTCTTGGT GAAGGTTAGCGGAATGAACG	3	100–200	0.436
15	CAMS 040	TATAGCCTGTGGGTGCCTTC TGGGGTGAACAATAGCATGT	2	150–175	0.164
	Mean				0.426

Table 9
Clustering of chilli genotypes on the basis of SSR data (NTSYS 2.02)

Cluster No.	Sub-cluster	No. of genotypes in each cluster	Genotypes
A	A1	7	'Him Palam Mirch-1', 'Surajmukhi', 'DPCH-6-2', 'DPCH-29-11', 'DPCH-29-12', 'DPCH-13-1' and '2019/CHIVAR-5'
	A2	20	'DPCH-501', 'DPCH-US-2', 'DPCH-US-1', 'DPCH-39-12',, 'DPCH-21',, 'DPCH-33-2', 'DPCH-22C',, 'DPCH-40-2', 'DPCH-39-11', '2019/CHIVAR-1', 'DPCH-32-11', 'DPCH-104-1', 'Him Palam Mirch-2',, 'DPCH-32-21', 'DPCH-9Y',, 'DPCH-38-121', 'DPCH-38-122', '2019/CHIVAR-6', '2019/CHIVAR-9' and '2019/CHIVAR-7'
	A3	2	'DPCH-38-22', 'DPCH-28-13'
	A4	2	'DPCH-10PP', 'DPCH-28-12'
B		1	'DPCH-502'

Table 10
Grouping of chilli genotypes into different clusters on the basis of SSR data (DARwin software)

Cluster No.	Sub-cluster	Number of genotypes in each cluster	Genotypes
I		5	DPCH-39-12', 'DPCH-40-2', 'DPCH-US-1', 'DPCH-38-122' and 'DPCH-38-121'
II	IIA	7	'Surajmukhi', 'Him Palam Mirch-1', 'DPCH-502', 'DPCH-38-22', 'DPCH-29-11', 'DPCH-6-2' and '2019/CHIVAR-5'
	IIB	8	'DPCH-39-11', 'DPCH-21', 'DPCH-501', 'DPCH-US-2', 'DPCH-13-1', 'DPCH-28-13', 'DPCH-10PP' and 'DPCH-28-12'
III		12	'DPCH-9Y', 'Him Palam Mirch-2', 'DPCH-32-21', 'DPCH-104-1', 'DPCH-29-12', 'DPCH-33-2', 'DPCH-22C', 'DPCH-32-11', 2019/CHIVAR-1', '2019/CHIVAR-6', '2019/CHIVAR-9' and '2019/CHIVAR-7'

AMOVA depicts high proportion of variability within population *i.e*., 89% of total variation, whereas only 11% genetic variation among population (Table 11). The PCoA grouped 32 chilli genotypes into two different populations (Fig. 7) and showed that accessions are more diverse based on the genetic constitution. Using SSR markers, first principal coordinate explained 17.13% of the total variation followed by second (13.64%) and third (11.35%) coordinate. The STRUCTURE analysis divided the population into three main groups (Table 12), but the differentiations at K = 3 were almost consistent with pedigree knowledge having few variations. The P1, P2 and P3 consist of seven, 18 and seven genotypes, respectively (Table 12). With the help of structure bar plot (Fig. 8), P1 showed maximum of 100% and minimum of 45% membership; P2 showed maximum of 99% and minimum of 70% membership and P3 showed maximum of 99% and minimum of 40% membership.

Table 11
AMOVA partition of molecular variance for two clade models suggested by the UPGMA derived dendrogram for chilli pepper genotypes

Marker system	Source of variations	*Df*	SS	MSS	Variation	% of total variation
SSR	Among populations	1	16.017	16.017	0.952	11%
	Within populations	30	239.452	7.982	7.982	89%
	Total	31	255.469		8.934	100%

Table 12
Grouping of chilli genotypes using STRUCTURE analysis

Population groups	Number of genotypes	Genotypes
Group 1	7	‘DPCH-10PP’, ‘DPCH-28-12’, ‘DPCH-21’, ‘DPCH-501’, ‘DPCH-28-13’, ‘DPCH-13-1’ and ‘DPCH-US-2’
Group 2	18	‘DPCH-104-1’, ‘2019/CHIVAR-1’, ‘Him Palam Mirch-2’, ‘DPCH-32-11’, ‘DPCH-32-21’, ‘DPCH-33-2’, ‘DPCH-40-2’, ‘DPCH-9Y’, ‘DPCH-22C’, ‘2019/CHIVAR-6’, ‘DPCH-29-12’, ‘2019/CHIVAR-9’, ‘DPCH-39-11’, ‘DPCH-39-12’, ‘DPCH-38-121’, ‘DPCH-US-1’ ‘DPCH-38-122’ and ‘2019/CHIVAR-7’
Group 3	7	‘Him Palam Mirch-1’, ‘DPCH-502’, ‘DPCH-6-2’, ‘2019/CHIVAR-5’, ‘DPCH-29-11’, ‘DPCH-38-22’ and ‘Surajmukhi’

Comparison of genetic diversity through D^2 statistic and molecular markers

When molecular and morphometric clusters were compared, over 37.5 percent (12) of genotypes were found to be common in major cluster I, namely 'DPCH-13-1', 'DPCH-501', 'DPCH-39-12', 'DPCH-33-2', 'DPCH-104-1', 'Him Palam Mirch-2', 'DPCH-32-21', 'DPCH-38-121', 'DPCH-38-122’, ‘2019/CHIVAR-6’, ‘DPCH-38-22’ and ‘DPCH-28-13’ (Table 13).

Table 13

Comparison of clustering patterns using D^2 analysis and SSR data (NTSYS 2.02)

D^2-statistic			SSR analysis				Number of genotypes common in both analysis (12)
Cluster number	**Number of genotypes**	**Genotypes**	**Cluster number**	**Sub-cluster**	**Number of genotypes**	**Genotypes**	
I	**13**	**DPCH-38-121', 'DPCH-38-122', 'DPCH-501', 'DPCH-32-21', 'DPCH-38-22', 'Him Palam Mirch-2', 'DPCH-39-12', 'DPCH-502', 'DPCH-13-1', 'DPCH-28-13', 'DPCH-104-1', '2019/CHIVAR-6' and 'DPCH-33-2'**	**A**	**A1**	**7**	**'Him Palam Mirch-1', 'Surajmukhi', 'DPCH-6-2', 'DPCH-29-11', 'DPCH-29-12', 'DPCH-13-1' and '2019/CHIVAR-5'**	**'DPCH-13-1', 'DPCH-501', 'DPCH-39-12', 'DPCH-33-2', 'DPCH-104-1', 'Him Palam Mirch-2', 'DPCH-32-21', 'DPCH-38-121', 'DPCH-38-122', '2019/CHIVAR-6', 'DPCH-38-22' and 'DPCH-28-13'**
				A2	**20**	**'DPCH-501', 'US-2', 'US-1', 'DPCH-39-12', 'DPCH-21', 'DPCH-33-2', 'DPCH-22C', 'DPCH-40-2', 'DPCH-39-11', '2019/CHIVAR-1', 'DPCH-32-11', 'DPCH-104-1', 'Him Palam Mirch-2',, 'DPCH-32-21', 'DPCH-9Y',, 'DPCH-38-121', 'DPCH-38-122', '2019/CHIVAR-6', '2019/CHIVAR-9' and '2019/CHIVAR-7'**	
				A3	**2**	**'DPCH-38-22', 'DPCH-28-13'**	
				A4	**2**	**'DPCH-10PP', 'DPCH-28-12'**	
II	5	'DPCH-10PP', 'DPCH-28-12', 'DPCH-9Y', 'DPCH-21' and '2019/CHIVAR-1'	B		1	'DPCH-502'	
III	5	'DPCH-6-2', 'DPCH-22C', '2019/CHIVAR-7', 'DPCH-39-11' and 'DPCH-29-11'					
IV	1	'DPCH-29-12'					
V	6	'US-1', 'Surajmukhi', 'DPCH-32-11', 'Him Palam Mirch-1', 'US-2' and 'DPCH-40-2'					
VI	1	'2019/CHIVAR-9'					
VII	1	'2019/CHIVAR-5'					

Discussion

Variability among different attributes is of immense significance as it offers vision and possibility for selection and improvement. For many years, morphological features have been employed to estimate genetic diversity and relationships among chilli genotypes; however these traits are often influenced by environmental conditions. Therefore, integrating molecular markers with traditional agro-morphological evaluation offers a more reliable understanding of genetic variation within the genetic resources. Since SSR markers are polymorphic, highly reproducible, co-dominant in nature and abundant in plant genomes, they are valuable molecular markers for establishing connections and genetic diversity[32, 33]. In the current project, genetic diversity among chilli genotypes based on morphological descriptors revealed variance across genotypes (Fig. 1) for various morphological descriptors with the greatest Shannon diversity index for anthocyanin color intensity of nodes (0.95) and divided genotypes into seven groups with a genetic similarity of 0.81 (Table 2), reflecting moderate to high morphological diversity within the population. These findings underscore the utility of morphological descriptors in effectively distinguishing and classifying chilli genotypes. Such characterization is crucial for the selection of diverse parental lines in hybridization programs, ultimately contributing to the development of improved chilli varieties with desirable agronomic traits. Sixteen (50%) genotypes exhibited upright plant growth habit (Table 2) allows maximum and uniform exposure of all the leaves and other vegetative parts to get better sunlight as upright plant height and medium and strong branching leads to higher yields. The dark green color of leaves which is exhibited by 30 (94%) genotypes, generally due to presence of high chlorophyll content in the leaves which ultimately leads to increased yield. Attractive fruit color and fruit shape are the factors which determine consumer acceptability of the product hence, these traits become a good selection criterion for a breeder. The study suggested that the genotypes exhibited a wide range of variations indicated that they can be used for further breeding programme for the development of improved varieties or hybrids of chilli. The crosses involving the diverse genotypes would be expected to manifest maximum heterosis and are more likely to evolve desirable recombinants in segregating generations.

D^2 analysis based 35 morphological traits grouped the genotypes into seven clusters following Tocher's method (Table 3 & Fig. 2), with maximum genotypes in cluster I indicated high homogeneity within this group while overall clustering pattern showed no clear link with geographic origin. This suggests that the genetic constitution rather than geographic distribution primarily governs diversity patterns, highlighting the existence of significant intra-regional variability[34]. Such diversity is advantageous for breeding programmes aimed at broadening the genetic base of chilli. Accessions in cluster IV showed superior performance for both marketable green and red ripe fruit yield per plant, making them valuable candidates for yield improvement (Table 5). In contrast, Cluster VII was characterized by early flowering, indicating its potential use in breeding for earliness. The selection of parents based on inter-cluster genetic divergence can enhance the probability of obtaining desirable recombinants and higher heterosis. Accordingly, best performing genotypes *viz.*, 'DPCH-29-12' from cluster IV, 'DPCH-US-1', 'DPCH-32-11', 'DPCH-US-2' and 'DPCH-40-2' from cluster V, 'DPCH-38-121', 'DPCH-501', 'DPCH-32-21', 'DPCH-38-22', 'DPCH-502', 'DPCH-28-13', 'DPCH-104-1', 'DPCH-33-2' from cluster I and 'DPCH-29-11' from cluster III offer promise for their direct use as varieties and as potential parents in hybridization programmes. In addition to clustering, PCA revealed high variability captured within the first nine-principal components (Table 7 & Fig. 3), indicating the complex nature of trait variation among genotypes[35, 36]. The loading plot drawn for PC1 and PC2 also indicated the importance of different morphological traits and genotypes for explaining the variance among accessions and understanding genotype relationship (Fig. 3). Scattered diagram revealed that maximum genotypes were unique as they fall in different corners of biplot. None of the variable falls in the left corner of the lower half of the biplot.

To overcome the limitations associated with morphological characterization, SSR markers were employed to obtain a more precise and environment-independent assessment of genetic diversity among chilli genotypes[21]. Genetic diversity analysis based on DNA markers may complement the conventional procedures by eliminating duplicates from the genotypes to get superior recombinants and is more reliable. It helps in the development of crop that is suitable and adaptable to rapid climate change through the introduction of foreign genes. In the present study, out of sixty SSR primers screened, fifteen produced clear and polymorphic amplification (Table 8), indicating their suitability for diversity analysis. The detection of 41 alleles with an average of 2.73 alleles per locus along with 100% polymorphism across markers, which depicted those genotypes under study were genetically diverse and attributable to alien introgression or genetic recombination. The identification of a considerable number of alleles in the present investigation emphasizes their vital role in strengthening the genetic diversity of the germplasm. The polymorphic information content (PIC) values ranged from 0.154 to 0.847 with an average value of 0.426 pointing towards good

polymorphism and allelic richness level among the chilli genotypes. Notably, marker BM 61910 exhibited the highest PIC value, indicating its effectiveness in distinguishing genotypes. Higher polymorphic information content (PIC) was also reported by Aguilar RT [37]et al. with values ranging from 0.07 to 1.00, by Sharmin A[38] et al. with a range of 0.255 to 0.500 and by Brilhante BDG[39] et al. reporting values between 0.43 and 0.83. Estimation of the genetic distance play an important role for effective utilization of the diverse genotypes for hybridization programs[40] which ranged from 0.45 to 1.00 with an average of 0.72 (Fig. 5). The distance is higher than the earlier report of Cupic T [41] et al. who found genetic distance ranged from 0.24 to 0.84. Cluster analysis is one of the most important methods for breeders to know the relationships and genetic diversity of parents for efficient hybridization program[41, 40] and it divided chilli genotypes into distinct clusters using SSR markers (Table 9 & Fig. 5). The results of cluster analysis based on the morphological and molecular markers are not similar that may be due to the environmental influence on the morphological traits. The UPGMA clustering analysis grouped chilli genotypes into two clusters that were further subdivided into sub-clusters (Fig. 5) while clustering using DARwin software grouped genotypes into three major clusters (Fig. 6) indicating the genotypes are more similar in the same cluster and are different from another cluster. The variation between the two systems *viz.*, NTSYS version 2.02 and DARwin 5 is might be due to differences in the method of clustering by two systems *i.e.*, by computing similarity and dissimilarity coefficients, respectively.

The analysis of molecular variance (AMOVA) indicated high proportion (89%) of variability within population while the remaining variation was attributed to differences among genotypes. Such genetic differences may be determined by evolutionary forces that include selection, genetic drift, gene flow, ecotype variation and cross-fertilization mechanism. Earlier workers, Castilla L[42] et al. and Munoz GYA[43] et al. also reported such variations in population based on AMOVA in chilli. PCoA is one of the multivariate approaches for grouping of the genotypes based on similarity coefficients which provide more important information about major groups in comparison to the cluster analysis. Thirty-two chilli pepper genotypes divided into three group by PCoA. The PCoA examines relationships between several quantitative variables by constructing a small number of linear combinations that retain as much information in the original data. PCoA grouped 32 chilli genotypes into two population and percentage of variation explained by three principal axes. Castilla L[42] et al. also reported percentage of variation explained by PCoA. Population structure analysis divided the chilli genotypes into three distinct sub-population where the genetic composition of each individual genotype represented as a vertical bar. The different colours within a bar pointed towards introgression or genetic admixture from multiple population and thereby explain the grouping better than a dendrogram. The introgression is quiet obvious as the factors such as mutation, recombination, cross-breeding, genetic drift and natural selection have contributed to the genetic diversity[44]. Lee PY[45] et al. and Cristov NK[46] et al. have also reported the introgression in chilli genotypes.

The D^2 statistic and SSR analysis were used in this study to indicate substantial genetic diversity among distinct genotypes. On the basis of morphological and molecular evidence, there was parallelism among cluster components. Although genetic diversity analysis based on morpho-physiological features is a useful method for identifying genotypes with similar or distinct qualities, it is frequently modified by the environment. On the other hand, a molecular technique effectively eliminates the blurring effect and reveals structural/molecular similarities between genotypes. The ability to investigate the degree of variety that exists within and across genotypes and construct an indicator of genetic similarity among genotypes has been made feasible by thorough characterization of crops following molecular analysis. Furthermore, combining SSR markers with agro-morphological descriptors provides a valuable tool for characterizing chilli germplasm for optimal genetic improvement and effective conservation of genetic resources.

Conclusion

The findings revealed substantial genetic diversity and a well-defined population structure among the chilli genotypes, underscoring their significance as valuable genetic resources for future breeding programs and germplasm conservation efforts. Furthermore, the observed intra- and inter-population variability among genotypes highlights their importance in breeding programs, as this genetic diversity provides a vital foundation for developing resilient cultivars capable of withstanding future challenges posed by climate change and evolving environmental conditions. Using both morphological and SSR analyses, substantial genetic diversity among distinct genotypes was discovered in this study. On the basis of morphological and molecular evidence, there was parallelism among cluster components. Although genetic diversity analysis based on morpho-physiological

features is a useful method for identifying genotypes with similar or distinct qualities, it is frequently modified by the environment. On the other hand, a molecular technique effectively eliminates the blurring effect and reveals structural/molecular similarities between genotypes. The ability to investigate the degree of variation that exists within and across genotypes and construct an index of genetic similarity among genotypes has been made feasible by thorough characterization of genotypes following molecular analysis. Future studies should aim to incorporate a wider range of germplasm from diverse geographical regions and investigate the genetic associations with important agronomic traits, thereby enhancing the effective utilization of chilli genetic resources in sustainable crop improvement programs.

Declarations

Conflicts of interest:

The authors declare no competing financial or non-financial interests.

Ethical approval:

This article does not contain any studies with human participants or animals performed by any of the authors.

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Author Contribution

A.S. conceptualized, planned and executed the experiments, data interpretation, writing of manuscript; N.B. executed the experiments; P.S. helped in manuscript editing; N.K. analyzed the data; S. helped in tables preparation and review- editing of manuscript; A.S. and A.P. helped in tables and figures preparation. All authors reviewed and approved the final manuscript.

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Data Availability

All relevant data are within the manuscript and its additional files. The data presented in this study are available on request from the corresponding author.

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Figures

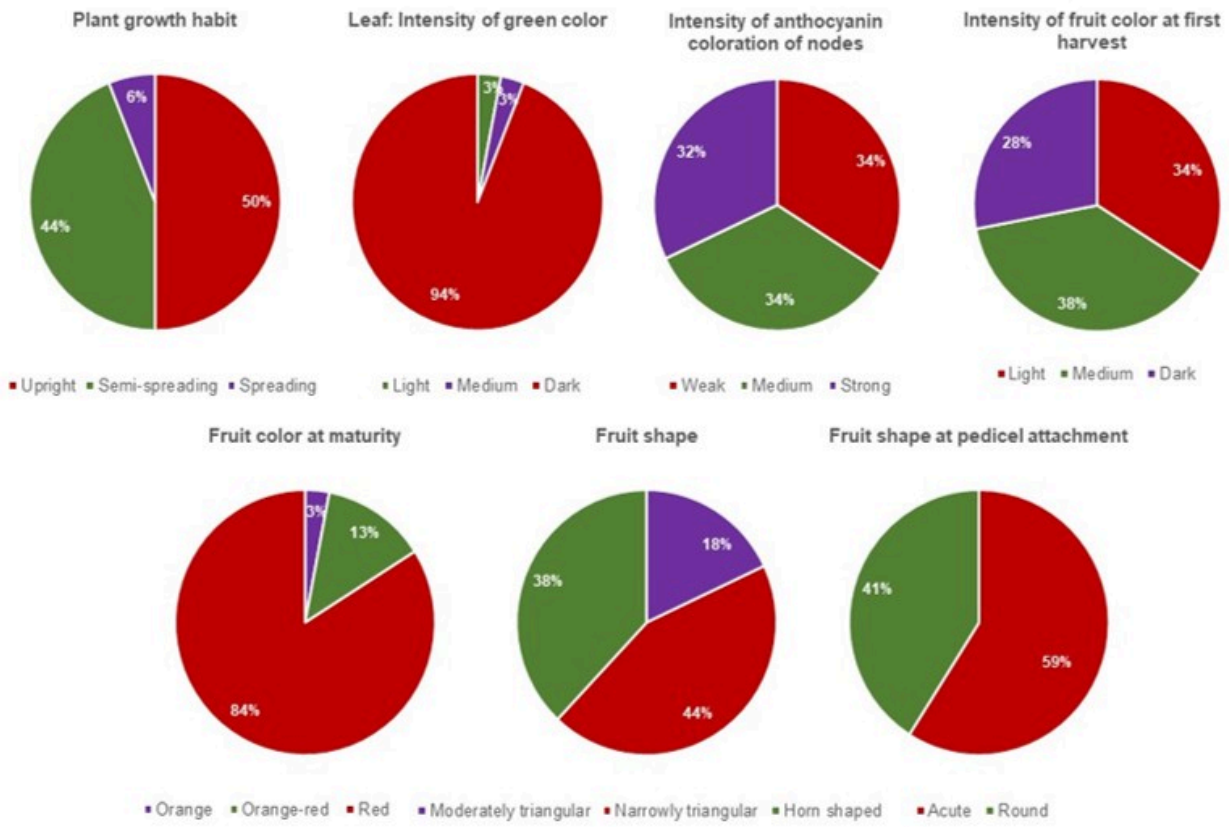


Figure 1

Frequency of 32 diverse chilli genotypes based on morphological descriptors

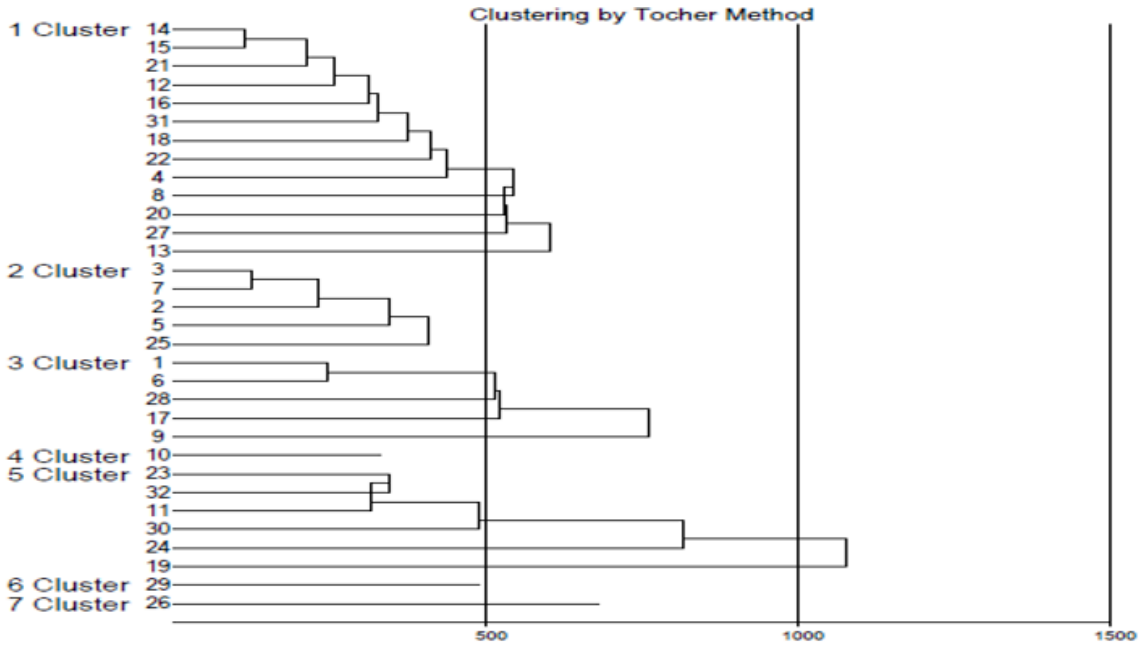


Figure 2

Dendrogram showing grouping of thirty-two chilli genotypes based on D^2 statistics using Tocher's method

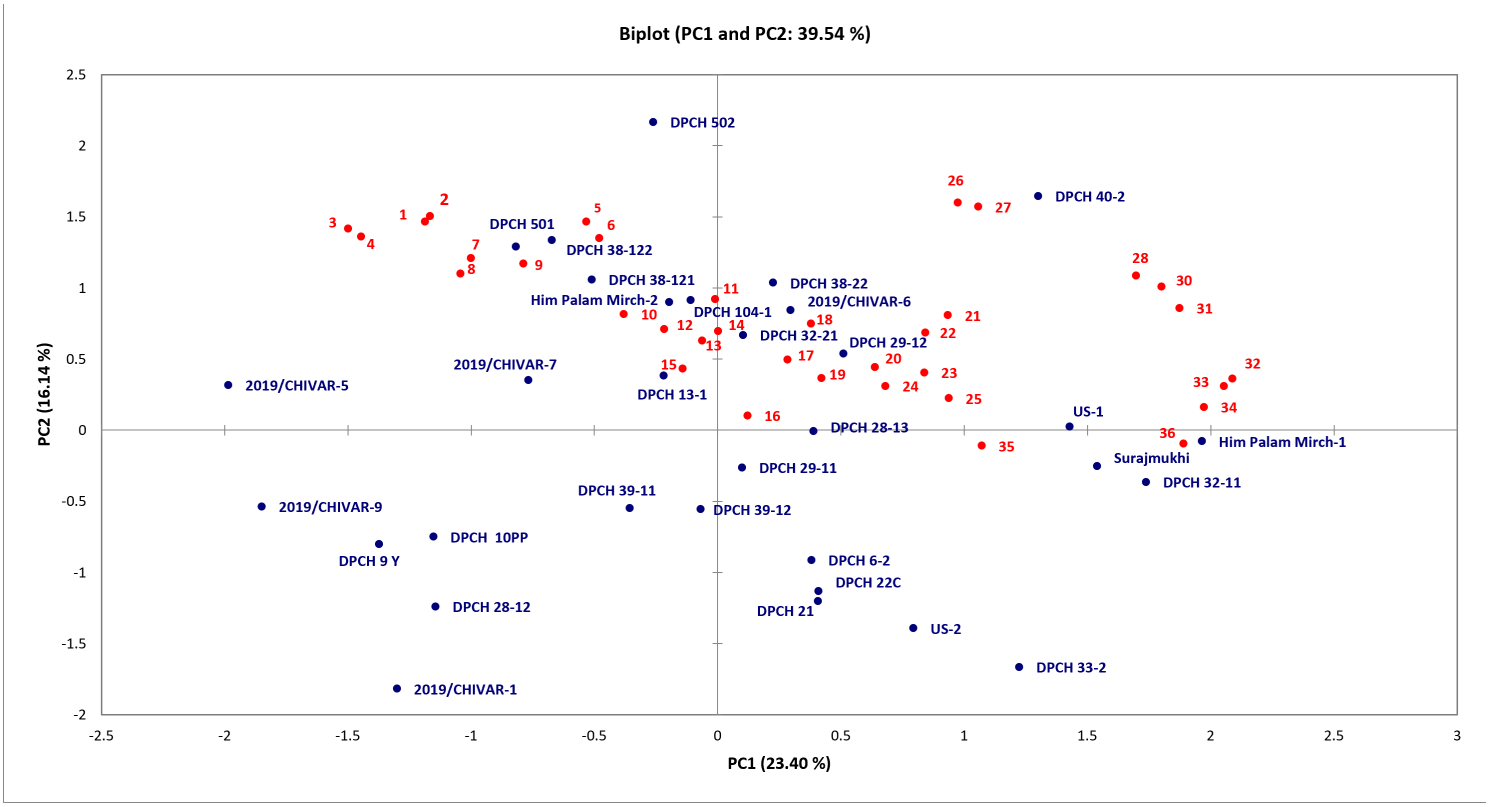


Figure 3

Principal component analysis (PCA) showing distribution of chilli pepper genotype collection with agro-morphological traits

Where, 1= fruit length (green), 2**=** fruit length (red), 3= average green fruit weight, 4= average red fruit weight, 5= number of seeds, 6= average dry fruit weight, 7= fruit girth (green), 8= fruit girth (red), 9= pedicel length (green), 10= pedicel length (red), 11= plant height (red), 12= primary branches (green), 13= primary branches (red), 14= plant height (green), 15= leaf petiole, 16= harvest duration (green), 17= harvest duration (red), 18= days to flowering, 19= days to first picking, 20= capsaicin content, 21= ascorbic acid, 22= secondary branches (red), 23= secondary branches (green), 24= leaf length, 25= leaf width, 26= marketable green fruit yield per plant, 27= marketable red ripe fruit yield per plant, 28= dry fruit yield per plant, 29= percent marketable red fruits per plant, 31= percent marketable fruits (green), 32= marketable red fruits per plant, 33= marketable green fruits per plant, 34= total green fruits per plant, 35= oleoresin content, 36= total red fruits per plant

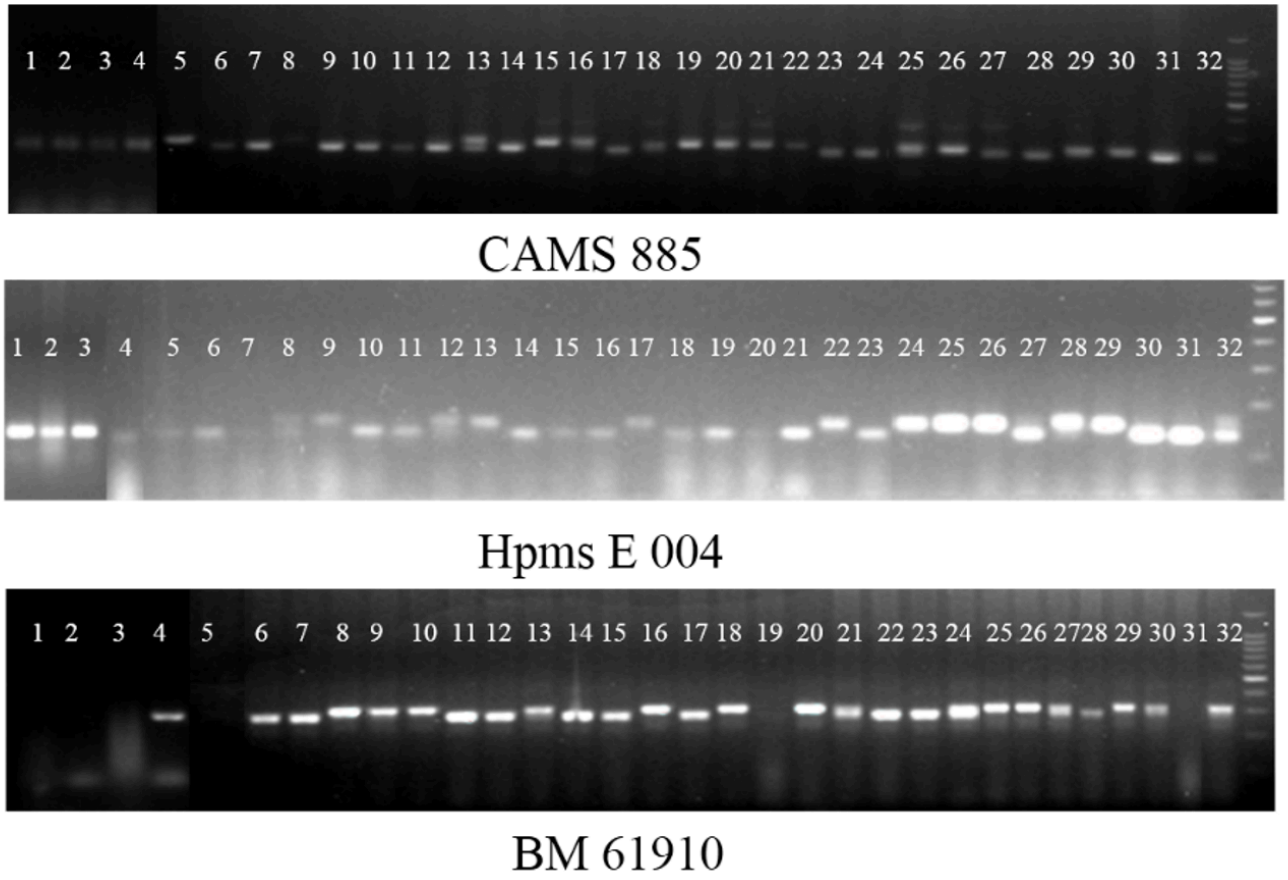


Figure 4

PCR amplification profile of genomic DNA of 32 genotypes of chilli pepper with SSR markers

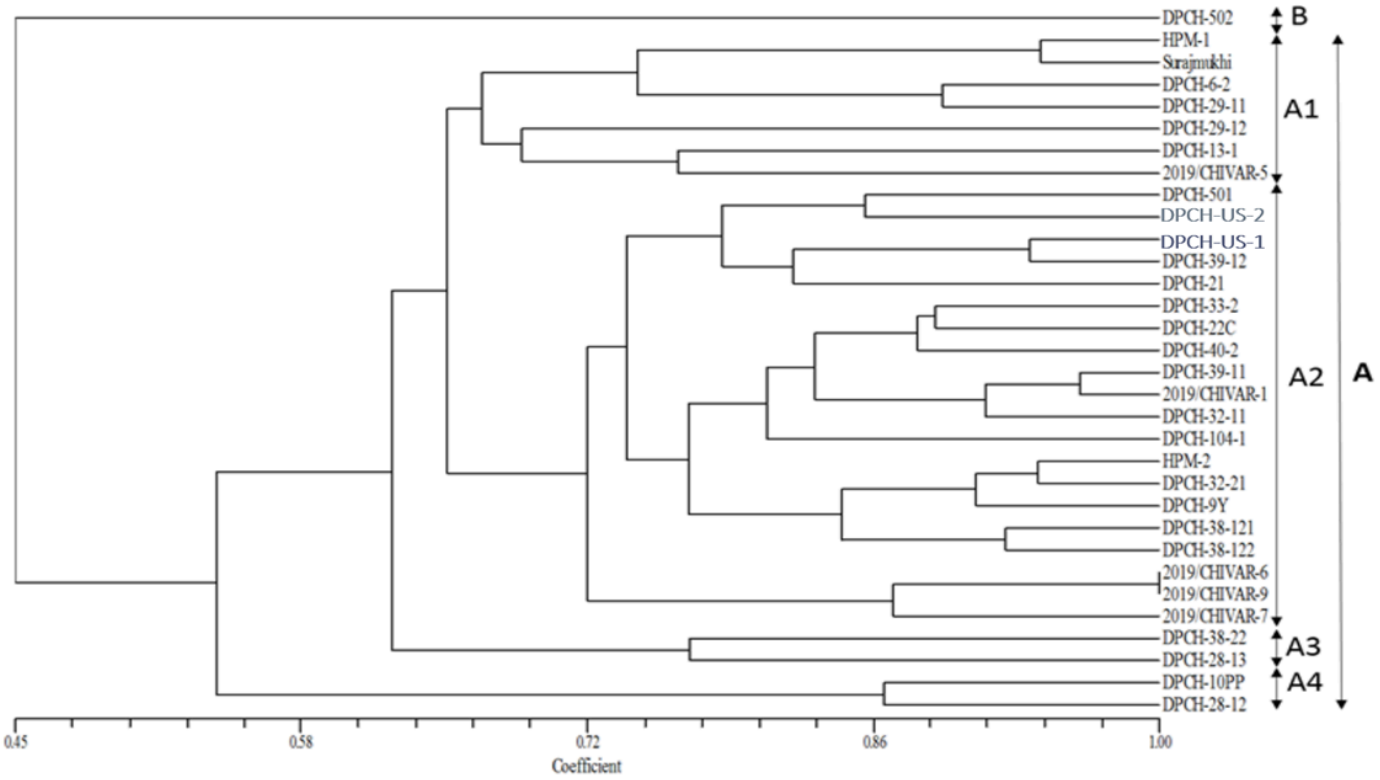


Figure 5

Dendrogram showing the genetic relationships among the 32 chilli pepper genotypes as revealed by SSR markers

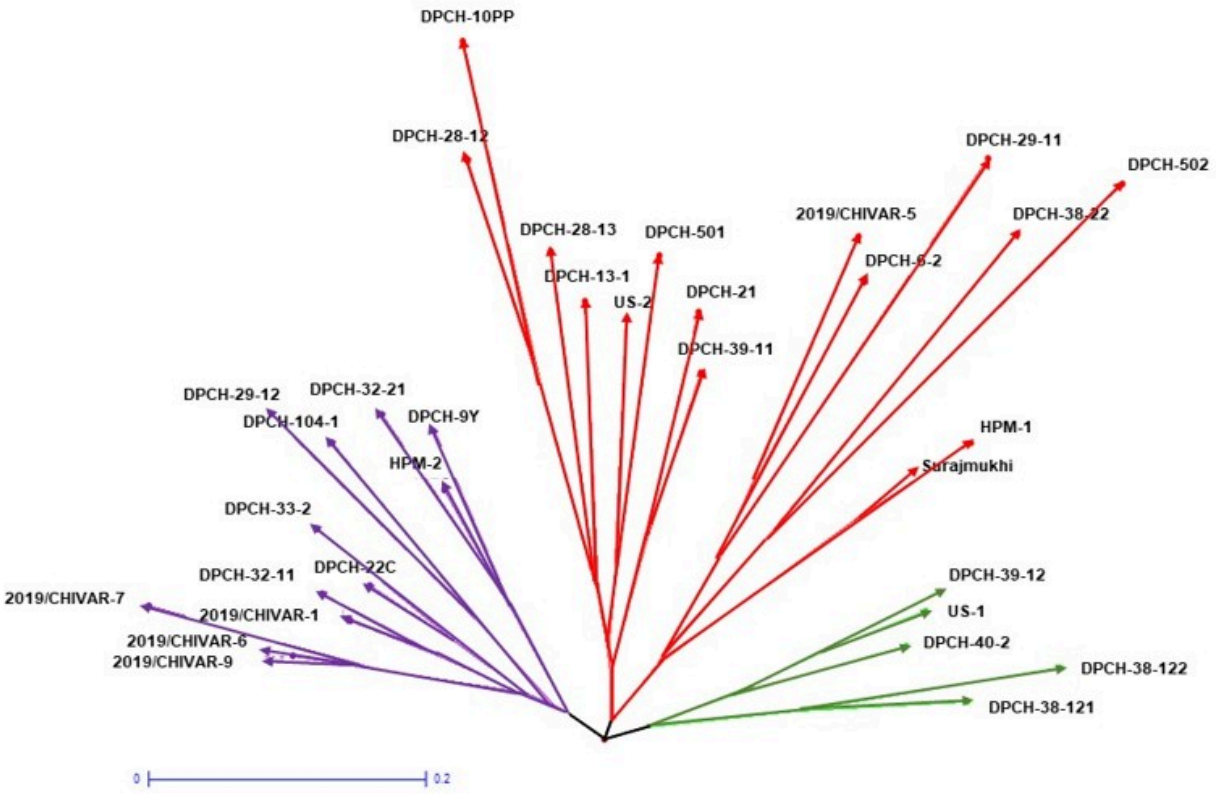


Figure 6

Neighbor joining tree of chilli pepper genotypes based on 15 polymorphic SSR markers

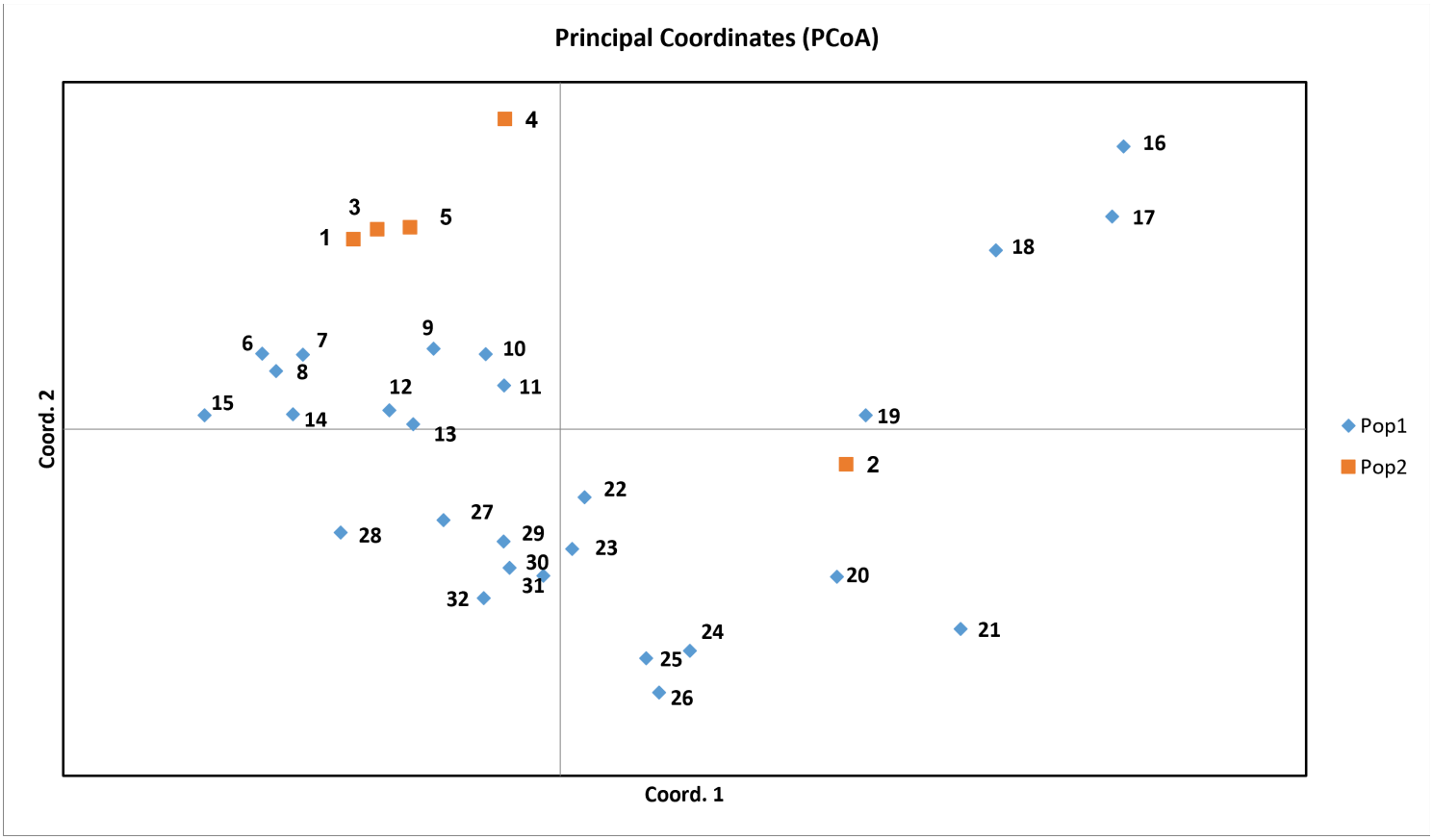


Figure 7

Principal coordinate analysis (PCoA) used to analysis substructures of chilli genotypes

Where, 1= 2019/CHIVAR-1, 2= 2019/CHIVAR-5, 3= 2019/CHIVAR-6, 4=2019/CHIVAR-7, 5=2019/CHIVAR-9, 6= DPCH-33-2, 7= DPCH-32-11, 8= DPCH-22C, 9= Surajmukhi, 10= Him Palam Mirch-1, 11= US-2, 12, 12= DPCH-29-12, 13= DPCH-40-2, 14= Him Palam Mirch-2, 15= DPCH-104-1, 16= DPCH-502, 17= DPCH-29-11, 18= DPCH-38-22, 19= DPCH-6-2, 20= DPCH-38-122, 21= DPCH-10PP, 22= DPCH-38-121, 23= DPCH-501, 24= DPCH-21, 25= DPCH-28-13, 26= DPCH-28-12, 27= DPCH-32-21, 28= DPCH-9Y, 29= DPCH-39-12, 30= US-1, 31= DPCH-39-11, 32= DPCH-13-1

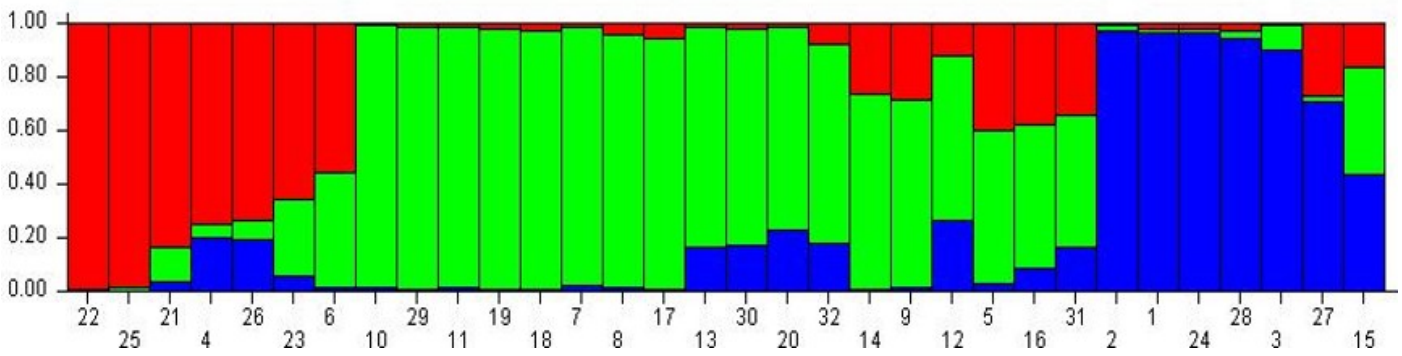


Figure 8

Analysis of substructure of the chilli pepper genotypes using STRUCTURE software at *K* = 3

Supplementary Files

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- 4SupplementaryTable.docx