Exploring the genetic makeup and population structure among *Capsicum* accessions for crop improvement and breeding curriculum insights

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**Abstract**  
**Background:** *Capsicum* or chilli is an important crop in India which exhibits immense structural and genetic variations reflecting their intra- and inter-specific relationships. The aim of this study was to establish relationships amongst 54 *Capsicum* accessions through analysis of genetic and population structure using ISSR markers.  
**Results:** Out of 19, successful DNA amplifications were shown by 7 ISSR primers and a total of 80 bands were identified ranging between 8 and 14 with an average of 11.43 bands/primer. A significant degree of polymorphic information content (PIC), discriminating power (DP), resolving power (RP), effective multiplex ratio (EMR), and marker index (MI) were identified as 0.39, 0.70, 6.40, 5.88, and 2.30, respectively, using ISSR markers in chillies. The cross-transferability ranged from 8.0 to 72.15% with an average of 52.63% among chillies. Amongst genetic information, grand mean values were 0.264, 0.180, 0.376, 0.296, and 0.180, which correspond to Shannon’s information index (I), expected heterozygosity (He), Nei’s gene diversity, total diversity among species (Ht), diversity within species (Hs), respectively. Further, the coefficients of gene differentiation (Gst) and gene flow (Nm) were 0.393 and 0.773, representing higher genetic variation among the population which was confirmed by analysis of molecular variance (AMOVA).  
**Conclusion:** ISSR markers represented a potent system for the estimation of relationships or variation studies and generated information useful for planning crop management and improvement strategies in chilli breeding.  

**Keywords:** *Capsicum* accessions, Inter-Simple Sequence Repeats (ISSR), DNA finger printing, Genetic diversity

**Background**  
Chilli or hot pepper is an important vegetable spice crop with widespread cultivation in the tropical and subtropical areas globally. The *Capsicum* genus represents a wide genetic diversity comprising 38 species [1] out of which, *C. annumm*, *C. frutescens*, *C. baccatum*, *C. chinense*, and *C. pubescens* are domesticated species worldwide [2]. Among these, *C. annumm* is a largely cultivated species, used as vegetable and spice globally. Regarding to nutritive value, chilli is a rich source of many essential vitamins, minerals, and nutrients that have a great importance for human health and consumption [3]. Besides this, chilli finds its use in pharmaceuticals and cosmetics, as natural coloring additive and in defense repellents [4]. In Solanaceae family, chilli harbors most complex and largest plant genome sizes, varying from 3.3 to 3.6 GB and usually with chromosome numbers 2n = 24 [5, 6]. Repetitive DNA elements are frequently found in its genome and constitute above 80% of the genome [4, 7]. Subsequently, it is envisaged that transposable elements would possibly have a role in repeat evolution and genome size variation of *Capsicum* species.
elements are driving evolutionary forces often causing species diversification and rearrangement in chilli genomes [8]. In addition, the development of new genes by gene duplication are important for the generation of functional diversity between the species and selection of superior ones for further crop improvement or breeding processes [9, 10].

*Capsicum* species also exhibits a huge variation in morphological features, biochemical properties, and at molecular level; thus, these differences make divergences amongst species [11–13]. Also, the immense genetic diversity displayed by *Capsicum* species is an important factor that provides the information about conservation of genetic resource, breeding practices, evolutionary transitions, adaptation under biotic and abiotic pressures, and ecology and environmental relationships [14, 15]. This diversity unveils the level of delineation within or between species or populations, and these variations are very important to identify the connection between species or cultivars which apprise us about the kind of crop evolution that took place and is very supportive in the breeding programmes. Proper assessment and pattern of genetic diversity in plants or corps are invaluable for knowing the genetic variability within or across the cultivar, development of segregating progenies with maximum genetic variability from the analysis of parental combinations for further selection, and transfer of desirable genetic information from diverge germplasm into existing genetic design [16]. Hence, the assessment of genetic diversity is the key step which aid in the practices of crop improvement and breeding practices for the development of superior cultivars [17].

The last few decades have witnessed the utility of molecular marker technologies especially DNA-based marker systems in various genetics studies mainly due to their ease, quickness, and economic feasibility along with their well discriminatory potential within and across species or varieties [18]. Simultaneously, the introduction of new principles has strengthened a molecular marker technology for sophisticated exploration of genetic variation analysis that have provided simple and easy platform for determining morphological, ecological, conservatory, and evolutionary relationship within and across species [19, 20]. Molecular markers are more decisive and preferable for identifying genetic variation because they are inert to environmental pressure and have the capacity to distinguish a variation at genome level making them more suitable to assess genetic diversity [3]. At present, frequently used DNA-based markers or molecular markers are restriction fragment-length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment-length polymorphisms (AFLPs), inter-simple sequence repeats (ISSRs), simple sequence repeats (SSRs), single-nucleotide polymorphisms (SNPs), start codon-targeted polymorphism (SCoT), etc. [21]. Using these marker technologies, numerous studies have been conducted in different plant species depending on specific genetic applications desired by various research groups [3, 22–26].

In the present study, assessment of genetic variation was done using ISSR markers to sketch a comparative overview of degree of genetic polymorphisms, primer efficiency, cross-transferability, and genetic and structural plasticity among 54 *Capsicum* accessions. ISSR marker system offers quick, easy handling, reliable, cost-effective, and highly informative method for a variety of genetic applications [26]. ISSR markers are highly reproducible that target microsatellites which are densely distributed throughout the plant or eukaryotic genome and reveal increased level of polymorphisms due to their higher annealing temperature and longer primer sequence length along with no requirement of prior information of flanking sequence like SSRs [27]. The advantages with this marker system comprise that they are present in both nuclear and organelle genomes, and their segregation follows the Mendelian rule as dominant markers and are highly polymorphic [28, 29]. Also, ISSR markers have proven their supremacy in variety of applications such as cultivar identification, genetic diversity, gene tagging, genome mapping, molecular ecology, phyllogenetic studies, plant breeding, and evolutionary analysis [30–34].

**Materials and methods**

**Plant materials and growing conditions**

A set of 54 accessions of *Capsicum* was procured from various research centres in India namely: Agriculture Research Station, Jodhpur; Indian Institute of Vegetable Research, Varanasi (ICAR-IIHR); School of Life Science, Jawaharlal University, New Delhi; National Bureau of Plant Genetic Resources, Hyderabad; and National Seeds Corporation, Hyderabad. These chilli accessions comprised 49 varieties of *C. annumm*, 3 varieties of *C. baccatum*, and 2 varieties of *C. frutescens* (Table 1). Seeds were planted in a seed tray and kept in a plant growth chamber under controlled growth environments 26 ± 1°C temperature, 16 h photoperiod, and 300 μmol/m² s⁻¹ photosynthetic photon fluxes according to the method explained by Gupta [3].

**DNA extraction and purification**

DNA extraction was carried out from fresh young leaves (5g) using CTAB method (Doyle & Doyle, 1990) with minor modifications. The leaves were grinded in extraction buffer [1 M Tris (pH 8.0), 0.5 M EDTA, 5 M NaCl, and 200 μM β-mercaptoethanol] and incubated at 65°C
Table 1 Capsicum accessions used for the genetic assessment study

| Sr. No. | Species | Accessions     | Sr. No. | Species | Accessions     |
|---------|---------|----------------|---------|---------|----------------|
| 1       | C. annuum | EC-596878      | 28      | C. annuum | Panjab Lal up |
| 2       | C. annuum | EC-596920      | 29      | C. annuum | Pant C-1 up   |
| 3       | C. annuum | EC-596940      | 30      | C. annuum | Arka Abhir    |
| 4       | C. annuum | EC-599955      | 31      | C. annuum | Kashi Anmol   |
| 5       | C. annuum | EC-599977      | 32      | C. annuum | Jayanti       |
| 6       | C. annuum | IC-328725      | 33      | C. annuum | LCA-423       |
| 7       | C. annuum | IC-361989      | 34      | C. annuum | LCA-402       |
| 8       | C. annuum | IC-372043      | 35      | C. annuum | EC-391075     |
| 9       | C. annuum | IC-565081      | 36      | C. annuum | LCA-440       |
| 10      | C. annuum | IC-572470      | 37      | C. annuum | LCA-443       |
| 11      | C. annuum | IC-572481      | 38      | C. annuum | LCA-434       |
| 12      | C. annuum | IC-572491      | 39      | C. annuum | LCA-422       |
| 13      | C. annuum | Pusa Sada Bahar | 40      | C. annuum | LCA-403       |
| 14      | C. annuum | Pusa Jivala    | 41      | C. annuum | LCA-353       |
| 15      | C. annuum | Pant Chilli-1   | 42      | C. annuum | LCA-335       |
| 16      | C. annuum | Chilli G-4      | 43      | C. annuum | LCA-427       |
| 17      | C. annuum | Chilli G-5      | 44      | C. annuum | LCA-435       |
| 18      | C. annuum | Chilli G-6      | 45      | C. annuum | LCA-206       |
| 19      | C. baccatum | EC-382035    | 46      | C. annuum | LCA-334       |
| 20      | C. baccatum | IC-315759    | 47      | C. annuum | AKC-89/38UP   |
| 21      | C. baccatum | EC-382035    | 48      | C. annuum | EC-341094     |
| 22      | C. frutescens | NMCA-40008 | 49      | C. annuum | EC-518968     |
| 23      | C. frutescens | COO-309     | 50      | C. annuum | EC-566320     |
| 24      | C. annuum | Phule Jyoti    | 51      | C. annuum | EC-622085     |
| 25      | C. annuum | Byadigi Kaddi  | 52      | C. annuum | EC-596958     |
| 26      | C. annuum | Byadigi Dabbi  | 53      | C. annuum | EC-497632     |
| 27      | C. annuum | Kashi Gaurav   | 54      | C. annuum | NIC-268216    |

Finally, 7 ISSR primers were selected for analysis among 54 chilli accessions due to their sharp and clear banding profiles. All PCR reactions were performed in the final volume of 10 μl each using thermal cycler (BioRad, UK). Each reaction mixture contained 1 μl of DNA template (25 ng), 1.0 μl Taq buffer (10X) with 2.5 mM of MgCl₂, 1 μl of primer (10 pmole/ μL), 0.25 μl of dNTPs (100 mM), and 0.1 μl of Taq DNA polymerase (0.5 U). PCR amplification conditions included initial denaturation at 94°C for 3 min followed by 35 cycles which included denaturation at 94°C for 1 min followed by annealing at 45 to 51°C for 1 min depending upon primers and then extension at 72°C for 2 min with final extension at 72°C for 7 min. All amplified products were separated through agarose gel electrophoresis using 1.2% agarose gel (Himedia) in 0.5× TBE (Tris-Borate- EDTA) buffer for ~1.5 h at 70 V. Gel was stained with ethidium bromide dye, and BioRad gel doc system was used for visualization of DNA bands and further analysis.

Estimation of ISSR marker efficiency

Clear and reproducible amplified bands obtained from the DNA amplifications profile for each ISSR primer were used for the experiment and scored as binary matrix, 1 for the presence and 0 for the absence. The efficiency of ISSR markers was calculated as described by polymorphic information content (PIC), discriminating power (DP), resolving power (RP), effective multiplex ratio (EMR), and marker index (MI) using iMEC platform [36]. The relative primer polymorphisms and cross-transferability were measured within and across different chilli accessions using ISSR markers [25].

Genetic and structural measurement in the population and statistical analysis

Parameters such as the number of different allele (Na), number of effective allele (Ne), Shannon’s information index (I), expected heterozygosity (He), unbiased expected heterozygosity (uHe), analysis of molecular variance (AMOVA), and principal coordinate analysis (PCoA) were evaluated through GenALEX 6.5 program [37]. Further, the factors namely Nei gene diversity, total species diversity (Ht), diversity within population (Hs), coefficient of gene differentiation (Gst), and gene flow (Nm) were examined to evaluate genetic flow using the POPGENE 1.32 software [38]. The genetic similarity among different chilli accession was identified by FreeTree software which generated a similarity matrix based on Jaccard’s similarity coefficient [39], and this matrix was further utilized to generate dendrogram based on UPGMA (Unweighted Pair Group Method Using
Arithmetic Averages) algorithm using TreeView X software [40].

Structural plasticity in the different chilli accessions was further evaluated by Euclidean similarity index and correlation matrix, and both were characterized according to Fruchterman-Reingold algorithm which computes the biological data according to space filling curve manner [41] using PAST 4.02 statistical software [42]. In order to confirm subpopulation (K) numbers in *Capsicum* accessions, the genetic makeup was further explored by STRUCTURE software version 2.3.4 based on Bayesian model-based clustering analysis [43]. To identify putative subpopulation (K), each chilli accession was tested for K = 1 to K = 10 with admixture model and correlated allele frequencies. The five independent runs were assessed for each fixed K with a burn-in period of 10,000 and 100,000 Markov chain Monte Carlo (MCMC) iterations. The optimum K value was examined by ΔK statistic and L (K) [44] using Structure Harvester program [45].

**Results**

**ISSR markers and GC content**

Initially, 19 ISSR markers were used for the analysis amongst which 7 ISSR markers were retained due to their successful amplification amongst different *Capsicum* accessions. The GC-content of the 7 selected primers were belonged to 47% and 53% whereas 4 primers revealed 53% GC content and other 3 primers displayed 47% GC content. All the selected primers anchored with different dinucleotide repeat microsatellites with each having 17 bp long sequence, and their annealing temperatures ranged from 45 to 51°C (Table 2).

**ISSR-PCR amplification in Capsicum accessions**

Out of 19 primers, 7 primers showed a successful DNA amplification at different annealing temperature and rest of them were unable to retain any PCR amplification amongst different *Capsicum* accessions. A total of 80 DNA amplicons or bands were obtained ranging from 8 to 14 bands with an average of 11.42 bands per primer. Amongst *Capsicum* accessions, the maximum banding patterns were observed in *C. annum* followed by *C. baccatum* and *C. frutescens* (Fig 1). The primer UBC 808 (14 bands) and UBC 818 (8 bands) showed increased and reduced DNA banding profile, respectively, and the size of DNA amplicons ranged from 141.15 to 2265.32 bp with an average of 583.38 bp in size (Table 2).

**Efficiency of ISSR marker in Capsicum accessions**

The efficiency of ISSR markers amongst different *Capsicum* accessions was identified through the estimation of various parameters such as polymorphic information content (PIC), discriminating power (DP), resolving power (RP), effective multiplex ratio (EMR), and marker index (MI). The PIC ranged from 0.37 (UBC 813) to 0.42 (UBC 810) with an average of 0.39. Moreover, the differential DNA banding pattern amongst chilli accessions was defined by DP and the average value was 0.7 which ranged from 0.45 (UBC 10) to 0.89 (UBC 808). Distribution of DNA banding among chilli accessions was calculated by RP which ranged from 4.9 (UBC 813) to 7.9 (UBC 809) with an average of 6.40 RP (Table 2). Further, an average EMR was 5.88 ranging from 4.43 (UBC 818) to 8.26 (UBC 807) and MI ranging from 1.67 (UBC 818) to 3.36 (UBC 807) with an average of 2.30 was observed. Thus, ISSR markers revealed significant genetic polymorphism amongst different chilli accessions taken for this study.

**Primer polymorphism and cross-transferability in Capsicum accessions**

Depending upon the banding profile amongst chilli accessions, the primer polymorphism falls in a range from 79.62% (UBC 818) to 100% (UBC 810) with an average of 91.80% polymorphism. Moreover, the cross-amplification potential or cross-transferability of primers was further identified amongst different chilli accessions and it ranged from 8.0 to 72.15% with an average of 52.63% (Fig. 2). Accessions EC-497632 and AKC-89/38UP showed a reduced and increased cross-transferability, respectively, and some other accessions IC-361989, Chilli G-4, and Pant C-1 UP also revealed significantly increased cross-transferability amongst *C. annum*. However, significantly reduced cross-transferability was also identified in EC-596958, EC-596878, NIC-268216, and EC-391075 and moderate level of cross-amplification also observed in *C. annum* accessions. All the accessions belonging to *C. baccatum* namely EC-382035, IC-315759, and PBC-81 showed improved cross-transferability. Elevated level of cross-amplification was also observed in EC-382035 and IC-315759 accession belonging to *C. frutescens*.

**Characterization of genetic structure in Capsicum species**

The genetic information ascertained at ISSR marker level among various chilli accessions revealed that *Na* was common for all the ISSR markers followed by *Ne* which varied from 1.442 (UBC 7) to 1.779 (UBC 8) and *I* ranged from 0.428 (UBC 7) to 0.616 (UBC 8), while *He* ranged from 0.275 (UBC 7) to 0.428 (UBC 8) and *uHe* were in between 0.277 and 0.432 for UBC 7 and UBC 8, respectively. *Nei’s* gene diversity ranged from 0.283 (UBC 808) to 0.432 (UBC 807) with an average of 0.383 while *Ht* ranged from 0.231 (UBC 807) to 0.354 (UBC 818) with an average of 0.297 and an average *Hs*: 0.178, which ranged from 0.146 (UBC 807 and UBC 813) to
Table 2  Depiction of primers used in the study and their amplification efficacy

| S.N. | ISSR primers | Sequence (S’ – 3’) | GC-content | Annealing temperature | Total bands | Range (bp) | Polymorphism information content (PIC) | Discriminating power (DP) | Resolving power (RP) | Effective multiplex ratio (EMR) | Marker index (MI) |
|------|--------------|-------------------|------------|-----------------------|-------------|------------|-------------------------------------|--------------------------|----------------------|-------------------------------|-----------------|
| 1    | UBC 807     | (AG)₈T           | 47         | 51°C                  | 12         | 153-883    | 0.407                              | 0.527                    | 5.778                | 8.26                          | 3.36            |
| 2    | UBC 808     | (AG)₈C           | 53         | 51°C                  | 14         | 187-941    | 0.401                              | 0.891                    | 7.704                | 4.63                          | 1.85            |
| 3    | UBC 809     | (AG)₈G           | 53         | 51°C                  | 13         | 168-1999   | 0.375                              | 0.780                    | 7.926                | 5.63                          | 2.11            |
| 4    | UBC 810     | (GA)₈T           | 47         | 45°C                  | 10         | 225-1434   | 0.414                              | 0.495                    | 5.185                | 7.11                          | 2.95            |
| 5    | UBC 811     | (GA)₈C           | 53         | 45°C                  | 11         | 141-892    | 0.376                              | 0.704                    | 6.222                | 5.44                          | 2.04            |
| 6    | UBC 813     | (CT)₈T           | 47         | 49°C                  | 12         | 324-2265   | 0.374                              | 0.779                    | 7.148                | 5.65                          | 2.12            |
| 7    | UBC 818     | (CA)₈G           | 53         | 45°C                  | 8          | 162-1238   | 0.376                              | 0.694                    | 4.852                | 4.43                          | 1.67            |
| Average |            |                   | 50.43      | 48.14                 | 11.42      | 583.38     | 0.389±0.017                        | 0.696±0.142              | 6.402±1.216          | 5.88±1.36                     | 2.30±0.62       |
0.219 (UBC 808), while the average of \( \text{Gst} \) was 0.337 which varied from 0.228 (UBC 808) to 0.449 (UBC 818) and \( Nm \) varied from 0.712 (UBC 818) to 6.171 (UBC 808) with an average of 3.261 (Table 3). Hence, the grand mean values of genetic parameters; Nei gene diversity, \( Ht \), \( Hs \), \( \text{Gst} \), and \( Nm \) were 0.376, 0.296, 0.180, 0.393, and 0.773, respectively (Table 4).

**Population structure of Capsicum accessions**

A significant genetic differentiation was observed within and across chilli accessions using AMOVA \((P < 0.001)\) which is useful for partitioning of the overall variation. The results indicated that 89% of total variance occurred within chillies and 11% among chillies (Fig. 3). The structural plasticity in the *Capsicum* population
Table 3. Characterization of a genetic plasticity at a marker level among chilli accessions

| Primers | Na        | Ne        | I         | He        | uHe       | Nei gene diversity | Ht         | Hs         | Gst        | Nm*       |
|---------|-----------|-----------|-----------|-----------|-----------|--------------------|------------|------------|------------|-----------|
| UBC 807 | 1.167±0.081 | 1.267±0.052 | 0.208±0.041 | 0.145±0.028 | 0.147±0.029 | 0.432±0.092         | 0.231±0.077 | 0.146±0.029 | 0.324±0.143 | 1.546±1.612 |
| UBC 808 | 1.476±0.084 | 1.384±0.038 | 0.337±0.030 | 0.225±0.021 | 0.256±0.025 | 0.282±0.154         | 0.315±0.182 | 0.219±0.114 | 0.228±0.189 | 6.171±9.663 |
| UBC 809 | 1.282±0.080 | 1.353±0.040 | 0.295±0.032 | 0.201±0.022 | 0.222±0.024 | 0.372±0.130         | 0.295±0.154 | 0.201±0.125 | 0.276±0.225 | 5.605±9.473 |
| UBC 810 | 1.233±0.078 | 1.275±0.048 | 0.217±0.038 | 0.151±0.026 | 0.156±0.027 | 0.400±0.130         | 0.237±0.126 | 0.151±0.072 | 0.299±0.168 | 3.450±7.024 |
| UBC 811 | 1.233±0.107 | 1.338±0.047 | 0.280±0.037 | 0.191±0.025 | 0.204±0.027 | 0.402±0.142         | 0.338±0.151 | 0.191±0.083 | 0.353±0.253 | 3.747±7.068 |
| UBC 813 | 1.194±0.085 | 1.249±0.042 | 0.216±0.036 | 0.146±0.024 | 0.153±0.025 | 0.370±0.110         | 0.309±0.159 | 0.146±0.068 | 0.430±0.250 | 1.597±2.038 |
| UBC 818 | 1.417±0.073 | 1.344±0.055 | 0.277±0.041 | 0.191±0.029 | 0.208±0.032 | 0.422±0.101         | 0.354±0.093 | 0.191±0.043 | 0.449±0.110 | 0.712±0.473 |
| Average | 1.286±0.084 | 1.316±0.046 | 0.262±0.036 | 0.179±0.025 | 0.383±0.027 | 0.383±0.123         | 0.297±0.134 | 0.178±0.076 | 0.337±0.191 | 3.261±5.336 |

*Na*, observed number of alleles; *Ne*, effective number of alleles; *I*, Shannon’s Information Index; *Ht*, total diversity; *Hs*, diversity within population; *Gst*, coefficient of gene differentiation; *Nm* *, gene flow
### Table 4 Characterization of genetic plasticity at species level among chilli accessions

| Species             | $Na$      | $Ne$       | $I$      | $He$       | $uHe$      | Nei gene diversity | $Ht$      | $Hs$      | $Gst$      | $Nm^*$    |
|---------------------|-----------|------------|----------|------------|------------|---------------------|-----------|-----------|------------|-----------|
| Capsicum annumm     | 2.000 ± 0.000 | 1.656 ± 0.034 | 0.549 ± 0.018 | 0.373 ± 0.015 | 0.377 ± 0.015 |                     |           |           |            |           |
| Capsicum baccatum   | 1.114 ± 0.079 | 1.223 ± 0.042 | 0.181 ± 0.032 | 0.124 ± 0.022 | 0.149 ± 0.027 |                     |           |           |            |           |
| Capsicum frutescens | 0.747 ± 0.071 | 1.072 ± 0.024 | 0.061 ± 0.021 | 0.042 ± 0.014 | 0.056 ± 0.019 |                     |           |           |            |           |
| Grand mean          | 1.287 ± 0.049 | 1.317 ± 0.025 | 0.264 ± 0.019 | 0.180 ± 0.014 | 0.194 ± 0.015 | 0.376 ± 0.132       | 0.296 ± 0.021 | 0.180 ± 0.008 | 0.393      | 0.773     |

$Na$, observed number of alleles; $Ne$, effective number of alleles; $I$, Shannon's Information Index; $Ht$, total diversity; $Hs$, diversity within population; $Gst$, coefficient of gene differentiation; $Nm^*$, gene flow.
was further identified by Jaccard's similarity coefficient, UPGMA clustering, Euclidean similarity, correlations, and principal coordinate analysis (PCoA) among the different Capsicum accessions. The Jaccard’s similarity coefficient fluctuated from 0.02 to 0.89, and maximum similarity was observed between different Capsicum accessions in the order: 0.89 between C23 & C21, 0.85 in C21 & C22, 0.84 in C22 & C23 and C11 & C16, 0.81 in C10 & C13 and C7 & C19, and 0.80 in between C20 & C21 and C42 & C44 (Fig. 4). The genetic relatedness analyzed amongst Capsicum accessions by UPGMA cluster analysis. Broadly, two major (I and II) groups were found with 18 and 13 chilli accessions which were placed into distinct branches in the dendrogram along with several other loose clusters containing few chilli accessions. It was observed that all the C. baccatum and C. frutescens accessions represented closeness and were placed in group I; on the other hand, they also showed association of several C. annumm accessions in the present study (Fig. 5).

The two major groups along with several loose associations were also observed through Euclidean similarity index associated with Fruchterman-Reingold algorithm which explained an intuitive and efficient representation of different Capsicum accessions into space-filling curves manner and provided a new way for the computation of biological data (Fig. 6). Similarly, the representation of relatedness and grouping were further supported by correlation matrix associated with Fruchterman-Reingold algorithm amongst different chilli accessions (Fig. 7). Major finding of present study indicates that broadly two major set of associations with inter- and intra-locking relation within chilli accessions with some reduced networks which were revealed within chillies.

In addition, principal coordinate analysis (PCoA) was performed to visualize population structure for 54 different chilli accessions and the results of first three PCoA
accounted 39.18% of total variation. Based on the PCoA outcomes, two groups were differentiated majorly with densely concentrated *Capsicum* accessions besides several other clustering which disclosed few members of *Capsicum* accessions (Fig. 8). Hence, the results of PCoA were found to be consistently similar with those accomplished by UPGMA clustering, and Euclidean similarity index and correlation matrix with Fruchterman-Reingold algorithm.

In order to confirm reliability of most likely grouping in 54 *Capsicum* accessions, an analysis was performed using Structure software. The maximum $\Delta K$ was observed at $K = 2$ with accessions falling into two groups, and the overall proportion of the samples in each of the two groups were 0.529 and 0.471 (Fig. 9). The inferred
Fig. 6  Euclidean similarity index with Fruchterman-Reingold algorithm data-based population characterization among 54 different Capsicum accessions

Fig. 7  Representation of correlation analysis with Fruchterman-Reingold algorithm among 54 different Capsicum accessions
Fig. 8  Principal coordinate analysis (PCoA) of 54 Capsicum accessions. Horizontal and vertical scales correspond to the first and second principal axes of variation, respectively, which represents the degree of variations among various chilli accessions.

Fig. 9  Inferred population structure of 54 Capsicum accessions based on ISSR markers using STRUCTURE program which observed at K = 2. The maximum value of ΔK was determined at K=2 by STRUCTURE HARVESTER.
population structure for $K = 2$ showed that 89% of the accessions have a membership coefficient ($q_i$) to one of the subpopulations higher than 0.8, while the rest could be considered as admixed ($q_i \leq 0.8$). Thus, the outcome obtained from structure analysis revealed that all the accessions were categorized into two groups, which is in consistency with results retrieved from aforesaid mention in UPGMA, Euclidean similarity, correlation matrix, and PCoA results.

**Discussion**

*Capsicum* is one of the most important crops in India, comprising of agro-morphologically distinct *Capsicum* varieties, and India is known to be the biggest contributor for both production and consumption of *Capsicum*. Aside from variation in growing areas, *Capsicum* fruits also displayed a variation in the size, shape, color, taste, shelf life, and chemical composition [46]. Moreover, knowledge of variation in the genome size, genetic plasticity, level of adulteration, fruit quality, pungency, size, and color is very important parameters for breeding advancement programs in chillies. For deciphering variation in *Capsicum* species, morphological indicators have played a big role, among which flower and fruit characteristics are most important [47–50], in which biochemical, physiological, and molecular aspects are also extensively investigated [3, 51–54]. Though morphological and biochemical characters are credible scores for evaluating variation in *Capsicum* species but are also subject to change under different environmental conditions [55, 56], therefore accessing genetic diversity using molecular markers is more advantageous because molecular markers are phenotypically neutral and not regulated by environmental conditions. Several workers have attempted to unveil genetic diversity in *Capsicum* species using various molecular markers, such as AFLP [57], SSRs [58], RAMPO [59], and RAPD [60], but extensive studies using ISSR markers are sparsely available [61]. ISSR markers are dominant markers comprising of polymorphic arbitrary primers with high reproducibility and requiring high stringency in PCR conditions compared to RAPD markers system. Following Mendelian fashion of inheritance, this technique includes microsatellite repeats (di, tri, tetra, or penta nucleotides) unit bearing oligo-nucleotide primers, non-anchored, or anchored at the 5' or 3' end with 1 to 4 degenerate nucleotides and generally 16 to 25 nucleotides long [62, 63].

The efficiency of ISSR markers have been utilized in various plant such as *Solanum lycopersicum* [64], *Jatropha curcas* [23], *Cymbopogon germplasms*, [63] *Citrullus colocynthis* [26], *Arabidopsis thaliana* [65], and *Triticum durum* [66]. The aforesaid ISSR accomplishments in various plants paved the way to undertake the present study to establish the genetic correlation among 54 different *Capsicum* accessions comprising three distinct *Capsicum* species (*C. annuum, C. baccatum* and *C. frutescens*). The ISSR marker technique is a well-established significant approach for exploring the varieties for useful applications such as germplasm identification, parentage inquiry, genetic diversity, gene mapping, QTL (quantitative trait loci) analysis, evolutionary strategy, and taxonomic studies [30, 67–70].

In the present study, an average GC-content of the 7 selected ISSR markers was 54.28% which is in consistent with previous reports [31, 71]. A total of 80 bands were generated from 7 ISSR primers selected in the present study, and the average frequency of banding pattern was 11.43 bands per primer, while in another study in *Capsicum*, 2 ISSR primers amplified a total 38 bands with an average of 19 bands per primer [61]. Enhanced marker efficacy indices such as PIC, DP, RP, EMR, and MI reflect the discriminant potential of ISSR markers [36]. PIC is a measure of quality or informativeness of polymorphism which is defined by the number and frequency of the alleles generated by given molecular marker, and thus, values in between 0 and 0.5 correspond to dominant marker while in between 0 and 1 correspond to co-dominant marker [36, 72]. In the present study, PIC ranged from 0.37 to 0.42 with an average of 0.39 PIC which is in compliance with 0.40 PIC reported while evaluating genetic diversity based on fruit pericarp in *Capsicum annuum*. An average PIC value of 0.156 was reported for 237 accessions of *C. baccatum, C. annuum, C. chinense*, and *C. frutescens* using AFLP markers [57], whereas deciphering genetic diversity in chilli germplasm, average PIC was observed to be 0.69 using SSR markers and 0.63 using RAMPO markers in 48 Chilli accessions [59] and 0.77 PIC was observed using two ISSR markers in chilli accessions [61].

Likewise, the RP value corresponds to the effectiveness of the marker for identification of variation, and in the present study, an average RP of 6.40, ranging from 4.9 to 7.9, was recorded, which is similar to genetic analysis done in different plant species [26, 73–75], but contrary to this, 16.08 Rp value was recorded using two ISSR primers in 12 *Capsicum* accessions [61]. A significant polymorphism within the accession is measured by DP while lower and higher values of DP represent highly and reasonably polymorphic nature of marker between the accessions. In this study, DP ranged from 0.45 to 0.89 with an average of 0.7 DP in different chilli accessions which is in consensus with outcome of several analysis performed with ISSR markers in different plant species [36, 76, 77], whereas in chilli germplasm using 7 SSR primers an average DP value of 0.40 was observed [58]. On the basis of allelic frequency, the informativeness of
makers may vary between the gene pool but the most informative remark is designated to those makers which exhibits increased DP value which corresponds to high discriminatory power in gene pools [36, 78]. Furthermore, a significant level of EMR and MI was observed which revealed the success of ISSR markers among Capsicum accessions. Therefore, the selected 7 ISSR markers reflected a significant genetic polymorphism and genetic information, indicating their effectiveness to differentiate various chilli accessions.

Primer polymorphism ranged from 79.62 to 100% with an average of 91.80% polymorphism amongst different chilli accessions in the present study which is close to 91.3% polymorphism as depicted in chilli accessions using ISSR markers [61]. In another study, primer polymorphism ranged from 50 to 100% with an average of 81.52% amongst various Capsicum accessions using SCoT markers [3]. The extent of cross- amplification or cross-transferability of ISSR maker ranged from 8.0 to 72.15% with an average of 52.63% amongst Capsicum accessions in the present study, and the values of which are quite comparable with that of different chilli accessions using different marker system [3, 59, 79]. Thus, the results of both the primer polymorphism and cross-transferability confirmed the extent of primer efficiency amongst chilli accessions through DNA fingerprinting process.

The techniques that measure the genetic polymorphism at genomic level are indispensable for identifying genetically and ecologically distinct populations and which can be used for the genetic improvement and breeding program in the desired populations. Therefore, the identification of genetic information such as Na, Ne, I, He, and uHe are very crucial for genetic characterization of populations using molecular markers. At species level, the increased level of genetic variation was observed within Capsicum annuum than in Capsicum baccatum and lowest was seen in Capsicum frutescense. A species with higher genetic variation owes it to its widespread ecological distribution, robust environmental adaptation, survivability, and evolutionary consequences [80]. Among the Capsicum population, the mean value for Nei’s gene diversity, Ht, and Hs were 0.376, 0.296, and 0.180, respectively, which is similar to previous reports of genetic structure analysis in populations involving other plants [81–83]. Such correlation studies using molecular markers have not been reported in Capsicum species, though correlation studies involving fruit characteristics with that of fruit diseases are reported [48]. Thus, a significant level of genetic variation was identified amongst different Capsicum accessions using ISSR markers and this genetic differentiation is influenced by several factors such as population size, reproduction patterns, cross-pollination or out crossing, genetic drift, and gene flow which are associated in the rise of genetic diversity within the population [84–86].

The coefficients of gene differentiation (Gst) and gene flow (Nm) are important indices for genetic differentiation within and across the population. Gst values are classified into low (Gst<0.05), median (0.05< Gst), and high (Gst>0.15) for genetic differentiation in the population [87]. Likewise, the values of Nm also varied from greater than 1 to less than 0.1 for determining the qualitative analysis for genetic differentiation within and across the population [88]. In present study, values of Gst and Nm were found to be 0.393 and 0.773, respectively, in Capsicum population, but such studies are yet not reported in chilli. However, several earlier reports have been documented on population genetics in other plant species such as [Gst (0.381) and Nm (0.835)] in Dipteroniadyerana surveyed by ISSR marker [89], and in another study on genetic structure of Jatropha curcas by microsatellite-based marker (ISSR and DAMD) system, Gst and Nm were reported to be 0.4053 and 0.8085, respectively [90].

In the present study, the increased Gst value indicates an enhanced genetic differentiation within the population but dropdown in the value of Nm represented low level of gene flow or allelic migration among the population due to genetic drift [80, 89] which indicates random fluctuations in the allelic frequency or gene variants in a population developed by chance over the time during evolution. Genetic mutations are responsible for creating allelic diversity and forces of such genetic drift and gene flow also add to genetic variations and are known to be an essential component in the framework of genetic diversity information. Mutation, drift and selection pressure make a dynamic balance in the amount of allelic diversity in the species that allow individuals to adapt into different environmental conditions. It is observed that a small size of the population is coupled to genetic drift which causes loss of rare alleles and decreases the gene pool which might play an influential role in the evolution of new species [91, 92]. The small population structure or absence of population structure often exhibit low genetic diversity due to genetically similar populations [93], common origin, restricted distribution of population, restricted gene flow, and homogenous reproduction [94]. Thus, the natural selection, genetic drift, and gene flow or allelic migrations are very important phenomenon that are coupled with the changes in the allele frequencies over time, and if population encountered one or more of these forces, it can result in the violation of Hardy-Weinberg assumptions, and evolution occurs [95].

The plasticity in the population structures was evaluated by AMOVA and Jaccard’s similarity coefficient, and
the result of AMOVA represented a significant genetic variation within the population of Capsicum accessions with 89% of total variability and 11% among the population of chillies. Such analysis have been reported for Glycyrrhiza uralensis [96], Parkia timoriiana [97], Trachyspermum ammi [98], Melocanna baccifera [69], and Solanum species [71]. The result of the ANOVA is in consistent with Gst and Nm values wherein increased genetic differentiation was observed within population and reduced gene flow among the population. This increased variation within different chilli accessions may be due to distinct ecological conditions, adaptations, and variations in morphological characteristics in chillies. Also, polymorphism of different microsatellite repeats offers a great efficacy to identify inter- and intra-specific genetic polymorphism [99].

According to Jaccard’s similarity coefficient and UPGMA clustering analysis, the varied level of relationships revealed with low, moderate, and extensive genetic association among the different Capsicum accessions. Wherein two major groups of associations were observed in the present study along with a few loosen connection. Alike pictorial representations of population structure were supported by Euclidean similarity index or correlation matrix with Fruchterman-Reingold algorithm, principal coordinate analysis (PCoA), and structure analysis which exhibited consistency of results in the characterization of Capsicum accessions. Due to varied genome size, morpho-physiological variation, and distinct agroecological environments, the result of the present study represented a significant genetic relationship among chilli accession. Important factors which explain these results regarding harmony and discordance among the chilli accessions are the nature of marker system used, level of polymorphism, the number of detected loci, and region coverage of genome by each marker [100], occurrence of distributions either local or geographically distinct spawning groups, natural selection as well as adaptation, survivability, and evolution in changing environments [101, 102].

Thus, the effect of each factor or combined effects of multiple factors have an impact on mechanism that shapes the population genetic structure while extensively related and dissimilar genetic variations might be associated with increased and reduced amount of genetic information respectively. Therefore, evaluation of genetic diversity is an important factor for explicating the connection among various chilli accessions which is essential component of germplasm characterization. Identification and characterization of new variations from the germplasm will help to develop new cultivars with improved agronomic trait, useful for crop improvement, and breeding program in chillies.

Conclusion
The study reveals valuable information about genetic polymorphism, cross-transferability, and genetic and structural plasticity among 54 accessions of chilli using ISSR markers. A significant amplification profiles were obtained which reflects marked genetic polymorphism and cross-transferability among chilli accessions indicating efficiency of ISSR markers for genetic discrimination and conservation among chilli accessions. A significant level of genetic information was revealed by the estimation of various factors (Na, Ne, I, uHe, Hs, Ht, and Nei’s statistics) which highlighted the molecular variability among different chilli accessions. Thus, the knowledge obtained through genetic variability could be used in the management of chilli germplasms. High level of coefficient of gene differentiation (Gst) represented restricted gene flow (Nm) due to genetic drift which can be correlated with high or low rate of allelic acquisition, adaptation, mating nature, interaction with different ecological conditions, and changes in morphological distinctiveness during the course of evolution. The present study provides a fundamental insight for germplasm characterization, genetic arrangement, and population structure of chilli germplasm which could be utilized in the effective management and selection of superior germplasm for breeding purposes.

Abbreviations
ISSR: Inter-simple sequence repeats; PCR: Polymerase chain reaction; CTAB: Cetyl trimethyl ammonium bromide; PIC: Polymorphism information content; I: Shannon’s information index; H: Expected heterozygosity; DP: Discriminating power; RP: Resolving power; GST: Coefficient of gene differentiation; Nm: Gene flow; PCoA: Principal coordinate analysis (PCoA); AMOVA: Analysis of molecular variance; UPGMA: Unweighted pair group method with arithmetic average; PAST: PAleontological statistics.

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Authors’ contributions
Dr. Sumita Kachhwaha and Prof. S. L. Kothari supervised and helped in writing manuscript. Shamshadul Haq conducted the experiment, data analysis and interpretation, and writing manuscript. Shikha Dubey generated the genotyping data and helped in drafting the manuscript. Prerna Dhingra, Kumar Sambhav Verma, and Deepa Kumari assisted and supported in the genotyping data and helped in drafting the manuscript. Prerna Dhingra, Kumar Sambhav Verma, and Deepa Kumari assisted and supported in the genotyping data and helped in drafting the manuscript.

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References
1. USDA-ARS (2011) Grin species records of Capsicum. National 2063 Germplasm Resources Laboratory, Beltsville
2. Csilléry G (2006) Pepper taxonomy and the botanical description of the species. Acta Agron Hung 54(2):151–166. https://doi.org/10.1556/AAGr.54.2006.2.5
3. Gupta V, Jatav PK, Haq SU, Verma KS, Kaul VK, Kothari S, Kachhawa S (2019) Translation initiation codon (ATG) or SCOT-markers-based polymorphism study within and across various Capsicum accessions: insight from their amplification, cross-transferability and genetic diversity. J Genet 98(2):1–12. https://doi.org/10.1080/00221757.2019.1699705
4. Kim S, Park M, Yeom S-I, Kim Y-M, Lee JM, Lee H-A, Seo E, Choi J, Cheong K, Kim K-T (2014) Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. Nat Genet 46(3):270. https://doi.org/10.1038/ng.2877
5. Arumuganathan K, Earle E (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9(3):208–218. https://doi.org/10.1007/BF02672069
6. Moscone EA, Baranyi M, Ebert I, Greilhuber J, Ehrendorfer F, Hunziker HM (2003) Analysis of nuclear DNA content in Capsicum (Solanaceae) by flow cytometry and Feulgen densitometry. Ann Bot 92(1):21–29. https://doi.org/10.1093/aob/mcg105
7. Qin C, Yu C, Shen Y, Fang X, Chen L, Min J, Cheng J, Zhao S, Xu M, Luo Y (2014) Whole-genome sequencing of cultivated and wild peppers provides insights into Capsicum domestication and specialization. Proc Natl Acad Sci 111(14):5135–5140. https://doi.org/10.1073/pnas.1409751.11
8. Oliver KR, McComb JA, Greene WK (2013) Transposable elements: powerful contributors to angiosperm evolution and diversity. Genome Biol Evol 5(10):1886–1901. https://doi.org/10.1093/gbe/evt141
9. Flagel LE, Wendel JF (2009) Gene duplication and evolutionary novelty in plants. New Phytol 183(4):557–564. https://doi.org/10.1111/j.1469-8137.2009.02923.x
10. Panchy N, Lehti-Shiu M, Shiu S-H (2016) Evolution of gene duplication in plants. Plant Physiol 171(4):2294–2316. https://doi.org/10.1002/pp.16005.23
11. Dagnoiko S, Yaro-Diarisso N, Sanogo PN, Adetula O, Dolo-Nantoume A, Gamby-Touré K, Traoré-Théra A, Katilé S, Diallo-Ba D (2013) Overview of pepper (Capsicum spp) breeding in West Africa. Afr J Agric Res 8(13):1108–1114. https://doi.org/10.5897/AJAR2012.1758
12. Rehavem JR, Patel JN, Kumar S, Acharya RR (2019) Morphological, biochemical and molecular characterization for genetic variability analysis of Capsicum annum. Vegetos 32(2):131–141. https://doi.org/10.1007/s42355-019-00106-5
13. Ridzuan R, Raffi MY, Mohammad Yusoff M, Ismail SJ, Miah G, Usman M (2019) Genetic diversity analysis of selected Capsicum annuum genotypes based on morphophysiological, yield characteristics and their biochemical properties. J Sci Food Agric 99(1):269–280. https://doi.org/10.1002/jsfa.9169
14. Calisihan M (2012) Genetic diversity in plants. Published by InTech, p 510. https://doi.org/10.5772/26460
15. Meyer RS, Purugganan MD (2013) Evolution of crop species: genetics of domestication and diversification. Nat Rev Genet 14(12):840–852. https://doi.org/10.1038/nrg3605
16. Mohammadi SA, Prasanna B (2003) Analysis of genetic diversity in crop plants—salient statistical tools and considerations. Crop Sci 43(4):1235–1248. https://doi.org/10.2135/cropsci2003.1235
17. Islam AF, Ali MR, Gregorio GB, Islam MR (2012) Genetic diversity analysis of stress tolerant rice (Oryza sativa L.). Afr J Biotechnol 11(85):15123–15129
18. Azeem S, Khan AI, Awam FS, Riaz A, Bahadur S (2012) Genetic diversity of rose germplasm in Pakistan characterized by random amplified polymorphic DNA (RAPD) markers. Afr J Biotechnol 11(47):10650–10654. https://doi.org/10.5897/AJBJ10.1375
19. Haq S, Jain R, Sharma M, Kachhawa S, Kothari S (2014) Identification and characterization of microsatellites in expressed sequence tags and their cross transferability in different plants. Int J Genomics 2014:12. https://doi.org/10.1155/2015/863948
20. Haq SU, Dhingra P, Sharma M, Kothari SL, Kachhawa S (2021) Plasticity of tandem repeats in expressed sequence tags of angiospermic and non-angiospermic species: insight into cladistic, phenetic, and elementary explorations. J Appl Biol Biotechnol 9(2):36–59. https://doi.org/10.7324/JABB.2021.9204
21. Agarwal A, Gupta V, Haq SU, Jatav PK, Kothari S, Kachhawa S (2018) Assessment of genetic diversity in 29 rose germplasms using SCoT marker. J King Saud Univ Sci 31:780–788. https://doi.org/10.1016/j.jksus.2018.04.022
22. Amar MH, Biswas MK, Zhang Z, Guo W-W (2011) Exploitation of SSR, RAP and CAPS-SNP markers for genetic diversity of citrus germplasm collection. Scientia Horticulturae 128(3):220–227. https://doi.org/10.1016/j.scienta.2011.01.021
23. Khurana-Kaul V, Kachhawa S, Kothari S (2012) Characterization of genetic diversity in Jatropha curcas L germplasm using RAPD and ISSR markers. Indian J Biotech 11:54–61
24. Varshney RK, Chabane K, Hendre PS, Aggarwal RK, Gnanar A (2007) Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barley. Plant Sci 173(6):638–649. https://doi.org/10.1016/j.plantsci.2007.08.010
25. Haq SU, Kumar P, Singh R, Verma KS, Bhatt R, Sharma M, Kachhawa S, Kothari S (2016) Assessment of functional EST-SSR markers (sugarcane) in cross-species transferability, genetic diversity among poaceae plants, and bulk segregation analysis. Genet Res Int 2016:16. https://doi.org/10.1155/2016/705233
26. Verma KS, UI Haq S, Kachhawa S, Kothari S (2017) RAPD and ISSR marker assessment of genetic diversity in Citrullus colocynthis (L.) Schrad. a unique source of germplasm highly adapted to drought and high-temperature stress. 3 Biotech 7(5):1–24. https://doi.org/10.1007/s13205-017-0918-z
27. Debnath SC (2008) Inter simple sequence repeat (ISSR) markers and pedigree information to assess genetic diversity and relatedness within raspberry genotypes. Int J Fruit Sci 7(4):1–17. https://doi.org/10.1080/1533836802003159
28. Peng X, Liu J, Xiang Y, Huang S (2006) A practical handbook of plant molecular biotechnology. Chemical Industry Press, Beijing
29. Bhawna AM, Arya L, Saha D, Sureja A, Pandey C, Verma M (2014) Population structure and genetic diversity in bottle gourd [Lagenaria siceraria (Mol.) Standl] germplasm from India assessed by ISSR markers. Plant Syst Evol 300(4):767–773. https://doi.org/10.1007/s00606-014-1000-9
30. Alansi S, Tarroum M, Al-Qurainy F, Khan S, Nadeem M (2016) Use of ISSR markers to assess the genetic diversity in wild medicinal Ziziphus spina-christi (L.) Willd. collected from different regions of Saudi Arabia. Biotechnol Biotechnol Equip 30(5):942–947. https://doi.org/10.1080/17448438.2016.119928
31. Kumar A, Mishra P, Baskaran K, Shukla AK, Shasany AK, Sundaresan V (2016) Higher efficiency of ISSR markers over plastid psbA-trnH region in resolving taxonomical status of genus Ocimum L. Ecol Evol 6(21):7671–7682. https://doi.org/10.1002/eece.2483
32. Uzun A, Gulsen O, Yesiloglu T, Aka-Kacar Y, Tuzcu O (2013) Distinguishing grapefruit and pummelo accessions using ISSR markers. Czech J Genet Plant Breed 46(4):170–177. https://doi.org/10.17221/89/2010-cjgbp

33. Nadeem MA, Navaz MA, Shahid MQ, Dogan Y, Comertpay G, Yildiz M, Hatipoğlu R, Ahmed F, Aliaheh A, Labhane N (2018) DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnol Biotechnol Equip 32(2):261–285. https://doi.org/10.1080/13120588.2017.1400401

34. López Castillo LC, Garaña Hernández R, Cárdena CA, Martínez-Hernández A, Ortiz-García MM, Anduesa-Noh RH (2019) Structure and genetic diversity of nine important landraces of Capsicum species cultivated in the Yucatan Peninsula, Mexico. Agronomy 9(7):376. https://doi.org/10.3390/agronomy9070376

35. Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

36. Amiriyousef A, Hyvönen J, Pocza P (2018) JMEC: online marker efficiency calculator. Appl Plant Sci 6(6):e01159. https://doi.org/10.1002/aps3.1159

37. Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28(19):2537–2539. https://doi.org/10.1093/bioinformatics/bts445

38. Yeh F, Yang R, Boyle T (1999) Microsoft Windows-based freeware for population genetic analysis (POPGEN), ver. 1.1. University of Alberta, Canada

39. Pavlicka A, Hrda S, Flegr J (1999) Free-tree—freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of genus Frenkelia. Folia Biologica 45(3):97

40. Page R (1996) TREEVIEW: an application to display phylogenetic trees and their bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of genus Frenkelia. Folia Biologica 45(3):97

41. Gajdoš P, Ježowicz T, Uher V, Dohnálek P (2016) A parallel Fruchterman–Reingold algorithm optimized for fast visualization of large graphs and swarms of data. Swarm Evol Comput 26:56–63. https://doi.org/10.1016/j.swevo.2015.07.006

42. Hammer Ø, Harper DA, Ryan PD (2001) PAST: palaeontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1)

43. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure. Genetics 155(2):945–955. https://doi.org/10.1534/genetics.116.195164

44. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters and validation of a new male sex-specific ISSR marker in pointed gourd (Trichosanthes dioica Roxb.). Sci World J 2014:6.ppages. https://doi.org/10.1155/2014/216898

45. Albrecht E, Zhang D, Saftner RA, Stommel JR (2012) Genetic diversity and population structure of Capsicum baccatum genetic resources. Genet Resour Crop Evol 59(4):517–538. https://doi.org/10.1007/s10722-011-9707-0

46. Page R (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358 Macintosh.

47. Jha TB, Saha PS, Nath S, Das A, Jha S (2017) Morphological and cytotgenetical characterization of 'Dalle Khursani': a polyploid cultivated Capsicum of India. Scientia horticulturae 215:80–90. https://doi.org/10.1016/j.scienta.2016.12.005

48. Dutta S, Singh S, Saha S, Akojam R, Boopathi T, Banjeeey A, Roy S (2017) Diversity in birds eye chili (Capsicum frutescens L) landraces of north-east India in terms of antioxidant activities. Proc Natl Acad Sci, India Section B: Biol Sci 87(4):1317–1326. https://doi.org/10.1007/s40011-016-0707-1

49. Jha TB, Bhowmick BK (2021) Unravelling the genetic diversity and phylogenetic relationships of Indian Capsicum through fluorescent banding. Genet Resour Crop Evol 68(1):205–225. https://doi.org/10.1007/s10722-020-00980-x

50. Gangadhar BH, Mishra RK, Pandian G, Park SW (2012) Comparative study of color, pungency, and biochemical composition in chili pepper (Capsicum annuum) under different light-emitting diode treatments. HortScience 47(12):1729–1735. https://doi.org/10.21273/HORTSCI.47.12.1729

51. Earl DA, Von Holdt BM (2012) Structure harvester: a website and calculator. Appl Plant Sci 6(6):e01159. https://doi.org/10.1002/aps3.1159

52. Lee J-H, An JT, Siddique M, Han K, Choi S, Kwon J-K, Kang B-C (2017) Identification and molecular mapping of Chili vein mottle virus (ChMV) resistance genes in pepper (Capsicum annuum). Mol Breed 37(10):1–10. https://doi.org/10.1007/s10811-017-0717-6

53. Albrecht E, Zhang D, Saftner RA, Stommel JR (2012) Genetic diversity and population structure of Capsicum baccatum genetic resources. Genet Resour Crop Evol 59(4):517–538. https://doi.org/10.1007/s10722-011-9707-0

54. Dutta S, Singh S, Saha S, Akojam R, Boopathi T, Banjeeey A, Roy S (2017) Diversity in birds eye chili (Capsicum frutescens L) landraces of north-east India in terms of antioxidant activities. Proc Natl Acad Sci, India Section B: Biol Sci 87(4):1317–1326. https://doi.org/10.1007/s40011-016-0707-1

55. Jha TB, Bhowmick BK (2021) Unravelling the genetic diversity and phylogenetic relationships of Indian Capsicum through fluorescent banding. Genet Resour Crop Evol 68(1):205–225. https://doi.org/10.1007/s10722-020-00980-x

56. Gangadhar BH, Mishra RK, Pandian G, Park SW (2012) Comparative study of color, pungency, and biochemical composition in chili pepper (Capsicum annuum) under different light-emitting diode treatments. HortScience 47(12):1729–1735. https://doi.org/10.21273/HORTSCI.47.12.1729

57. Lee J-H, An JT, Siddique M, Han K, Choi S, Kwon J-K, Kang B-C (2017) Identification and molecular mapping of Chili vein mottle virus (ChMV) resistance genes in pepper (Capsicum annuum). Mol Breed 37(10):1–10. https://doi.org/10.1007/s10811-017-0717-6

58. Albrecht E, Zhang D, Saftner RA, Stommel JR (2012) Genetic diversity and population structure of Capsicum baccatum genetic resources. Genet Resour Crop Evol 59(4):517–538. https://doi.org/10.1007/s10722-011-9707-0

59. Dutta S, Singh S, Saha S, Akojam R, Boopathi T, Banjeeey A, Roy S (2017) Diversity in birds eye chili (Capsicum frutescens L) landraces of north-east India in terms of antioxidant activities. Proc Natl Acad Sci, India Section B: Biol Sci 87(4):1317–1326. https://doi.org/10.1007/s40011-016-0707-1
