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Species diversity of genus *Capsicum* using agromorphological descriptors and simple sequence repeat markers

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Sustainability of crops in most demand depends upon their genetic diversity. *Capsicum*, commonly called chilli, is one such crop with its fruits extensively used as vegetable across the world. Knowledge on various traits is important for genetic improvement of such species. Here, we assessed the genetic diversity among 10 genotypes of six *Capsicum* species, namely *Capsicum annuum*, *C. chinense*, *C. chacoense*, *C. frutescens*, *C. tovarii* and *C. galapagoense*. *C. annuum* MS-12 is a genetic male sterile line. We used morphological descriptors and simple-sequence repeat (SSR) molecular markers for this study. Out of 60 SSR screened, 22 markers (36.66%) showed polymorphism. Alleles number per locus varied from 3 to 7. Average PIC value for 22 polymorphic markers was 0.69, and ranged from 0.54 for the primer Hpms 1-139 to 0.85 for the primer CAMS-072. Ten genotypes of *Capsicum* species were grouped into three major clusters such that genotypes in a single cluster had less dissimilarity matrix values among themselves than which belongs to other clusters. Range of fruit weight and pericarp thickness varied from 0.1 g ('PAU-621') to 2.3 g ('MS-12'), and from 0.29 mm ('PAU-621') to1.09 mm ('MS-12'), respectively. These two genotypes can be used in hybridization or in recombinant breeding program for obtaining higher heterotic effects/ heterosis or for transgressive segregants in chilli pepper.

Keywords: Chillies, Microsatellite markers, Molecular characterization, Spices, SSR markers

The cultivated *Capsicum* fruits are utilized as a source of vegetables (sweet pepper), spice (pungent pepper), natural colouring agents, and for medicinal applications. The *Capsicum* genus is native to South and Central America, and comprises 32-34 species[1]. India is treated as the secondary center of diversity for many species of *Capsicum* genus, especially *C. annuum*. A wide range of genetic variability with respect to morphoagronomic attributes, especially in fruit morphology (shape, size, colour and aroma), levels of pungency, fruit bearing habit (pendent, intermediate or erect), and plant type is observed within and among the cultivated species of the genus *Capsicum*, which enables their use in crop improvement program[2]. The exploitation of cultivated and wild landrace genotypes of chilli offers an opportunity to identify possible sources of resistance to various abiotic and biotic stresses.

For proper utilization of genetic resource, it is necessary to understand how the genetic variation is distributed, and which characteristics of the species and environment influence this distribution[3]. Study on genetic diversity in the *Capsicum* genus is essential, because it provides criteria for the selection of suitable parents that produce higher heterotic effects on the progeny, and increase the probability of obtaining superior genotypes in segregating populations, and for landraces management and conservations[4]. Morphoagronomic markers are simplest approach for the assessment of genetic diversity in crop plants. However, level of polymorphism, for morphological and agronomic traits in elite genotypes is sometimes too limited and inadequate to allow for genotype discrimination[5]. In recent years, molecular markers have proved to be useful in assessing genetic diversity analysis. Among molecular markers, simple-sequence repeats (SSR) have high reproducibility and better use in germplasm characterization, and genetic diversity analysis in cultivated spp. which have low level of variation[6].

We have earlier mapped the genetic male sterile gene *ms10* in *C. annuum*[7]. Two SSR markers 'AVRDC-PP12' and 'AVRDC_MD997*' were found linked to gene, however markers tightly linked to the GMS *ms10* gene are still lacking. Hence, for marker assisted selection to be very effective, fine mapping of

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the gene *ms10* is important. Genotyping-by-sequencing (GBS) has proven to be technology of choice for generating ultra density maps and precise mapping of traits[8]. Secondly, interspecific cross selected based on the diversity analysis can be used to generate the mapping population. In this study, we tried to assess genetic diversity among the genotypes of chilli belonging to different *Capsicum* species and to characterize them using SSR markers. The variation in agronomic performance of the *Capsicum* species was assessed with 45 morphological descriptors.

Materials and Methods

Plant materials and SSR markers

In this study, we evaluated a total of 10 genotypes belonging to different *Capsicum* species collected from North American and Asian countries (Table 1). For genetic diversity analysis, the genotypes were screened using 60 SSR markers. Table 2 showed a total 22 polymorphic markers. The SSR markers were selected from genetic maps developed by Lee *et al.*[9], Minamiyama *et al.*[10] and Yi *et al.*[11]. The investigation was carried out at the Vegetable Research Farm and Molecular Breeding Laboratory of the Department of Vegetable Science, Punjab Agricultural University (PAU), Ludhiana, Punjab, India during 2017. The experimental site lies at 30° 54' N, 75° 48' E and 248 m above main sea level.

Morphological evaluation

Morphological evaluation of 10 genotypes of *Capsicum* species was carried out during 2016-17. The genotypes were sown in finely prepared nursery beds of 0.15 m height and 1.0 m wide. Treated seed

Table 1 — List of ten *Capsicum* species genotypes used in the study

Genotype	Species	Source
IHR-616	*Capsicum frutescens*	Indian Institute of Horticultural Research, Bengaluru
TC-07246	*Capsicum tovarii*	AVRDC- The World Vegetable Center, Taiwan
TC-07245	*Capsicum galapagoense*	AVRDC- The World Vegetable Center, Taiwan
Perennial	*Capsicum frutescens*	USA
PAU-621	*Capsicum frutescens*	Meghalaya, India
PAU-624	*Capsicum chinense*	Meghalaya, India
CO-4390	*Capsicum chacoense*	AVRDC- The World Vegetable Center, Taiwan
IHR-583	*Capsicum chacoense*	Indian Institute of Horticultural Research, Bengaluru
PC-1	*Capsicum frutescens*	USA
MS-12 (S)	*Capsicum annuum*	Punjab Agricultural University, Ludhiana, India

Table 2 — List of polymorphic SSR primers, their linkage group, product size, alleles amplified and polymorphism information content (PIC) among 10 *Capsicum* species genotypes

SSR primer	Forward sequence	Reverse sequence	Linkage group	Product size (bp)	No. of alleles amplified	PIC value
Hpms 1-1	TCAACCCAAT5ATTAAGGTCACTTCC	CCAGGCGGGGATTGTAGATG	1	283	3	0.66
Hpms 1-5	CCAAACGAACCGATGAACACTC	GACAATGTTGAAAAAGGTGGAAGAC	6	311	4	0.71
Hpms 1-69	CGGTGGCATGTAGTTTCTGGAG	AAGACATGAAATCCACAAGTTTTC	4	217	4	0.73
Hpms 1-139	CCAACAGTAGGACCCGAAAATCC	ATGAAGGCTACTGCTGCGATCC	1	299	3	0.54
Hpms 1-148	GGCGGAGAAGAACTAGACGATTAGC	CCACCCAATCCACATAGACG	1	197	3	0.61
Hpms 1-214	TGCGAGTACCGAGTTCTTTCTAG	GGCAGTCCTGGGACAACTCG	1	100	4	0.73
Hpms 2-13	TCACCTCATAAGGGCTTATCAATC	TCCTTAACCTTACGAAACCTTGG	1	259	3	0.66
Hpms 2-23	CCCTCGGCTCAGGATAAATACC	CCCCAGACTCCCACTTTGTG	5	126	5	0.63
Hpms 2-24	TCGTATTGGCTTGTGATTTACCG	TTGAATCGAATACCCGCAGGAG	9	205	3	0.64
Hpms 2-26	GGGATGTAGGAACAACCCTAACC	TGCATCTTTTCTTCATCCCCTTTC	1,3,5	217	5	0.70
Hpms AT2-20	TGCACTGTCTTGTGTTAAAATGACG	AAAATTGCACAAATATGGCTGCTG	6	148	4	0.75
Hpms CaSIG-19	CATGAATTTCGTCTTGAAGGTCCC	AAGGGTGTATCGTACGCAGCCTTA	7	218	4	0.68
CAMS-020	CAGCAGTAACAGAGGCAGGTC	CACAAGTGAGTTTATTCATATCACCA	5	171	3	0.66
CAMS-072	CCCGCGAAATCAAGGTAAT	AAAGCTATTGCTACTGGGTTCG	5	153	7	0.85
CAMS-101	TCAGCAATTAACATGCCAAAA	TGGATTGGGAGAAGATCGAC	6	217	4	0.68
CAMS-162	GGACCGTTCAGGAGGTTACA	GCCATCATTCAAAACCGAAT	1	210	5	0.72
CAMS-311	GGTGCGCTAGAGATGGAGAG	TTTGAGTGTTCGGGACTGGT	6	234	4	0.72
CAMS-378	GAAATCGACGCGTTTCTAGC	TGTGGGGAGAGAGAGGAAGA	1	168	3	0.67
CAMS-644	CGCATGAAGCAAATGTACCA	ACCTGCAGTTTGTTGTTGGA	4	206	4	0.69
CAMS-647	CGGATTCGGTTGAGTCGATA	GTGCTTTGGTTCGGTCTTTC	3	221	5	0.77
CAMS-806	TGTCACAAGTGTCAAGGTAGGAG	CCCCAAAAATTTTCCCTCAT	10	227	4	0.67
CAMS-864	CTGTTGTGGAAGAAGAGGACA	GCTTCTTTTTCAACCTCCTCCT	7	222	4	0.71
Total					88	15.18
Average					4.0	0.69

with Captan @ 2-3 g/kg of seed were sown on 15th October, 2016 at a depth of 5 cm. Before transplanting, seedlings were hardened by withholding water 5 days before transplanting. The seedlings were transplanted to the field on 20th February, 2017 on ridges at a spacing of 75 cm between rows × 45 cm among plants. The experiment was laid out in a randomized complete block design (RCBD) with two replications. There were ten plants of each genotype in each replication on a ridge. Cultural practices such as fertilization, irrigation, weed control, disease and insect pest control were performed as per the standard agronomic practices recommended by PAU, Ludhiana[12].

Agromorphological data were collected from five randomized selected plants of each genotype. Forty-five characters correlated to both the plant, flower, and the fruit were evaluated on the basis of the descriptors proposed by the Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) for chilli (hot pepper), bell (sweet) pepper and paprika (*C. annuum* L.), New Delhi, India: plant growth habit, length of main stem (cm), length of first internode (on primary branches in cm), anthocyanin colouration of nodes, plant height (cm), plant spread (cm), stem pubescence (hairiness), stem intensity of pubescence (hairiness), stem shape, leaf length of blade, leaf width of blade, leaf colour, leaf intensity of green colour, leaf shape, leaf undulation of margin, leaf pubescence (hairiness), leaf intensity of pubescence (hairiness), flower petal colour, anther colour, flower/ fruit orientation, fruit bearing habit, fruit colour (at mature unripe stage), fruit intensity of colour (at mature unripe stage), fruit length (cm), fruit weight (g), fruit diameter (mm), fruit shape in longitudinal section, fruit curvature, fruit neck at basal end, fruit cross sectional corrugation (at level of placenta), fruit sinuation of pericarp, fruit texture of surface, fruit colour (at ripe maturity), fruit intensity of colour (at maturity), fruit color transition, fruit glossiness, fruit shape at the base, fruit shape of apex, pericarp thickness (mm), fruit stalk length (cm), fruit calyx cover, fruit calyx margin, fruit calyx constriction, fruit pedicel attachment, and fruit blossom end appendage.

Molecular marker analysis

Isolation and purification of genomic DNA

Genomic DNA of 10 genotypes was isolated from the young leaf tissues of each genotype as per the method described by Singh *et al.*[13]. DNA was extracted from five randomly selected plants in each genotype and then bulked for subsequent analysis. The quantity and quality of extracted DNA sample was determined by NanoDrop 1000 spectrophotometer (Thermo Scientific Inc., Waltham, MA, USA) using 2 µL of genomic DNA. The samples showing adequate DNA concentration (50ng.µL^{-1}, and above) and quality (260/280 nm= 1.7 to 2.0) were selected for PCR amplification.

PCR amplification of SSRs

PCR was performed in a total reaction volume of 25 µL, which contained 2.0 µL (50 ng/µL) of genomic DNA, 1.5 µL (5 µM) of the forward primer, 1.5 µL (5 µM) of the reverse primers, 0.5 µL (10 mM) of the dNTPs mix, 1.5 µL (25 mM) $MgCl_2$, 5 µL (5X) of the PCR buffer, 0.12 µL (5 U.µL^{-1}) of Taq polymerase and 12.8 µL of nuclease free water. All the PCR reagents were procured from Promega, Madison, WI USA. The DNA amplifications were performed in an Eppendorf Mastercycler. A touchdown PCR programme was followed to amplify the DNA fragments, that is an initial denaturation at 94°C for 3 min followed by 10 cycles of denaturation at 94°C for 30 s, annealing at 60°C (the annealing temperature for each cycle being reduced by 1 °C per cycle) for 1 min and extension at 72°C for 1 min, and subsequently, 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 5 min. The amplified product was separated on 2.5% agarose gel. The slabs were casted in a horizontal gel frame; products were visualized by incorporating 1 µL (10 mg.mL^{-1}) ethidium bromide (HiMedia Labs. Pvt. Ltd, Mumbai, India) per 10 mL of gel, and visualized under the UV light in AlphaImager HP imaging system (Fisher Scientific Ltd, Loughborough, UK). The SSR amplicons were recorded in a binary matrix as 1 (band present) and 0 (band absent). Total numbers of alleles for each primer were then scored in all the genotypes under the study.

Statistical analysis

Plant growth and important fruit traits of *Capsicum* species genotypes were compared according to Fisher's Least Significant Difference (LSD) test using statistical software STAR (Version 2.0.1, IRRI, Manila, Philippines). Dissimilarity matrix was constructed using DICE's dissimilarity coefficient to measure the genetic inter relationship among the genotypes. The data was also subjected to Unweighted Pair Group Method with Arithmetic

Mean analysis to generate Neighbour Joining (NJ) tree dendogram using the software DARwin 6.0[14]. The polymorphic information content (PIC) values for all the primers were calculated using the formula given by Nei *et al.*[15].

PIC = $1- \sum(P_{ij})^2$, where P_{ij} is the frequency of the ith pattern revealed by the jth primer summed across all patterns revealed by the primers.

Results and Discussion

Morphological characterization

Total forty five agromorphological descriptors were used to characterize 10 *Capsicum* species landraces as depicted in Table 3. Of the 45 descriptors used in the agromorphological characterization, 14 descriptors were monomorphic for the 10 *Capsicum* species landraces characterized *viz*., plant anthocyanin

Table 3 — Morphological characterization of *Capsicum* species genotypes on the basis of Descriptors of *Capsicum* species (PPV&FRA, New Delhi, India)

Characteristics	IHR-616	TC-07246	TC-07245	Perennial	PAU-621	PAU-624	CO-4390	IHR-583	PC-1	MS-12 (S)
Plant descriptors										
Plant										
Habit	Semi-upright	Semi-upright	Semi-upright	Upright	Semi-upright	Semi-upright	Semi-upright	Semi-upright	Semi-upright	Semi-upright
Length of main stem (cm)	Short	Short	Short	Short	Medium	Short	Short	Short	Short	Short
Length of first internode(on primary branches in cm)	Long	Medium	Medium	Long	Short	Long	Long	Medium	Very long	Long
Anthocyanin colouration of nodes	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Height (cm)	Medium	Tall	Medium	Tall	Tall	Tall	Tall	Medium	Tall	Tall
Spread (cm)	Narrow	Medium	Narrow	Medium	Narrow	Narrow	Narrow	Narrow	Narrow	Medium
Stem										
Pubescence (hairiness)	Absent	Present	Absent	Absent	Present	Absent	Absent	Absent	Absent	Absent
Intensity of pubescence (hairiness)	-	Sparse	-	-	Medium	-	-	-	-	-
Shape	Round	Round	Round	Round	Round	Round	Round	Round	Round	Round
Leaf										
Length of blade	Short	Short	Short	Short	Short	Short	Short	Short	Short	Short
Width of blade	Narrow	Narrow	Narrow	Narrow	Narrow	Narrow	Narrow	Narrow	Narrow	Narrow
Colour	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Intensity of green colour	Dark	Dark	Light	Medium	Dark	Dark	Light	Light	Dark	Medium
Shape	Ovate	Lanceolate	Lanceolate	Lanceolate	Lanceolate	Lanceolate	Lanceolate	Ovate	Lanceolate	Lanceolate
Undulation of margin	Strong	Weak	Weak	Medium	Weak	Weak	Weak	Weak	Weak	Weak
pubescence (hairiness)	Absent	Present	Absent	Present	Present	Absent	Absent	Present	Absent	Absent
Intensity of pubescence (hairiness)	-	Sparse	-	Sparse	Medium	-	-	Sparse	-	
Inflorescence descriptors										
Flower										
Petal colour	Yellowish green	White	White	White	White	White	White	White	White	White
Anther colour	Yellowish green	Pale blue	Pale blue	Pale blue	Pale blue	Pale blue	Yellow	Pale blue	Pale blue	Purple
Flower/Fruit: Orientation	Erect	Erect	Erect	Erect	Erect	Drooping	Erect	Drooping	Erect	Erect
Fruit: Calyx cover	Enveloping	Enveloping	Enveloping	Enveloping	Enveloping	Enveloping	Enveloping	Enveloping	Enveloping	Enveloping
Fruit: Calyx margin	Smooth	Smooth	Smooth	Smooth	Smooth	Dented	Dented	Smooth	Dented	Smooth
Fruit: Calyx constriction	Absent	Absent	Absent	Absent	Absent	Present	Absent	Absent	Absent	Absent
Fruit descriptors										
Fruit										
Bearing habit	Solitary	Solitary	Solitary	Solitary	Solitary	Solitary	Solitary	Solitary	Solitary	Solitary
Colour (at mature unripe stage)	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
Intensity of colour (at mature unripe stage)	Light	Dark	Light	Light	Light	Light	Light	Light	Medium	Light

(*Contd.*)

Table 3 — Morphological characterization of *Capsicum* species genotypes on the basis of Descriptors of *Capsicum* species (PPV&FRA, New Delhi, India)

Characteristics	IHR-616	TC-07246	TC-07245	Perennial	PAU-621	PAU-624	CO-4390	IHR-583	PC-1	MS-12 (S)
Plant descriptors										
Plant										
Length (cm)	Very short	Very short	Very short	Very short	Very short	Short	Short	Very short	Short	Short
Shape in longitudinal section	Cordate	Moderately triangular	Moderately triangular	Moderately triangular	Moderately triangular	Moderately triangular	Moderately triangular	Moderately triangular	Moderately triangular	Moderately triangular
Curvature	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Neck at basal end	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Cross sectional corrugation (at level of placenta)	Round	Round	Round	Round	Round	Round	Round	Round	Round	Round
Sinuation of pericarp	Weak	Weak	Weak	Weak	Weak	Weak	Weak	Medium	Weak	Strong
Texture of surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Slightly rough	Smooth	Smooth	Smooth
Table 3 (continued)										
Colour (at ripe maturity)	Red	Dark Red	Red	Red	Red	Red	Red	Red	Red	Red
Intensity of colour (at maturity)	Medium	Medium	Medium	Dark	Medium	Medium	Medium	Light	Medium	Light
Color transition	One stage	One stage	One stage	One stage	One stage	One stage	One stage	One stage	One stage	One stage
Glossiness	Medium	Medium	Medium	Strong	Medium	Strong	Weak	Medium	Medium	Strong
Shape at the base	Acute	Acute	Acute	Acute	Acute	Acute	Acute	Acute	Acute	Acute
Shape of apex	Acute	Acute	Acute	Acute	Acute	Acute	Acute	Acute	Acute	Acute
Pericarp thickness	Very thin	Very thin	Very thin	Very thin	Very thin	Very thin	Thin	Very thin	Thin	Thin
Stalk Length (cm)	Very short	Short	Very short	Short	Very short	Short	Short	Very short	Short	Short
Pedicel attachment	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Strong
Blossom end appendage	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

colouration of nodes (absent), stem shape (round), leaf length of blade (short), leaf width of blade (narrow), fruit calyx cover (enveloping), fruit bearing habit (solitary), fruit curvature (absent), fruit neck at basal end (absent), fruit cross sectional corrugation (round), fruit color transition (one stage), fruit shape at the base (acute), fruit shape of apex (acute), fruit pedicel attachment (strong) and fruit blossom end appendage (absent), means invariable or unable to discriminate the evaluated landraces. All the *Capsicum* species landraces which included in our study were semi-upright in plant growth habit, except for landrace 'Perennial', which showed upright growth habit. Stem pubescence was present in 'PAU-621', and 'TC-07246' with the intensity of stem pubescence was medium, strong, and sparse, respectively. The length of leaf blade was varied among all the genotypes evaluated, and ranged from 2.06 cm ('CO-4390') to 3.7 cm ('PC-1'). Leaf blade width ranged from 1.08 cm ('IHR-583') to 1.82 cm ('MS-12 (S)'). The genotypes namely, 'PAU-621', 'PAU-624', 'IHR-616', 'TC-07246' and 'PC-1' have dark green coloured leaves, whereas the genotypes 'CO-4390', 'TC-07245' and 'IHR-583' had light green coloured leaves. The genotype 'Perennial' and 'MS-12 (S)' had medium green coloured leaves. The genotype 'PAU-621' had medium leaf pubescence, and the genotypes 'Perennial', 'TC-07246', and 'IHR-583' showed sparse leaf pubescence, while other genotypes showed no leaf pubescence. The genotype 'IHR-616' had yellowish green petal colour with yellow green colour anther; the genotypes 'CO-4390'and 'MS-12 (S)' have white coloured petals with yellow and purple anthers, respectively, while rest of the genotypes have white petal colour with pale blue anther. Two types of flower and fruit orientations were observed *viz*., erect ('PAU-621', 'CO-4390' 'Perennial', 'IHR-616', 'TC-07246', 'PC-1', 'TC-07245' and 'MS-12 (S)', and drooping ('PAU-624' and 'IHR-583') with solitary fruit bearing habit among the genotypes. Fruit colour at mature unripe stage was light green ('PAU-621', 'CO-4390', 'PAU-624', 'Perennial', 'IHR-616', 'TC-07245', 'IHR-583' and 'MS-12 (S)', medium green ('PC-1'), and dark green ('TC-07246'). The light green fruits turned light red colour on ripe maturity stage, whereas the dark green fruits turned dark red colour. Cordate type of fruit shape in longitudinal section was observed in 'IHR-616', while other genotypes had moderately triangular fruit shape. The fruit of all the genotypes have a smooth surface, except for 'CO-4390', which had slightly rough fruit surface. The calyx cover was enveloping and calyx construction was absent among

all the genotypes, except 'PAU-624' (*C. chinense*) which had the calyx constriction. Our study is also verified that the presence of a calyx annular constriction at junction of calyx and pedicel being discriminative of *C. chinense*. According to Baral & Bosland[16], to differentiate among the *Capsicum* species, the inflorescence related descriptors are necessary, such as the flower position and the presence of calyx constriction, which are used to distinguish between *C. frutescens* and *C. chinense*.

Among all the agro-morphological descriptors, the fruit and plant growth related descriptors showed highest degree of polymorphism for the 10 *Capsicum* species landraces (Table 4). Data regarding to fruit weight and pericarp thickness showed wide variation among the genotypes. The mean values for fruit weight was ranged from 0.1 g ('PAU-621') to 2.3 g ('MS-12 (S)'. Similarly, the genotype 'MS-12 (S)' exhibited the highest pericarp thickness (1.09 mm), whereas the genotype 'PAU-621' showed lowest thickness of pericarp (0.29 mm). These two genotypes could use in hybridization or in recombinant breeding program for obtaining higher heterotic effects/ for transgressive segregants in peppers. A wide variation among the evaluated genotypes was also observed for fruit length. The genotype 'IHR-616' had the minimum fruit length (0.89 cm), while the genotype 'PAU-624' showed maximum fruit length with the mean value of 4.28 cm. The variation in fruit diameter was found to be higher among the tested genotypes. The highest fruit diameter was recorded in 'MS-12 (S)' (11.54 mm) and least in 'IHR-583' (5.27 mm). The plant height ranged from 55.5 cm ('TC-07245') to 117.5 cm ('Perennial') with an average of 87.3 cm. The variation for plant spread varied from 34.0 cm ('PC-1') to 72.5 cm ('TC-07246'). The variation among the *Capsicum* species landraces with respect to flower morphology were also noted earlier by many[17-20]; for leaf size by Yumnam *et al.*[18]; and for fruit traits by Yumnam *et al.*[18] and Meena *et al.*[21].

Molecular analysis

A total of 10 *Capsicum* species landraces/ genotypes maintained at Punjab Agricultural University, Ludhiana were characterized by using 60 SSR markers. The characteristics of the SSRs used in the present study are summarized in Table 2. The molecular markers are employed for improved taxonomic identification of landraces since morpho-agronomic characters used in the characterization or identification of *Capsicum* species are difficult to score. Out of 60 SSRs tested, 38 markers did not detect polymorphism and were not used in further analysis. Twenty two markers (36.66%) were thus used for genetic diversity analysis on the basis of scoreable amplified bands (Fig. 1). The number of bands amplified by each of the

Table 4 — Summary of variation for plant growth and fruit traits of *Capsicum* species genotypes

Genotypes	Plant height (cm)	Plant spread (cm)	Fruit weight (g)	Fruit length (cm)	Fruit diameter (mm)	Pericarp thickness (mm)	Fruit stalk length (cm)
IHR-616	70.5±5.0 cd	44.0±8.7 bc	0.14±0.04 e	0.89±0.08 e	5.58±0.57 de	0.84±0.05 b	1.09±0.05 d
TC-07246	109.0±4.9 ab	72.5±10.4 a	0.3±0.14 de	1.51±0.03 de	5.72±0.02 de	0.52 ±0.06 c	1.63±0.13 bcd
TC-07245	55.5±10.8 d	37.0±8.4 c	0.75±0.08 cd	2.29±0.02 c	7.47±0.06 c	0.71±0.03 b	1.58±0.10 cd
Perennial	117.5±18.3 a	62.5±5.1 a	0.5±0.14 cde	2.1±0.14 cd	6.45±0.29 d	0.69±0.11 bc	1.91±0.01 abc
PAU-621	78.5±8.0 cd	42.0±5.8 bc	0.1±0.06 e	1.14±0.03 e	5.9±0.78 de	0.29±0.11 d	1.56±0.47 cd
PAU-624	88.0±8.7 bc	36.5±7.1 c	1.4±0.24 b	4.28±0.03 a	8.14±0.65 c	0.74±0.01 b	2.35±0.24 ab
CO-4390	114.0±8.6 a	44.5±5.7 bc	0.16±0.06 e	3.96±0.83 a	9.32±1.23 b	1.04±0.07 a	1.82±0.52 abcd
IHR-583	72.0±8.6 cd	39.5±8.5 c	0.15±0.09 e	1.32±0.19 e	5.27±0.78 e	0.68±0.14 bc	1.3±0.57 cd
PC-1	80.0±12.0 c	34.0±9.4 c	0.95±0.11 bc	3.08±0.02 b	8.39±0.92 bc	0.75±0.03 b	2.05±0.01 abc
MS-12 (S)	87.5±15.1 bc	57.5±6.3 ab	2.3±0.68 a	3.22±0.04 b	11.54±0.22 a	1.09±0.01 a	2.52±0.39 a
Mean	87.3	47.0	0.68	2.38	7.38	0.74	1.78
LSD at p=0.05	23.91	16.71	0.58	0.65	0.94	0.18	0.77

[Data are expressed as the mean values ± standard deviation]

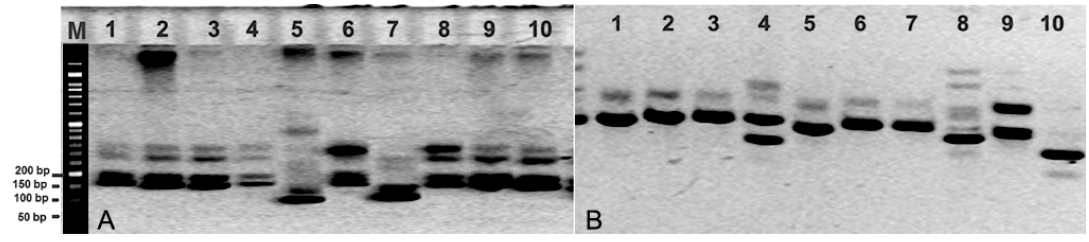

Fig. 1 — PCR amplification profiles of 10 genotypes of *Capsicum* species with SSR markers: (A) CAMS-864; and (B) Hpms 2-23. [Lanes 1-10: IHR-616; TC-07246; TC-07245; PAU-624; Perennial; PAU-621; IHR-583; MS-12 (S); CO-4390; and PC-1]

22 markers ranged from three to seven on superfine 2.5% agarose gel. A total number of 88 alleles were detected with an average of 4.0 alleles per locus in 10 genotypes (Table 2). The maximum number of alleles (seven) was observed for primer pair CAMS-072, and majority of the primers (17) amplified three to four alleles each. The polymorphic level of SSR markers is higher in our study when compared to other chilli pepper studies. Minamiyama *et al.*[10] used SSR markers to construct a genetic map of *C. annuum* in an intraspecific DH population. In their study, they found 26% polymorphism between the parental lines. Aulakh *et al.*[7] screened 558 SSR markers on parents for mapping of GMS *ms10* gene in chilli, and found 21.68% polymorphism. Rai *et al.*[22] using 106 SSRs found 24.51% polymorphism in 48 genotypes of *Capsicum* species. Yi *et al.*[11] used 513 SSRs primer pairs observed 29.2% polymorphism between *C. annuum* cv. 'TF68' and *C. chinense* cv. 'Habanero'. However, some other researchers documented higher level of polymorphisms. Meng *et al.*[23] screened a collection of chilli genotypes. Using SSRs, they reported 50% polymorphism. Colney *et al.*[24] used a set of 30 markers for amplification in 22 genotypes. About 66% markers (20) were found to be polymorphic between the genotypes. Guzman *et al*.[25] characterized 42 *Capsicum* accessions representing eleven species, out of 21 SSRs, all 21 (100%) were polymorphic in the set of five accessions of *C. frutescens*, 20 (95.2%) were polymorphic within the accessions of *C. baccatum* and *C. chinense*, 15 (71.4%) were useful to differentiate the ten accessions of *C. annuum*.

The different level of polymorphism in *Capsicum* observed by different workers could be attributed to the variation in genetic structures of populations screened, and the efficiency of the primer pairs in detecting polymorphism or the molecular techniques used. On the other hand, the slightly lower level of polymorphism may be due to self-pollination of *Capsicum* crops and sequence conservation of genic regions. Similarly, the obtained allele numbers per locus in our study is higher than previous authors who reported average values of 2.9[10], 3.5[26], 2.76[27], 3.04[22], 3.0[28] and 2.8 alleles per locus[24].

The PIC values provide an estimate of discriminating power of a primer by taking into account not only the allele numbers at a locus but also relative frequencies of these alleles. These values depend upon the genetic diversity among the genotypes. The lower PIC value implies a higher level of genetic similarity within the analyzed crop genotypes and the *vice-versa*. The PIC values was obtained in the range of 0.54 for Hpms 1-139 to 0.85 for CAMS-072 with an average PIC value for 22 polymorphic markers to be 0.69, highlighting the presence of genetic variability among the evaluated landraces (Table 2). Based on the PIC values, the most informative marker was CAMS-072, with a PIC value of 0.85, followed by marker CAMS-647 (0.77), Hpms AT2-20 (0.75), Hpms 1-69 (0.73), and Hpms 1-214 (0.73). Minamiyama *et al.*[10] reported average PIC value of 0.46 in their study of doubled-haploid population of pepper (*C. annuum* L.). Yumnam *et al.*[18] assessed genetic diversity among 53 genotypes of chilli belonging to different *Capsicum* species collected from North Eastern (NE) region of India by using 50 SSRs, and they observed 0.52 average PIC values. The results of our study were also comparable to those of Lee *et al.*[9] (0.75) and Rai *et al.*[22] (0.69).

The dendrogram showing genetic relationships among 10 genotypes based on SSR markers is presented in Fig. 2. The dendrogram obtained from the cluster analysis grouped the 10 *Capsicum* genotypes into three main clusters i.e., I, II and III. The first major cluster had five genotypes (50%), showed high homogeneity within the cluster or the least genetic variation. The Cluster I is further divided into two subclusters, Ia and Ib, with three ('PC-1', 'CO-4390', 'IHR-583') and two genotypes ('MS-12 (S)' and 'Perennial'), respectively. The second major cluster (30%) consisted of three genotypes ('TC-07246', 'IHR-616' and 'TC-07245'). The third major cluster was the smallest with two genotypes (20%) namely 'PAU-624' and 'PAU-621. The genotypes of *C. frutescens* and *C. chinense* grouped together in cluster III. The close association between

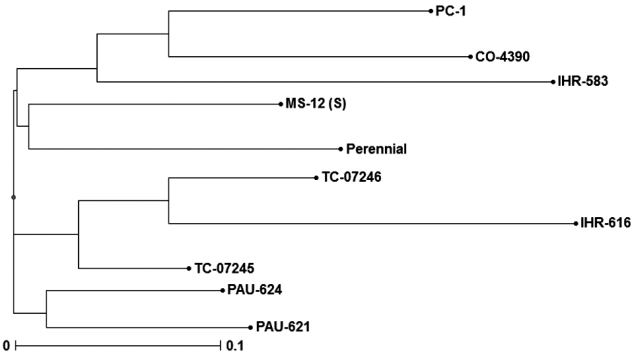

Fig. 2 — Dendrogram produced by UPGMA cluster analysis from SSRs data generated by 22 markers for 10 *Capsicum* species landraces

C. frutescens and *C. chinense* has been documented and described by Ince *et al.*[17], and this also supported by our study via genetic dissimilarity index. Eshbaugh[29] suggested that *C. frutescens*, in its primitive form might be the ancestor of *C. chinense*. Pattern of distribution of genotypes among various clusters reflected the significant genetic variability present in the genotypes tested. The clustering of the genotypes indicated no parallelism between genetic diversity and geographical diversity, since the landraces/ genotypes of various geographic regions were grouped in different clusters. This result was also corroborated by Moreira *et al.*[30].

The important observation was the grouping of the *C. frutescens* genotypes with other *Capsicum* species genotypes, indicating significant amounts of genetic diversity within the *C. frutescens* landraces/ genotypes. Chilli plants often show high level of cross-pollination[31-34], it may have lead to the transfer of some genes between the species. This is one of plausible explanation of the groupism of *C. frutescens* genotypes with other species (possibility of cross-fertilization between species in cultivation field). Other probable reason could be the present sets of molecular markers are not sufficient to detect differences between the species or due to the technical limitations like handling error. Similar observation has also been reported for a *C. chinense* cultivar suggesting interspecific origin for the cultivar[35]. The molecular markers developed specifically for *C. frutescens* might give better results or revalidate the genetic background of the genotypes namely 'IHR-616', 'Perennial', 'PAU-621', and 'PC-1' or more research is needed to reach a conclusion. In a previous study, Lee *et al.*[36] grouped the different *Capsicum* species accessions based on SNP markers. They reported that the species such as *C. frutescens, C. pubescens*, *C. chacoense*, and *C. baccatum* were not clearly separated from each other. Using high-throughput genotyping-by-sequencing (GBS) technique, Pereira-Dias *et al.*[37] characterized a total of 190 *Capsicum* spp. genotypes, including 183 of five cultivated species (*C. annuum*, *C. frutescens*, *C. chinense*, *C. pubescens* and *C. baccatum*) and seven of wild form *C. annuum* var. *glabriusculum*. Whole population was divided into seven clusters by discriminant analysis of principal components (DAPC), where *C. frutescens* genotypes were clustered together with *C. chinense* genotypes.

The average genetic dissimilarity index generated by SSR markers is presented in Table 5. The dissimilarity among the *Capsicum* genotypes was computed using the Dice coefficient. Dissimilarity coefficients of the 10 *Capsicum* species landraces ranged from 0.199 to 0.437. The genotype namely 'TC-07246' and 'TC-07245' were the closest genotypes with the lowest dissimilarity index (0.119) followed by pairs of the genotypes 'PAU-621' and 'PAU-624' (0.15), 'TC-07245' and 'PAU-624' (0.16), and 'TC-07245' and 'PAU-621' (0.167). Such pairs, for having the same or lowest similarity standards, are not recommended for use in breeding program, avoiding restriction in the genetic variability, in order to derail the gain to be obtained by selection. The genotypes 'Perennial' and 'IHR-583' exhibited the greatest dissimilarity (0.437) followed by 'IHR-616' and 'PC-1' (0.42), 'IHR-616' and 'CO-4390' (0.408), and 'IHR-616' and 'IHR-583' (0.389). With this high divergence, these pairs could be used in further breeding programs to developing new segregants. Lima *et al.*[38] reported the average value of dissimilarity between the studied pepper genotypes was 0.315. The average value of genetic distances between *C. baccatum* var. *pendulum* and other evaluated species, 0.68 with *C. annuum* and 0.64 with *C. chinense*[39]. Rabuma *et al.*[40] recorded the genetic distance between thirty two *Phytophthora capsici* resistance *C. annuum* genotypes and observed that the genetic dissimilarity index ranged from 0.05 to 0.51. Paliwal *et al.*[41] performed RAPD based genetic diversity among 21 sweet potato accessions and reported that the pairwise similarity between the accession varied from 0.58 to 0.76. In the set of

Table 5 — Average genetic dissimilarity index based on SSR markers patterns among 10 *Capsicum* species genotypes

Genotypes	IHR-616	TC-07246	TC-07245	Perennial	PAU-621	PAU-624	CO-4390	IHR-583	PC-1
TC-07246	0.243								
TC-07245	0.279	0.119							
Perennial	0.346	0.261	0.210						
PAU-621	0.371	0.25	0.167	0.204					
PAU-624	0.346	0.243	0.160	0.214	0.150				
CO-4390	0.408	0.314	0.310	0.302	0.234	0.245			
IHR-583	0.389	0.333	0.309	0.437	0.365	0.340	0.320		
PC-1	0.42	0.271	0.252	0.278	0.284	0.259	0.196	0.293	
MS-12 (S)	0.365	0.225	0.244	0.196	0.239	0.25	0.226	0.359	0.222

20 *Phaseolus vulgaris* L. genotypes, Kapadia *et al*.[42] reported the values of smiliarity coefficient ranged from 0.395 to 0.822. In the present study, a genetic male sterile (GMS) line namely, 'MS-12 (S)' was included. From the GMS line 'MS-12 (S)', the genotypes namely 'IHR-616' showed the highest dissimilarity (0.365) followed by 'IHR-583' (0.359), 'PAU-624' (0.25), 'TC-07245' (0.244), 'PAU-621' (0.239), and 'CO-4390' (0.226). Due to their genetic divergence, the identified genotypes are included in hybridization/ crossing program, and develop breeding populations. We have crossed the GMS line 'MS-12 (S)', as a female parent, with the diverse genotypes 'IHR-616', 'IHR-583', 'PAU-624', 'TC-07245', 'PAU-621', and 'CO-4390', but we were not gets much success except with the genotype 'PAU-621'. There were no fruit setting in the crosses between MS-12 (S) × IHR-616 and MS-12 (S) × CO-4390. The crossed fruits of MS-12 (S) × TC-07245 were does not reached maturity (lost at green stage). Fruit formation with abnormal seed was received in cross MS-12 (S) × IHR-583. In the cross involving MS-12 (S) × PAU-624, fruits with seed were obtained, from which however no seed could be germinated. Hence, we have preceded the developed F_1 hybrid by crossing 'MS-12 (S)' with 'PAU-621' to generate the breeding population for fine mapping of GMS *ms10* gene in chilli.

Conclusion

In conclusion, the analysis of 10 *Capsicum* species genotypes using SSR markers has provided information that can be utilized for associative relationship between morpho-agronomic traits and genetic level. Thus, morphometric and molecular techniques appeared to be harmonizing and useful for selection of potential parents in breeding program. Based on the molecular characterization of the *Capsicum* species, genotypes has been identified for developing breeding population and chilli improvement. On the basis of obtained results, we have proceeded the developed F_1 hybrid by crossing between 'MS-12 (S)' and 'PAU-621' to generate the breeding population for fine mapping of GMS *ms10* gene in chilli.

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