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RESEARCH ARTICLE

Genetic diversity analysis and molecular characterization of elite chilli (*Capsicum annuum* L.) germplasm lines for fruit quality traits

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Abstract

Around the world, chilli is the most important crop as a vegetable and spice. An essential step in plant breeding procedures to develop and enhance new varieties is the genetic characterization of resources and evaluation of genetic diversity. In the current study, 72 elite chilli genotypes were assessed along with four checks for fruit quality attributes in Augmented Design-II between 2021 and 2022. Ascorbic acid, phenols, oleoresin, dry matter content, peroxidase and capsaicin content were among the fruit quality criteria that were examined. The accessions' average capsaicin content was 0.42 %, with a range of 0.13 to 1.20 %. BCA-26 has maximum amount of capsaicin (1.20 %). For capsaicin concentration, higher estimations of the genotypic and phenotypic coefficients of variation (62.24 % and 67.20 %) were found, along with high heritability (85.79 %) and high GAM (118.93 %). Based on fruit quality and yield attributes, genetic diversity investigation depicted that clusters II and III had the greatest inter-cluster distance, followed by clusters II and V and II and IV, indicating greater genetic divergence. Moreover, 72 genotypes were characterized by molecularly using nine pungency-specific polymorphism SSR markers. The mean allele number was 2.77, with a range of 2 to 4. With an average value of 0.415, the heterozygosity was observed to be lowest for the markers HpmsE005 and HpmsE063 (0) and highest for the primer HpmsE031 (0.444). The PIC value averaged 0.415 and ranged from 0.298 (HpmsE005) to 0.538 (HpmsE022). High and low pungent chilli cultivars might be distinguished with the help of the HpmsE022 marker.

Keywords: chilli; chilli genotypes; genetic diversity; pungency; SSR markers

Introduction

Chilli peppers (*Capsicum* spp.) also called as hot peppers, paprika, cayenne are among the most significant vegetable and spice crops globally, valued for their economic significance, industrial application and health promoting properties (1). Chilli belonging to the family solanaceae is believed to have originated in Mexico and Central America around 7000 BC (2). Presently, five cultivated species (*C. annuum, C. baccatum, C. chinense, C. frutescens and C. pubescens*) are recognized and about 20-30 wild species are reported, all are being diploid with a chromosome number of 2n=2x=24 (3). The most widely grown species across the globe is *C. annuum* L. for both pungent and non-pungent fruits. It is a prominent source of minerals and vitamins including steam -volatile oil, fatty oils, carotenoids, protein and fiber (4, 5). Quality traits are highly emphasized in breeding, the prime focus is the enhancement of capsaicin, vitamin C and oleoresin contents. The pungency in chilli is the central quality attribute (6), which is owed to the crystalline, acrid, volatile alkaloid called capsaicin (8-methyl-N-vanillyl-6-enamide) existing in the placenta and pericarp of the fruit which has greater application in pharmaceutical and cosmetic industry (7) and having diverse prophylactic and therapeutic uses in Allopathic and Ayurvedic medicine system (8). Tremendous variability among genotypes for quality traits in hot peppers has been reported (9).

The genetic evolution of crop varieties is largely dependent on available genetic variability and quantification of this genetic diversity (1). The phenotypic variability observed has genetic as well as environmental components, which

necessitates to assess the genotypic coefficient of variation (GCV). Furthermore, variability estimate alone is not appropriate to govern the effectiveness of selection, since it does not deliver accurate evidence regarding heritability and gene action. Heritability assessment along with genetic gain are highly dependable in establishing the genotypic worth and the gene action involved in the expression of various polygenic traits (10). High heritability and discernible genetic progress contribute for enhanced selection of fruit quality traits (11, 12). Information gathered on genetic architecture *i.e.,* quantification of genetic variability, heritability of traits and understanding the genetic gain to be realized is the preliminary step in planning the path of breeding. Further, the knowledge of the genetic diversity available in the gene pool is essential to conserve the germplasm, to mould the genetic makeup and for the selection of parents to develop desirable hybrids (13). Estimation of genetic distance is done by several methods, the K-means clustering analysis is one among them (14). In addition, DNA markers that directly access genetic information and independent of environmental factors, have been conveniently used in molecular characterization and marker-assisted breeding (MAB) in chilli for selecting various important characters like high capsaicin (15). Among the various molecular markers used, the most alluring is Simple Sequence Repeats (SSR) known for their locus-specificity, co-dominance, PCR-based, multi-allelic nature, high level of polymorphism between different genotypes, abundance in genome and fast and easy approach in MAS (5, 16). This approach is specifically vital for difficult-to-phenotype traits like pungency, where phenotypic selection is often difficult and misleading (17).

The current study was conducted in this manner to quantify genetic diversity, determine the degree of genetic variability for quality variables and select elite chilli lines for fruit quality parameters through focused and consistent breeding efforts.

Material and Methods

Experimental details

The research was carried out in the research block of the College of Horticulture's Department of Biotechnology and Crop Improvement in Bengaluru between 2021 and 2022. The soil had a neutral pH of 6.2 to 6.4 and was a red sandy loam. The study's germplasm comprised four checks (Pusa Jwala, Pusa Sadabahar, Punjab Tej and Punjab CH-21) and 68 genotypes, including a few elite and advanced selections and genotypes gathered from all around India. Using Augmented block design II (18), these 72 genotypes were assessed for six distinct fruit quality parameters using four compact blocks, each of which had 17 genotypes and four checks spaced 60 cm apart.

Every cultural operation was completed in accordance with the set of guidelines proposed by the Bagalkot-based University of Horticultural Sciences. From every genotype, five plants were chosen at random to record observations on a range of yield and quality metrics. The quality parameters were measured using fruits that were picked from the chosen plants. Quality traits like Ascorbic acid (19), capsaicin content (20), oleoresin content (21), dry matter content (22), total phenols (23) and peroxidase assay (24) were conducted as per the standard protocols in 72 genotypes at the green pod harvest stage except for oleoresin estimation where red matured pods were used (Table 1).

Statistical analysis

Following an analysis of variance (18), the genotypic and phenotypic variances were used to compute the genotypic coefficients of variation (GCV) and phenotypic coefficients of variation (PCV) (25) respectively.

$$GCV\ (\%) = \frac{\sqrt{\text{Genotypic variance}}}{\text{Grand mean}} \times 100$$

$$PCV\ (\%) = \frac{\sqrt{\text{Phenotypic variance}}}{\text{Grand mean}} \times 100$$

Broad sense heritability (h^2_{bs}) was calculated as earlier reported (26).

$$h^2_{bs}\ (\%) = \left(\frac{\sigma^2 g}{\sigma^2 p}\right) \times 100$$

Where,

$\sigma^2 g$= Genotypic variance $\sigma^2 P$ = Phenotypic variance

Genetic advance for the characters studied with 5 % selection intensity that indicated the expected gain in the next generation by selecting the superior individuals was computed using the formula (27).

$$GA = i.\ \sigma p.h^2$$

Where,

i = Standard selection differential (2.06) at 5 % selection intensity

σp = Phenotypic standard deviation of the trait

h^2 = Heritability in broad sense

Genetic advance over mean was calculated bestowing to the following formula (10).

Table 1. Procedure for recording the observations for different fruit quality characters

Sl. No.	Traits	Code	Descriptions
1	Ascorbic acid	Vit. C	By using, 6-dichlorophenol indophenol dye through the volumetric method (19).
2	Capsaicin content	CAP	Colorimetric method (20) by using standard chemicals of dry acetone, NaoH solution and anhydrous sodium sulphate.
3	Oleoresin content	OLE	Acetone was used as a solvent to extract the oleoresin by the Soxhlet method (21).
4	Dry matter content	DM	Standard procedures were adopted to estimate the dry matter content by oven drying of chilli for twenty-four hours at 60 to 70 degrees Celsius in triplicate (22).
5	Total phenols	TP	A colorimetric method based on oxidation-reduction reaction using Folin-Ciocalteu reagent, chilled ethanol and tannic acid (23).
6	Peroxidase assay	POD	Potassium phosphate buffer used to estimate Peroxidase content by using a visible UV spectrophotometer Guaiacol oxidation method (24).

$$GAM\ (\%) = \frac{GA}{\overline{X}} \times 100$$

Where,

GA = Genetic Advance X = General mean of the trait

In order to describe an algorithm that places each item in the cluster with the closest means, a non-hierarchical Euclidean cluster examination on the basis of "K-means" clustering approach (28) was utilized to cluster the genotypes using the R Studio R 4.2.1 edition of the program.

Validation of chilli genotypes using pungency-specific SSR markers

The 72 genotypes of chillies were validated using nine pungency-specific SSR markers (17).

A changed CTAB protocol was utilized to genomic DNA extraction from healthy leaf tissue (29) and nanodrop instrument was used to assess the amount and quality of the DNA. The mined DNA was further diluted and used in a 10 µl volume for PCR amplification. This volume included 1 µl of DNA, 5 µl of Taq DNA 2X master mix, 0.5 µl of forward and reverse primer each and 3 µl of nuclease-free water. The first denaturation was performed at 94 °C for two minutes, followed by the following 35-cycle amplification process: denaturation for one minute at 94 °C, annealing for one minute at 54-65 ^{0}C, extension for one minute at 72 °C and last extension for six minutes at 72 °C (17). On a 3 % agarose gel, SSR amplification products were examined using TAE buffer. PCR amplification's banding pattern was examined under a UV lamp and captured on camera with a Gel documentation system. Based on the allele polymorphism of every primer, band scoring was carried out. The PIC was evaluated by means of power marker software and GeneLX software for molecular analysis of SSR data. The following formula (30) was used to calculate the PIC value.

$$PIC = 1 - \Sigma(pi)^2$$

where, pi is the frequency of i^{th} allele

The observed number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I) and expected heterozygosity (He) were used to compute the genetic diversity within the population (31).

The expected heterozygosity (He) for a genetic marker was considered as

$$He = 1 - pi^2/(n-1)$$

where pi is the allele frequency of i^{th} allele and n is the size of the sample

The observed heterozygosity (Ho) calculated as given by H_o= Number of genotypes harboring heterozygous genotypes at the i^{th} allele divided by total number of genotypes. Number of alleles per locus was computed as below.

$$\text{Number of alleles per locus} = \frac{\sum \text{Number of alleles}}{\text{number of loci}}$$

Results and Discussion

The analysis of variance among 72 genotypes of chilli as per Augmented Design-II (Table 2) showed highly significant variations among genotypes for the examined six fruit quality traits *viz*., capsaicin content, ascorbic acid, dry matter content, total phenols and peroxidase content.

Per se performance, genetic variability estimates for quality traits

The evaluation of chilli's performance for quality metrics would reveal the average performance of the assessed germplasm and aid in picking the best genotypes for a blend of quality and yield features. Selecting potential genotypes for the enhancement of desired attributes is strongly encouraged by the broad to moderate range of variation seen for fruit quality characters among the test genotypes. Table 3 lists the elite genotypes of chillies that were reported to have excellent qualities. These genotypes include BCA-26, BCA-24 and BCA-30 for high capsaicin content, PSB-Sel-1, KCA-17-1 and BCA-20 for high ascorbic acid content and the accessions KCA-17-6, BCA-27 and KCA-26-1 for oleoresin content. The lines identified for combination of quality traits can be used for numerous trait specific breeding, that include BCA-27 for both capsaicin and oleoresin content, KCA-17-1 for high amount of ascorbic acid and peroxidase level, KCA-21-1 with total phenols and peroxidase (Table 3). Furthermore, the accessions KCA-27-2, KCA-27-1 and KCA-17-6 were superior for yield and yield accrediting traits along with varied quality traits (Table 4).

Crop development initiatives rely on data regarding the distribution pattern and the notch of variability existing in the test germplasm for the desired traits. Because the phenotypic variance includes both genotypic and environmental factors, as well as the effects of their interactions, it does not provide a true picture of the heritable variation. Phenotypical and genotypical variability must be separated from environmental features, which were then examined as unit-independent indicators of variability in the form of phenotypic and genotypic amount of variation. Furthermore, in addition to the variability estimates for a character, the effectiveness of selection is also hooked on the heritability and genetic advancement that can be achieved. The effectiveness of selection is more meaningfully influenced by the assessment of heritability considered in combination with the anticipated genetic

Table 2. ANOVA for yield and quality parameters among the chilli germplasm accession

Sl. No.	Source	Block (ignoring treatments)	Treatment (eliminating blocks)	Treatment: check	Treatment: Test and Test vs. check	Residuals
	Df	3	71	3	68	9
1	Capsaicin content (%)	0.23 **	0.09 **	0.52 **	0.07 **	0.01
2	Oleoresin content (%)	12.01	6.39	29.02 *	5.38	5.12
3	Dry matter content (%)	5.12 *	11.76 **	22.72 **	11.28 **	1.10
4	Ascorbic acid (mg/100g)	13251.06 **	4838.89 **	9611.38 **	4628.34 **	55.37
5	Total phenols (mg/100g)	6245.55 **	1483.9 **	7348.09 **	1225.19 **	105.98
6	Peroxidase (activity/min/g)	0.15	1.03 **	5.168**	0.85**	0.056

*Significant at 5 % ** Significant at 1 % df= degrees of freedom

Table 3. Elite genotypes identified for fruit quality traits in chilli

Sl. No.	Genotypes	Characters
1	Capsaicin content (%)	BCA-26, BCA-24, BCA-30, BCA-25, BCA-27
2	Oleoresin content (%)	KCA-17-6, BCA-27, Pusa Jwala, KCA-26-1, BCA-33
3	Dry matter content (%)	BCA-34, GPM-60, KCA-24-3, KCA-2-2, Pusa Sadabahar
4	Ascorbic acid (mg/100g)	PSB selection-1, KCA-17-1, BCA-20, BCA-22, KCA-2-1
5	Total phenolics (mg/100g)	KCA-21-1-A, KCA-21-1, KCA-19-1-1, KCA-2-1, Punjab CH-21
6	Peroxidase (activity/min/g)	KCA-44-1-A, KCA-21-1, KCA-24-1-C, Pusa Sadabahar, KCA-17-1

Table 4. Elite fruit yielding genotypes of chilli along with their performance for fruit quality traits

Sl. No.	Genotype	Fruit yield/ plant (g)	Capsaicin (%)	Oleoresin (%)	Dry Matter (%)	Vitamin- C (mg/ 100g)	Total phenolics (mg/ 100g)	Peroxidase (activity/min/g)
1	KCA-27-2	657.95	0.48	8.20	15.06	190.20	94.25	0.43
2	KCA-27-1	582.12	0.25	10.60	18.36	151.04	56.50	0.42
3	KCA-17-6	478.80	0.18	11.20	17.51	81.11	75.16	0.50
4	CB-2	437.40	0.18	8.80	22.57	69.93	81.16	0.73
5	Pusa Jwala	437.00	0.36	15.20	17.39	137.48	132.50	0.49
6	BCA-27	432.76	1.13	16.20	19.30	130.06	62.66	2.79
7	KCA-2-2	390.41	0.49	11.70	23.19	188.81	68.66	0.88
8	KCA-33-2	389.48	0.16	13.40	15.04	174.82	86.66	0.47
9	KCA-21-1	387.60	0.18	12.40	17.26	120.34	191.08	3.42
10	BCA-34	385.00	0.36	13.80	24.56	134.80	62.33	1.43
	Pusa Sadabahar	288.10	0.27	12.50	23.07	116.08	81.91	3.25

advancement as previously proposed (10). Estimates of genetic advance give information on the gene action that contributes to the development of different polygenic traits; the higher the genetic advance, the more will be the additive gene action. Table 5 provides descriptive statistics for 72 genotypes for quality variables, including mean, range, GCV, PCV, heritability (h^2_{bs}) and genetic advancement as a percentage of mean (GAM). The average amount of capsaicin was 0.42 percent, with a range of 0.13 to 1.20 percent (Fig. 1). KCA-2-1 and GPM-40 (0.13 %) had modest capsaicin contents, however the genotype BCA-26 (1.20 %) was thought to be a very spicy cultivar.

KCA-17-6 had the highest reported oleoresin (17.50 %), with an overall mean of 12.19 percent. The dry matter content of accession BCA-34 was observed to be maximum (24.56 %). With a population mean of 154.04 mg/100g, the ascorbic acid levels in Fig. 2 ranged from 60.13 (Punjab CH-21) to 353.84 mg/100g (PSB selection -1). With an average of 88.80 mg per 100g, the total phenol content showed a significant range of variance, ranging from 29.00 mg per 100g (GPM-52) to 222.50 mg per 100g (KCA-21-1-A). The mean peroxidase activity measured was 1.65 $min^{-1}g^{-1}$, with a range of 0.31 $min^{-1}g^{-1}$(GPM -40) to 3.42 $min^{-1}g^{-1}$(KCA-44-1-A and KCA-21-1). Higher

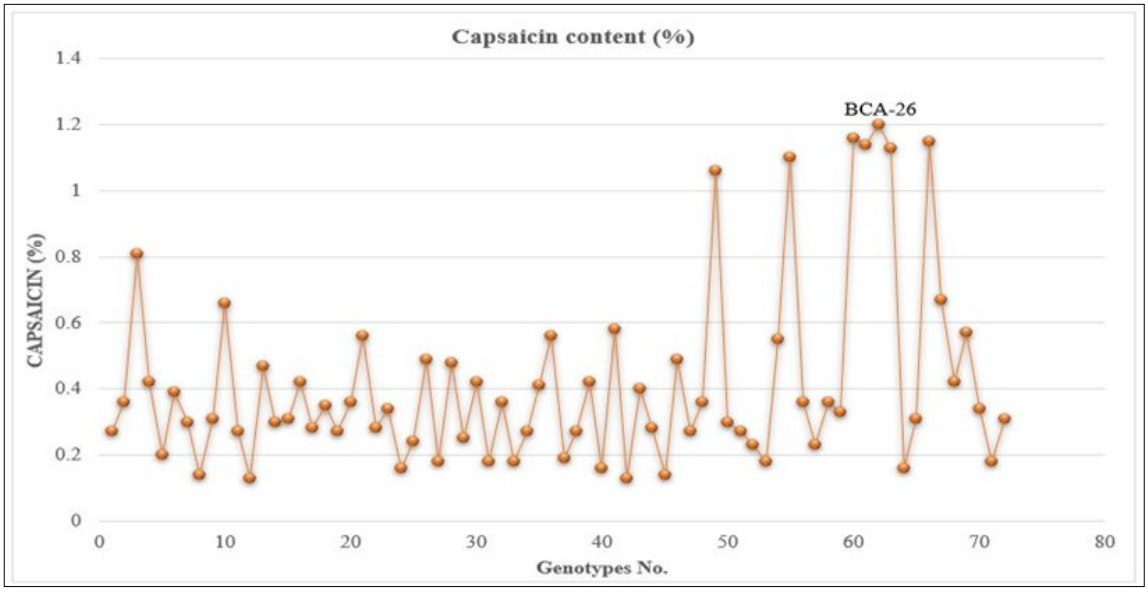

Fig. 1. Performance of chilli genotypes for capsaicin content.

Table 5. Descriptive statistics for fruit quality parameters among chilli genotypes

Sl. No.	Characters	Mean	Range		Coefficient of variation (%)		h^2_{bs} (%)	GAM (%)
			Min.	Max.	GCV	PCV		
1	Capsaicin content (%)	0.42	0.12	1.20	62.24	67.2	85.79	118.93
2	Dry matter content (%)	17.13	10.11	24.56	18.81	19.80	90.27	36.88
3	Ascorbic acid (mg/100g)	154.04	60.13	353.84	44.79	45.04	98.91	91.90
4	Total phenols (mg/100g)	88.80	29.00	222.50	42.59	44.22	92.78	84.64
5	Peroxidase (activity/min/g)	1.65	0.31	3.42	55.16	56.99	93.51	109.95

GCV - Genotypic co-efficient of variation PCV - Phenotypic co-efficient of variation

h^2_{bs} - Broad sense heritability GAM - Genetic advance as per cent of mean

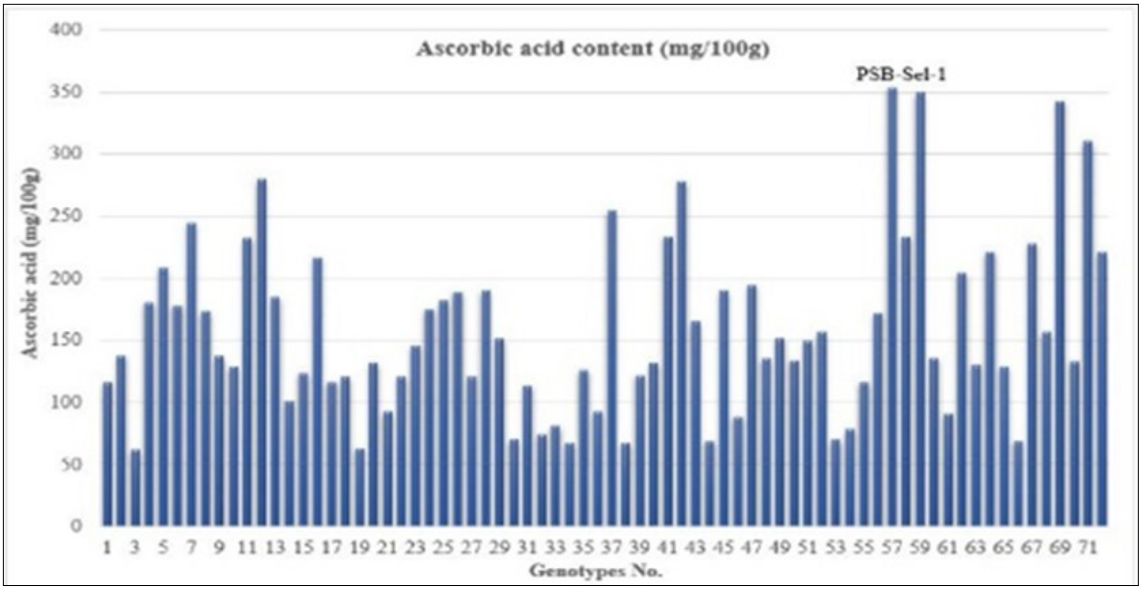

Fig. 2. Performance of chilli genotypes for ascorbic acid content.

estimates of GCV (62.24 %), PCV (67.20 %) along with high heritability (85.79 %) and high GAM (118.93 %) were observed for the capsaicin content. Similarly, the other parameters *viz.,* ascorbic acid (45.04 %, 44.79 %), total phenol content (44.22 %, 42.59 %), peroxidase activity (56.99 %, 55.16 %) revealed high values of PCV and GCV respectively coupled with higher evaluations of heritability and GAM. The prevalence of additive genes that respond to selection resulting in higher genetic gains could be interpreted for these types and earlier similar findings are in line with the results of this study (32, 33). The moderate values of GCV and PCV (18.81 % and 19.80 % respectively), but high heritability of 90.27 per cent and high GAM of 36.88 per cent were recorded for the dry matter content representing that the available moderate variation can be utilized for selection programs (8, 32, 33). Moderate PCV and GCV revealed for dry matter content specified to include some more wider, geographically divergent germplasm collections to enrich the variability for this trait. Such similar reports were also depicted (34-38). Estimates of PCV were comparable to GCV, which indicated the minimal influence of confounding environment on the expression of these characters (12, 32, 33, 34, 35).

Genetic diversity studies

Because of its extensive range and maximum rates of intervarietal and interspecific hybridization, chillies have a great contract of genetic variability that must be measured in to preserve and use germplasm in breeding. In crop breeding, the evolution of high-yielding genotypes is influenced by the population's level of genetic diversity. The cluster plot was created using the non-hierarchical method of clustering data based on K-means clustering, which was used to group the accessions based on data from the yield and quality parameters (Fig. 3). The genotypes that had common tendencies were grouped together in clusters, as the cluster plots demonstrated. Table 6 shows the clustering pattern and composition of the six clusters (13, 14, 39, 40) formed from the test genotypes.

Group I was the largest with 20 genotypes followed by group III (15), V & VI (11), IV (10), whereas group II (5

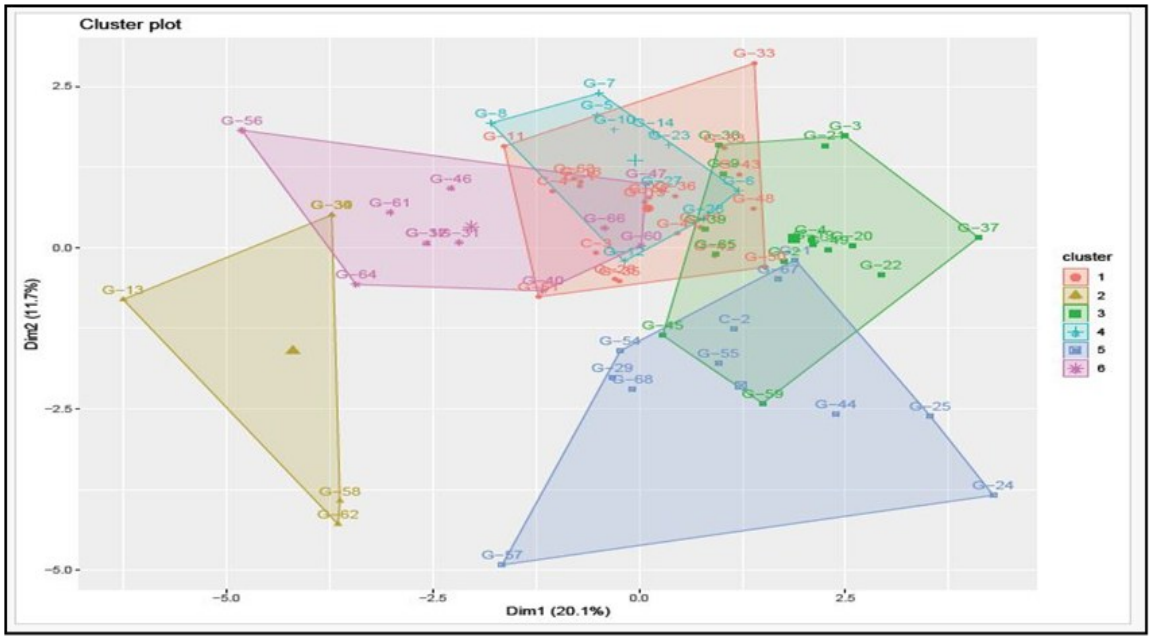

Fig. 3. Dendrogram depicting the distribution pattern of genotypes into different clusters.

Table 6. Grouping of 72 chilli genotypes into six clusters based on K-means clustering approach

Cluster	Number of genotypes	Cluster composition
I	20	Pusa Sadabahar, Punjab CH-21, Punjab Suvarna, KCA-33-1, KCA-21-2-A, KCA-24-3, Pusa Sadabahar, KCA-24-1-C, GPM-60, GPM-36-1, GPM-52, S-5-1, BCA-32, BCA-33, CB-1, CB-3, Pavgada chilli, Psb-seln-2-A, Psb-seln-1and Punjab -27-A
II	05	Punjab Tej, KCA-17-5, GPM-40-2, BCA-26, BCA-30
III	15	KCA-S-4, KCA-19-4, KCA-44, KCA-44-1-A, KCA-33-A, KCA-33-2, KCA-24-2, KCA-2-2, GPM-36-2, GPM-40, BCA-35, BJ-3, CB-2, BCA-27 and BCA-20
IV	10	KCA-20-1, KCA-19-1-1, KCA-19-3, KCA-2-1, KCA-21-1-A, KCA-9-2, KCA-21-2, KCA-21-1, KCA-26-2 and KCA-26-1
V	11	Pusa Jwala, KCA-17-1, KCA-27-2, KCA-27-1, KCA-17-6, BCA-34, BCA-31, KCA-17-4, BCA-25, BCA-22 and KCA-17-7
VI	11	KCA-24-3-A, GPM-54, GPM-55, EC-16, BJ-4, BJ-5, BCA-24, BCA-28, BCA-29, BCA-21 and BCA-23

genotypes) was the smallest. Average inter-cluster distances (Table 7) revealed extreme divergence between cluster II and III (9.084) followed by cluster II and V (8.725), cluster II and IV (8.089) and cluster I and II (8.020). Group II was the farthest cluster from all other clusters. Similar clustering pattern was reported in earlier studies (13, 32, 35, 41, 42). Cluster mean values for fruit yield and quality parameters in chilli (Table 8) revealed that the cluster V had the highest fruit yield (379.141 g/plant) with longer fruits (11.009 cm) and longer stalks (4.334 cm) and taller plant habit (67.239 cm). This group had high ascorbic acid content (192.017 mg/100g) but low peroxidase activity (1.005 activity/min/g sample). Maximum dry matter content (18.331 %) and peroxidase (1.987 activity/min/g sample) was shown in cluster I. Group II was having large fruits (18.02 mm fruit diameter) with high individual fruit weight (10.98 g) as well as high capsaicin content (0.633 %). Cluster III consisted of accessions with a greater quantity of fruits, a wider plant spread and more branches. Group VI consisted of early flowering and early maturing varieties. The greatest oleoresin mean value (12.736 %) was found in Cluster VI. Similar grouping and cluster mean comparison patterns have also been recognized (11, 12, 40, 42). Wider genetic divergence was observed amid the genotypes for all the features that were examined, according to the variance analysis, which also demonstrated very considerable differences between the genotypes. Plant height, individual fruit weight and quantity of fruits per plant all contributed the most to divergence (11, 12, 30, 39, 41, 42).

High heterotic hybrids and superior segregants would result from promising crosses between genotypes from these divergent clusters based on their performance for various combinations of yield and quality traits. Crosses between genotypes of divergent clusters with desirable gene combinations for traits are usually desired. This leads to the accumulation of advantageous genes in hybrids and transgressive segregants in subsequent generations (13, 14, 39, 40). When selecting genotypes for crossbreeding from clusters with large inter-cluster distance, it is crucial to consider the degree of divergence with respect to a particular character of interest and the combination of characters. When mean performance and heritable diversity were considered, it was discovered that clusters II and III, as well as

Table 7. Estimates of average inter and intra cluster distance for yield and quality traits in chilli germplasm

Clusters	I	II	III	IV	V	VI
I	5.068	8.020	5.602	5.686	6.464	5.927
II		7.350	9.084	8.089	8.725	7.899
III			5.125	5.982	6.231	6.569
IV				4.795	6.635	6.166
V					6.006	6.996
VI						4.599

Table 8. Cluster mean values for yield and quality parameters in chilli

Sl. No	Characters	Clusters					
		I	II	III	IV	V	VI
1	Plant height (cm)	66.454	37.160	66.753	61.860	67.239	52.427
2	Number of primary branches per plant	3.513	1.932	3.933	2.126	2.595	2.890
3	Number of secondary branches per plant	8.556	6.410	9.400	7.340	7.170	8.259
4	Stem girth (mm)	13.981	10.334	13.777	12.151	13.272	10.485
5	Plant spread in east-west (cm)	47.038	23.040	55.233	50.429	48.555	41.700
6	Plant spread in north-south (cm)	45.865	25.880	53.277	44.350	53.264	41.858
7	Days to 50 % flowering	61.125	62.900	60.867	62.750	57.364	51.182
8	Days to first harvest	81.470	82.500	77.533	81.600	76.216	68.545
9	Number of fruits per plant	32.854	20.610	55.658	54.330	38.357	29.232
10	Fruit length (cm)	7.343	7.100	8.907	8.620	11.009	8.836
11	Fruit diameter (mm)	12.574	18.020	11.340	11.220	15.059	10.182
12	Stalk length (cm)	3.661	3.200	3.480	3.740	4.334	3.682
13	Individual fruit weight (g)	6.455	10.980	6.147	4.470	9.905	4.564
14	Fruit yield per plant (g)	218.390	201.440	332.660	245.275	379.141	128.992
15	Capsaicin content (%)	0.405	0.633	0.439	0.316	0.370	0.434
16	Oleoresin content (%)	12.409	10.472	12.087	11.750	12.120	12.736
17	Dry matter content (%)	18.331	13.014	17.691	16.787	17.602	14.750
18	Ascorbic acid (mg/100g)	148.246	104.592	189.448	152.309	192.017	130.616
19	Total phenols (mg/100g)	75.788	76.224	82.811	148.510	77.413	69.531
20	Peroxidase estimation (activity/min/g)	1.987	1.863	1.637	1.654	1.005	1.724

II and V, were more genetically distinct. These clusters also contain superior genotypes for a combination of yield and quality parameters. Therefore, based on the inter-cluster distance and genotype performance for a variety of trait combinations from the divergent clusters, suitable parents for the crossover program have been suggested and are shown (Table 9). Similar findings were noted in (11, 13, 43-45).

Molecular characterization of 72 chilli genotypes using pungency-specific SSR marker

Trait assessment using linked molecular markers is more precise and effective than the morphological markers as the phenotype measured vary conferring to the methodology used as well as the prevailing environment. Phenotyping of pungency trait is a vital task and its measurement is found to be bottleneck in chilli breeding. In addition, pungency being complex trait in nature and significantly vary across environments, accurate quantification is difficult. So, selection based on molecular markers is highly effective approach in developing pungent genotypes by screening large germplasm. Simple Sequence Repeats (SSRs) are the attractive ones due to their locus-specificity, co-dominance, PCR based, multi-allelic nature and abundance in genome (15, 16).

Nine SSR markers that have been linked to pungency were used to molecularly characterize 72 genotypes of chilli (17). All the nine SSR markers exhibited good polymorphism and a consistent amplification pattern. Power Marker software was used to record and analyze marker parameters (Table 10). With an average of 2.77 (44 - 47), the allele number differed from 2 to 4, with the marker HpmsE017 recording the greatest number of alleles (4 alleles). With an average value of 0.631, the major allele frequency varied from 0.534 (HpmsE022) to 0.777 (HpmsE005). With a mean value of 2.018, the number of effective alleles varied from 1.542 (HpmsE005) to 2.537 (HpmsE022). Furthermore, it was discovered that HpmsE022 (1.013) had the highest Shannon's index, with an average value of 0.793. An average value of 0.493, gene diversity varied from 0.351 to 0.605 (HpmsE022). With a mean value of 0.415, the heterozygosity observed for the primer HpmsE031 marker (0.444) was high. Further with an average PIC value of 0.415, the PIC values ranged from 0.298 (HpmsE005) to 0.538 (HpmsE022). PIC value measures the ability of a marker to distinguish the genotypes by considering both the number of alleles at a locus and the relative frequencies of these alleles. This value depends on the genetic diversity of the population.

The polymorphism rate of a marker at a particular locus can be found with great ease using markers with a PIC value of 0.5 or above, which are very useful for genetic investigations. When identifying pungent and non-pungent varieties of chilli, the HpmsE022 marker proved to be highly successful. Fig. 4 displays the primer HpmsE022 gel image. The current results are fairly steady with a number of previous studies (46 - 48). The genotypes showed varying degrees of heterozygosity, depending on the polymorphism and the reproducibility of the marker. Because of the wide range of potential uses, breeding for quality in chillies-more especially, pungency-is the focus. A crucial first step in marker -assisted breeding is the identification of molecular markers unique to the desired trait and their validation across germplasm sets to speed up genetic improvement.

Table 9. Proposed parents for hybridization based on cluster distance and cluster means in chilli

Sl. No.	Diverse clusters	Inter-cluster distance	Genotypes for proposed crosses	Characters to be combined
1	II - III	9.084	KCA-19-4, KCA-24-2 and BCA-27 (cluster III)	Number of fruits per plant, primary and secondary branches and plant height
			BCA-26 and BCA-30, (cluster II)	Individual fruit weight, fruit diameter and high capsaicin content
2	II - V	8.725	KCA-27-2, KCA-27-1, KCA-17-6 (cluster V)	High yield, ascorbic acid content
			BCA-26 and BCA-30, (cluster II)	Individual fruit weight, fruit diameter and high capsaicin content

Table 10. Genetic parameters of SSR markers used for validation of 72 chilli genotypes for pungency

Marker	HpmsE005	HpmsE017	HpmsE022	HpmsE031	HpmsE054	HpmsE058	HpmsE062	HpmsE063	HpmsE0101	Mean
Major allele frequency	0.777	0.611	0.534	0.569	0.611	0.59	0.743	0.638	0.611	0.631
Genotype number	3	6	5	4	3	3	4	2	3	3.66
Sample size	72	72	72	72	72	72	72	72	72	72
Allele number	3	4	3	3	2	2	3	2	3	2.77
Gene diversity	0.351	0.525	0.605	0.582	0.475	0.483	0.414	0.461	0.539	0.493
No. of effective alleles	1.542	2.109	2.537	2.396	1.906	1.937	1.709	1.857	2.17	2.018
Shannon's Index	0.582	0.914	1.013	0.981	0.668	0.677	0.748	0.654	0.907	0.793
Heterozygosity	0	0.041	0.069	0.444	0.055	0.069	0.347	0	0.236	0.14
PIC	0.298	0.45	0.538	0.518	0.362	0.366	0.377	0.354	0.472	0.415

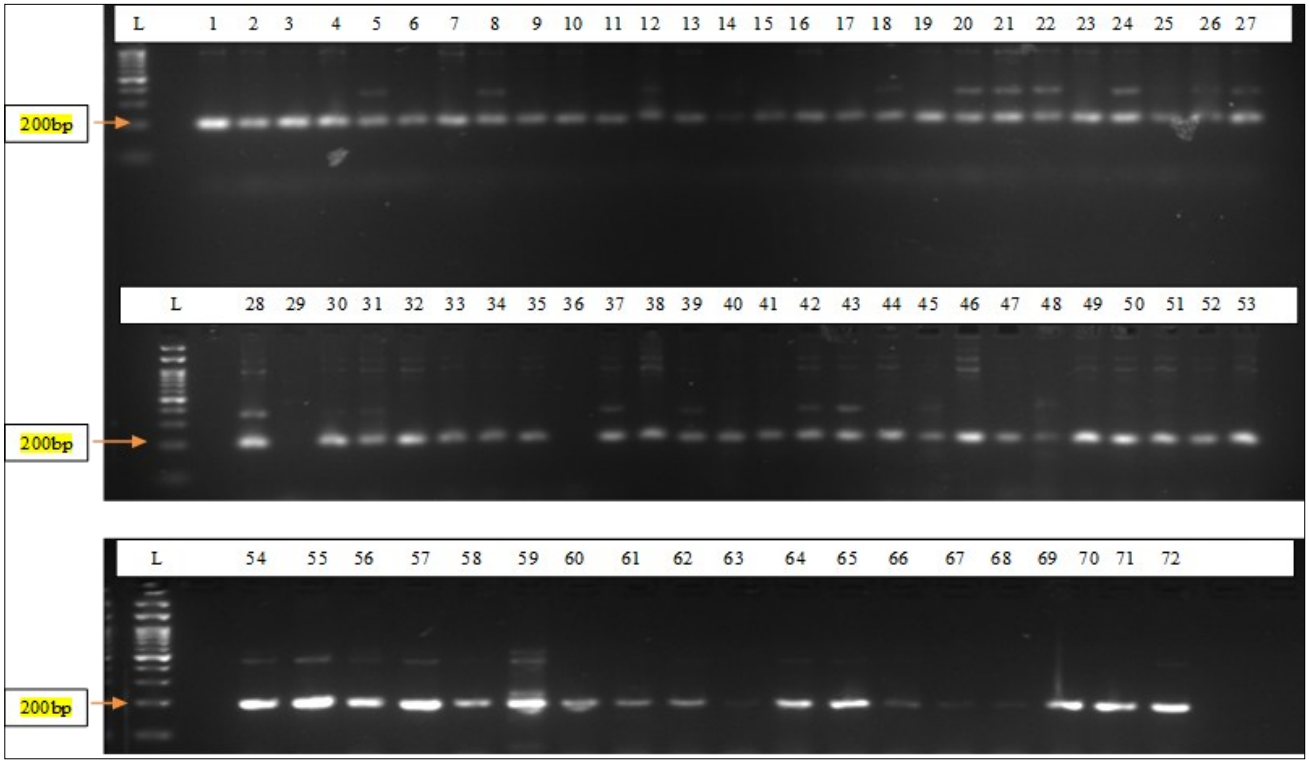

Fig. 4. Agarose gel showing validation of DNA markers associated with a pungency in chilli germplasm by PCR with HpmsE022 marker (Lanes M: 100bp ladder and 1-72 chilli genotypes).

Conclusion

Variability assessment in the test chilli germplasm provides more scope for selecting targeted genotypes as improved varieties as well as for heterosis breeding. In the current investigation, genetic diversity and variability data was measured using 72 chilli germplasm lines considering fruit quality traits.

This information would help chilli breeding programs in choosing elite breeding lines to improve quality. Therefore, estimating GCV, PCV, general heritability and genetic advancement aid in selecting the genotypes for precise exploitation. Capsaicin, peroxidase, total phenols and ascorbic acid content showed greater magnitudes of GCV, PCV, heritability and GAM, indicating high variability and additive gene action predominance for these variables. The data also sheds light on the genetic diversity, as the 72 accessions were divided into six different clusters, with cluster I having the most genotypes (20). Groups II and III were the farthest clusters, according to the inter-cluster distance and their genotypes were very different and suitable for hybridization. The development of molecular markers unique to the desired trait and their validation across germplasm sets supporting marker-assisted breeding are essential for accelerated genetic improvement. As per the highest PIC values recorded, the marker HpmsE022 is suitable for differentiating between genotypes of spicy and non-spicy chillies.

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Authors' contributions

LTN contributed to the design and execution of the research, as well as the recording, compilation and interpretation of the data. FB provided access to molecular biology laboratory facilities and assisted with manuscript revision. SKS provided scientific guidance throughout the research process, assisted in data compilation and performed multiple rounds of manuscript review. DP was involved in data collection and the subsequent analysis of the results. RR played a major role in conducting field experiment for healthy growth of the plants and played a key role in research article preparation. MS conceived the study, designed the experimental approach and played a leading role in the interpretation of the data and preparation of the research article. All authors reviewed and approved the final manuscript. JU guided to conduct biochemical analysis and assisted with manuscript revision.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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