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Establishment of a core collection of chilli germplasm using microsatellite analysis

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Abstract

Chilli (*Capsicum* spp.) is an economically important vegetable and spice. The Tropical Vegetable Research and Development Center, Kasetsart University, Kamphaeng Saen Campus has collected more than 2500 chilli germplasm accessions since 1989. Investment to maintain and evaluate such a large germplasm collection is high. For efficient germplasm management and to reduce the cost of germplasm maintenance, a core collection needs to be established. Therefore, this study aimed to investigate the genetic diversity of chilli germplasm using microsatellites. A total of 230 chilli germplasm accessions were evaluated using ten anchored *Capsicum* microsatellite loci. These loci generated 42 alleles with a size ranging from 87 to 323 bp. The average polymorphic information content was 0.57, ranging from 0.414 to 0.681, and the probability of identity (PI) ranged from 0.17 to 0.49. The combined PI of the ten microsatellite loci was 2.30×10^{-6}. The similarity index ranged from 0.29 to 1.00. The 230 chilli accessions were divided into two major groups. Group I comprised mainly *C. annuum*, and group II comprised all four *Capsicum* species with the majority being *C. frutescens*, *C. chinense* and *C. baccatum*. The matrix comparison showed that a cophenetic correlation of 0.798 indicated the best fit of the obtained dendrogram. The PowerCore program selected 28 representative chilli accessions to form a core collection, which maintained a similar level of diversity to that of the overall 230 chilli accessions. The representative alleles in those 28 core accessions equalled all the alleles present in the entire collection.

Keywords: *Capsicum*; chilli pepper; diversity; simple sequence repeat

Introduction

Chilli or chilli pepper (*Capsicum* spp.) is an economically important crop grown as a vegetable and spice worldwide. The 2010 world production of fresh and dried chillies has been reported to be 26.8 and 2.8 MT, respectively (FAOSTAT, 2012). Germplasm conservation is essential to maintain genetic diversity for food security and future uses. Given that chilli is one of the most important crops of Thailand, the Tropical Vegetable Research and Development Center (TVRC), Kasetsart University, Thailand has collected 2827 chilli accessions from around Thailand and overseas since 1989 (Mongkolporn and Taylor, 2011). Utilizing these

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germplasm accessions requires a good management programme including seed storage, seed rejuvenation and germplasm evaluation, which, in turn, requires a high maintenance cost. Consequently, a large germplasm collection without proper management will not enhance its utilization (Reed *et al*., 2004).

A core collection is a limited collection containing selected germplasm that represents a similar diversity level to that of the large collection (Frankel and Brown, 1984). Development of core collections has been encouraged as an essential activity in plant genetic resources conservation by the FAO since 1996. Establishing a core collection requires information on the genetic diversity of accessions and varieties that will serve as a basis for the development of the core collection. To a large extent, genetic diversity has been best identified using the molecular analysis of repetitive DNA sequences (microsatellites) of genomes.

Repeat sequences, also known as microsatellites, are well known to be hypervariable and highly conserved in plant genomes (Lagercrantz *et al*., 1993; Gupta *et al*., 1996). Several groups of researchers have developed *Capsicum* microsatellites that are available publicly (Huang *et al*., 2000; Lee *et al*., 2004; Portis *et al*., 2007; Nagy *et al*., 2007; Huang *et al*., 2011). The present study utilized ten microsatellite loci anchored to nine chilli chromosomes (Table 1) to investigate the genetic diversity of 230 chilli germplasm accessions collected by the TVRC, which were then used to form a core collection of chilli germplasm. These ten microsatellite loci were selected based on their best polymorphic information content (PIC) values ($>0.5–0.8$) reported in a preliminary study, whereby 23 microsatellites covering all 12 *Capsicum* chromosomes based on the published Pepper FAO3 map (Bombarely *et al*., 2011) were tested with 96 chilli accessions.

Materials and methods

Plant material

A total of 230 *Capsicum* accessions collected by the TVRC were selected to cover a wide range of morphological characteristics based on the morphological cluster analysis of 2827 chilli accessions (Wasee *et al*., 2005). Originally, the chilli accessions were divided into seven main groups by the NTSYS-pc software based on 16 morphological characteristics, which were known to be controlled by single genes, including hypocotyl colour, cotyledon colour and shape, nodal anthocyanin, plant growth habit, leaf colour and shape, leaf pubescence density, number of pedicel per axil, pedicel position at anthesis, corolla colour and spot, anther colour, filament colour, fruit shape, and neck at the base of the fruit. An additional six quantitative traits that included plant size, number of tilling, leaf size, days to first and last harvesting,

Table 1. Ten microsatellite loci used in this study

Locus	Chr[a]	Pos[b]	Inst[c]	Repeat motif	Primer sequence (5′–3′)[d]
Hpms1-148	1	45.71	SNU	$(GA)_{14}$	F: ggcggagaagaactagacgattagc R: ccacccaatccacatagacg
Hpms1-143	2	126.42	SNU	$(AG)_{12}$	F: aatgctgagctggcaaggaaag R: tgaaggcagtaggtggggagtg
CA524065	5	72.21	ABCH	$(CTT)_{12}$	F: tctctctctacatctctccgttg R: tgtcgttcgtcgacgtactc
CA516334	6	48.31	ABCH	$(CAA)_6$	F: acccaccttcatcaacaacc R: atttgtggcttttcgaaacg
BM067271	8	49.66	ABCH	$(CTG)_6$	F: gtatgctgcaaccatcgttg R: attggtttgggagacacagc
GPMS171	9	69.16	ABCH	$(TC)_6(TC)_5(TC)_6$	F: tccaccacaatatttcgaagg R: tggctgtccaacactgtgag
GPMS159	10	60.70	ABCH	$(TAA)_{20}$	F: aagaacatgaggaactttaaccatg R: ttcacccttctccgactcc
CA516439	10	94.08	ABCH	$(AG)_{11}$	F: gacagtctttcaagaactagagagag R: tggagcaaacacagcagaac
CA519548	11	49.44	ABCH	$(AT)_9$	F: tttcttctctggccctttg R: acgcctattgcgaatttcag
CA515275	12	28.97	ABCH	$(TC)_4(CT)_{13}$	F: ctctgccctcctcaaccc R: aaaatatggtcggagatccg

[a] The chromosome number located by the microsatellite locus. [b] The position of the microsatellite locus residing on the chromosome, expressed as the genetic distance (in cM) from the first marker mapped on that chromosome. [c] The institute that developed the microsatellites: ABCH – Istvan Nagy, Agricultural Biotechnology Center, Hungary; SNU – Byung-Dong Kim, Seoul National University, Korea. [d] F, forward primer; R, reverse primer.

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and fruit weight were subsequently evaluated within each main group for further grouping. The selection was made with the aim to obtain representative chilli accessions from all clusters and subclusters. Species identification was based on floral and fruit morphologies following the description of IPGRI *et al.* (1995). Of the 230 accessions, 184 were *C. annuum*, 30 were *C. frutescens*, eight were *C. chinense* and eight were *C. baccatum*.

For each chilli accession, five plants were grown. Total genomic DNA was separately extracted from each plant at the 30-d-old seedling stage, using a modified CTAB method following Mongkolporn *et al.* (2004). The DNA was equally pooled from each of the five plants within a chilli accession.

Microsatellite analysis

The ten anchored microsatellite loci (Table 1) were used to amplify the chilli DNA. A polymerase chain reaction (PCR) was set up in a total volume of 15 μl containing 50 ng genomic DNA, 1 × PCR buffer, 2.5 mM $MgCl_2$, 0.24 mM dNTPs (Promega, Madison, USA), 0.45 unit *Taq* DNA polymerase (Invitrogen Life Science Technologies, Sao Paulo, Brazil), and 0.2 mM each of forward and reverse microsatellite primers. DNA amplification was performed in a thermocycler (Biometra TProfessional Thermocycler, Goettingen, Germany) with an initial denaturation step at 94°C for 3 min, followed by 35 cycles of 30 s at 94°C for denaturation, 30 s at 50–65°C for annealing, and 1 min at 72°C for extension, with 7 min at 72°C for final extension. The annealing temperatures were optimized to suit each primer pair. Amplified PCR products were separated on a 4.5% polyacrylamide gel, and visualized by silver staining. A ϕX174/*Hin*f I (Fermentas, Ottawa, Canada) and *Capsicum* allelic ladder (developed by J. Chunwongse, unpublished data) were used as references for DNA size. DNA sizes were measured more accurately using the PhotoCapMw program version 99.03 (Vilber Lourmat, Cedex, France) and the AlleloBin software program (Idury and Cardon, 1997).

Genetic diversity and cluster analyses

Microsatellite variation was estimated as the total number of alleles and the number of alleles per locus. The PIC, a measure of allelic diversity at a locus (Botstein *et al.*, 1980), and the probability of identity (PI) or probability that two individuals in a population were the same genotype at multiple loci (Kaul *et al.*, 2001) were calculated.

Microsatellite allelic bands were scored as 1 or 0 for the presence or absence of the allelic band, respectively. The similarity index (SI) was calculated from the scoring data using Nei and Li's (Dice's) coefficient (Nei and Li, 1979). A dendrogram was constructed based on similarity matrix data using the unweighted pair-group method with arithmetic average (UPGMA) clustering using the NTSYS-pc software version 2.20e (Rohlf, 1997). Cophenetic correlation (*r*) and bootstrap were calculated using WinBoot (Yap and Nelson, 1996) to justify how reliable the groupings were.

Establishment of the core collection

PowerCore software version 1.0 (Kim *et al.*, 2007) was used for the selection of chilli accessions to form a core collection following the maximization strategy proposed by Schoen and Brown (1993), which maximized the number of the observed alleles in the dataset.

Results

Genetic diversity and cluster analyses

The ten anchored microsatellite loci generated a total of 42 alleles from the entire 230 chilli accessions, one of which is shown in Fig. 1. The number of alleles per microsatellite locus ranged from 3 to 6 with sizes ranging from 87 to 323 bp (Table 2). The average PIC was 0.57 (0.414–0.681), and the PI values ranged from 0.1725 to 0.4907. The combined PI of the ten microsatellite loci was 2.30×10^{-6}.

The UPGMA cluster analysis was carried out using the similarity matrix to construct a dendrogram. The SI of the entire chilli germplasm ranged from 0.29 to 1.00. The dendrogram contained two main clusters at a SI of 0.29 (Fig. 2). Cluster 1, the larger group, comprised 180 chilli accessions and was divided into 13 subgroups (A–M). Cluster 2 comprised 50 chilli accessions and was divided into four subgroups (N–Q). Almost all chilli members in cluster 1 (176 out of 180) were C. annuum, and the rest were *C. frutescens, C. chinense* and *C. baccatum*. Cluster 2 had a combination of all *Capsicum* species, most of which were *C. frutescens* (29 accessions), eight were *C. annuum*, seven were *C. chinense* and six were *C. baccatum*. Grouping reliability was predicted by cophenetic correlation (*r*), whose value was 0.798, indicating a highly reliable grouping.

Development of the core collection

The 42 alleles detected by the ten microsatellite loci were used to develop a set of core collections using the PowerCore software with a maximization approach.

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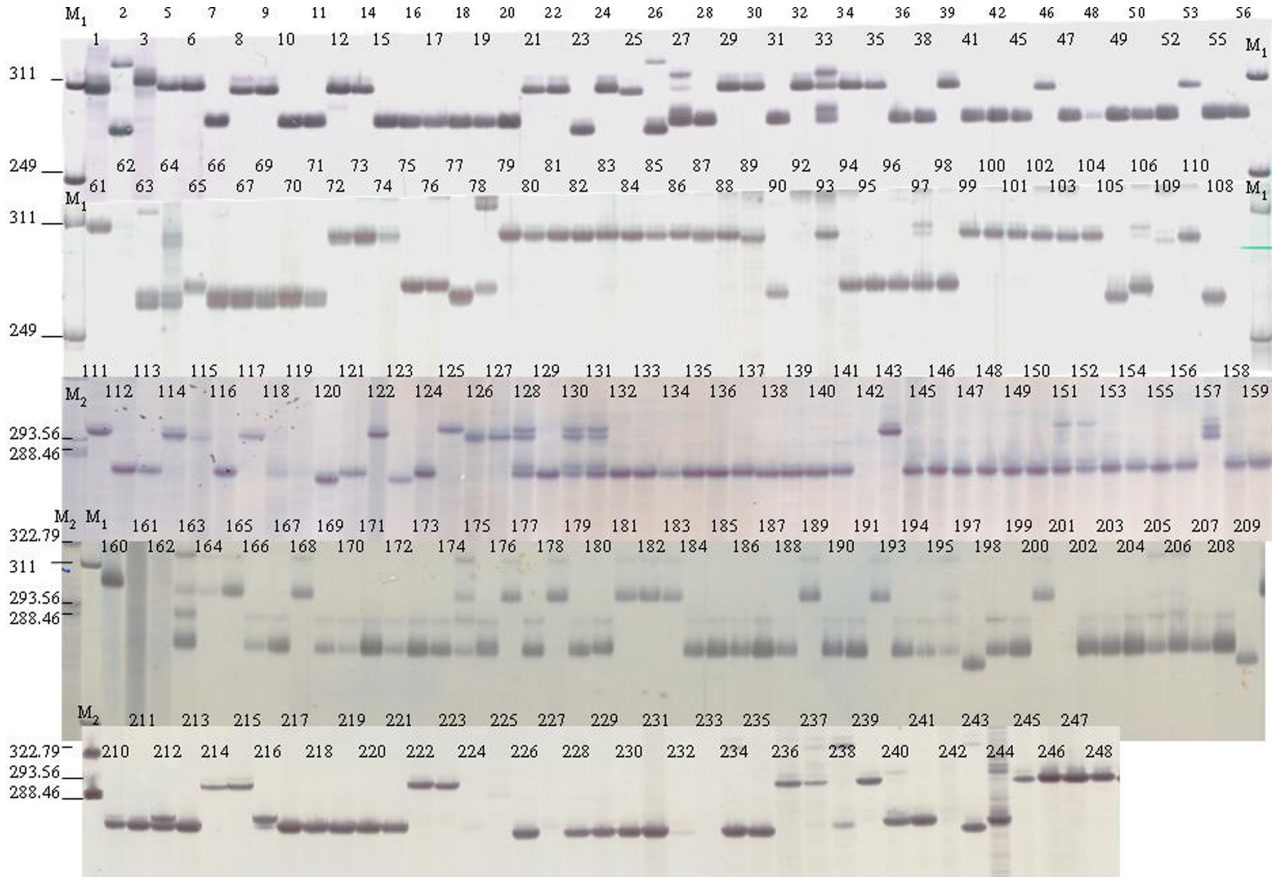

Fig. 1. *GPMS171* microsatellite alleles of 230 *Capsicum* accessions (codes 1–248). M_1 is the DNA marker ϕX174/*Hin*f I (Fermentas, Ottawa, Canada) and M_2 is the *Capsicum* allelic ladder (J. Chunwongse, unpublished data).

The PowerCore program selected 28 chilli accessions that captured all the 42 alleles from the entire 230 germplasm accessions (Fig. 2). The size of the derived core collection was accounted for 12% of the entire size (28 out of 230), with minimum redundancy. The 42 alleles revealed 77 genotypes in both the entire collection and the core collection (Table 3). The average genetic diversity index (*H*) (Nei, 1973) value was 0.754, ranging from 0.487 to 0.881. Compared with the entire collection, the average *H* value was 0.656, ranging from 0.505 to 0.757.

Table 2. Number and size of alleles, polymorphic information content (PIC) and probability of identity (PI) of the ten microsatellite loci assessed using the entire 230 *Capsicum* accessions

	Allele		Genetic diversity value	
Locus	Number	Size (bp)	PIC	PI
Hpms1-148	4	185, 195, 207, 211	0.630	0.3077
Hpms1-143	3	171, 219, 245	0.462	0.3330
CA524065	6	211, 225, 231, 237, 240, 243	0.635	0.1975
CA516334	4	231, 240, 249, 258	0.634	0.4907
BM067271	5	277, 295, 304, 310, 316	0.633	0.3457
GPMS171	4	289, 293, 319, 323	0.542	0.1788
GPMS159	3	282, 288, 312	0.414	0.1725
CA516439	4	143, 159, 167, 169	0.635	0.2529
CA519548	3	187, 189, 193	0.432	0.4567
CA515275	6	87, 91, 93, 99, 101, 105	0.681	0.1882
Total	42	87–323	5.698	2.30×10^{-6a}
Average	4.2	–	0.570	

[a] Combined PI of the ten microsatellite loci.

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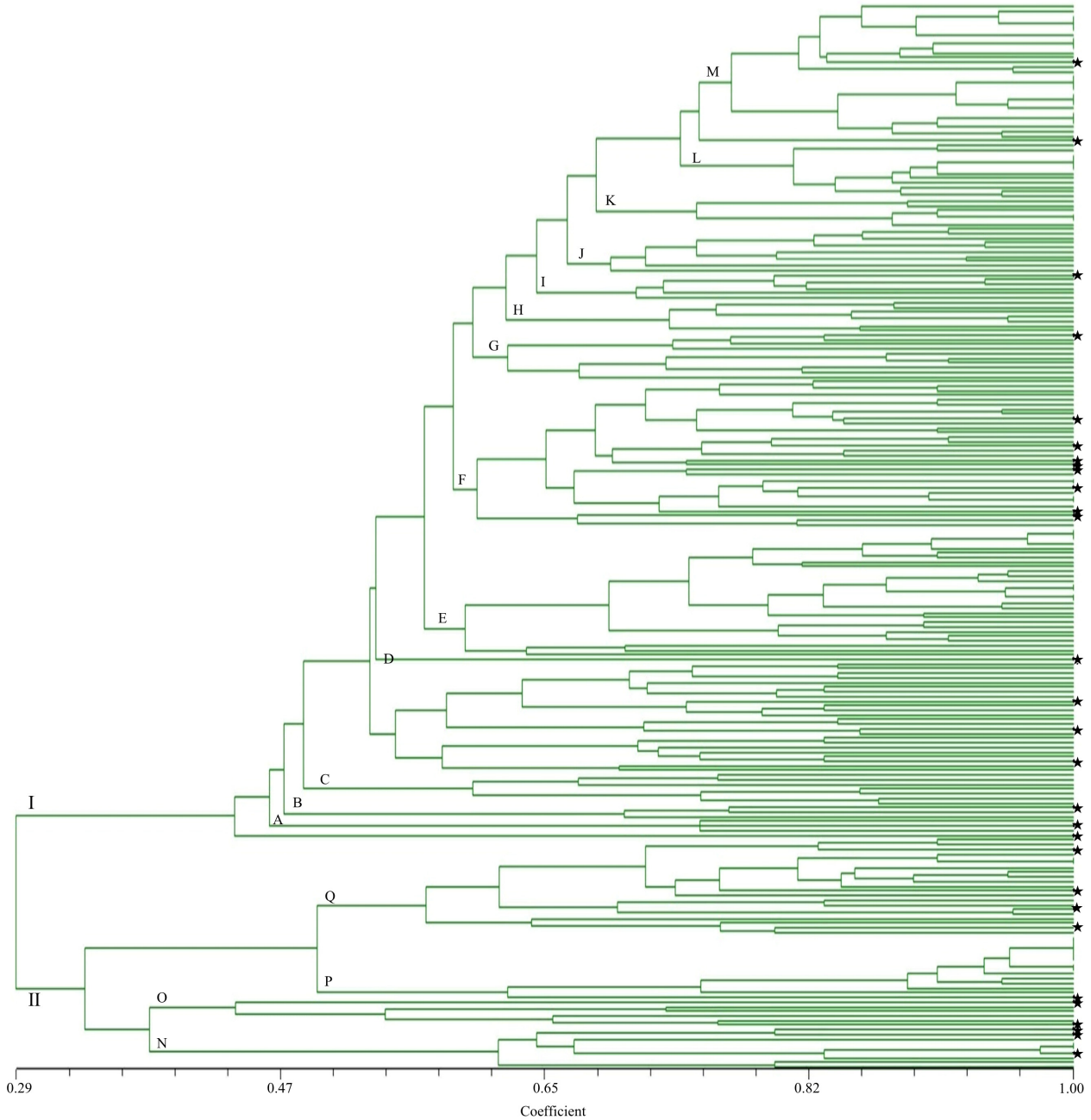

Fig. 2. UPGMA dendrogram illustrating the relatedness of 230 *Capsicum* accessions generated from ten anchored microsatellite loci, drawn by the NTSYS-pc software 2.2e using Nei and Li's similarity coefficient with a cophenetic correlation (*r*) of 0.789. The asterisks represent the 28 chilli accessions selected by the PowerCore software version 1.0 for the development of a core collection.

Discussion

Genetic diversity of chilli germplasm accessions

The genetic diversity of the 230 chilli accessions was found to be relatively high based on the average PIC of 0.570 with the SI ranging from 0.29 to 1.00. The high variability among the chilli accessions may have reflected the diverse species and the diverse origins, from where the accessions had been collected. Although most of the germplasm accessions were collected from Thailand, the rest were from other Asian, European and South American countries (data not shown). Briefly, 56% of the germplasm had Thai origins, while 33% had other

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Table 3. Genetic diversity index (*H*) and number of genotypes of the ten microsatellite loci in the entire 230 chilli germplasm and core collections

		Entire collection		Core collection	
Locus	No. of alleles	*H*	No. of genotypes	*H*	No. of genotypes
Hpms1-148	4	0.719	10	0.834	10
Hpms1-143	3	0.566	3	0.656	3
CA524065	6	0.709	12	0.881	12
CA516334	4	0.701	5	0.732	5
BM067271	5	0.642	8	0.825	8
GPMS171	4	0.635	9	0.792	9
GPMS159	3	0.581	6	0.661	6
CA516439	4	0.757	11	0.821	11
CA519548	3	0.505	4	0.487	4
CA515275	6	0.740	9	0.855	9
Total	42	–	77	–	77
Average	4.2	0.656	–	0.754	–

Asian origins (Taiwan, Japan, Laos, China, Vietnam, the Philippines and Bhutan), 5% had European and South American origins (Hungary, Costa Rica, Spain, Bolivia, Brazil, Peru, Columbia and Mexico) and 6% were unknown origins. The grouping did not seem to relate to the germplasm origins. In addition, these germplasm accessions were originally selected based on their diverse morphological traits.

The ten microsatellite loci that were used were highly informative based on the PI values. The PI of a locus generally suggested a probability that two individuals were identical at that locus (Kaul *et al.*, 2001). Based on the total of ten loci, the combined PI was 2.30×10^{-6}, which indicated that the chance that two chilli genotypes were the same was 1 in 434,500. Therefore, these ten anchored microsatellite loci appeared to be most suitable for the diversity analysis of 230 chilli accessions.

The clustering seemed to fairly relate to the grouping of the *Capsicum* species. Cluster 1, the larger group, contained mainly *C. annuum*, while cluster 2 contained all the four *Capsicum* species. Cluster 2 had four subgroups, i.e. N, O, P and Q (Fig. 2), whereby most *C. frutescens* belonged to P and Q, most *C. chinense* belonged to O and most *C. baccatum* belonged to N subgroups (data not shown). Species identification followed the *Capsicum* taxonomic key (IPGRI *et al.*, 1995), which was based on seed, flower and fruit characteristics. In addition, there were no species-specific microsatellite markers.

These microsatellites were developed from *C. annuum*; however, they were able to amplify all the other three *Capsicum* species. The four *Capsicum* species were domesticated species, and retained some degree of close relatedness among the species. *C. annuum*, *C. frutescens* and *C. chinense* are in the *C. annuum* complex (Mongkolporn and Taylor, 2011), which are crossable between different species. Artificial interspecific hybridization is often accomplished between the species within the *C. annuum* complex for disease-resistant breeding purposes. *C. baccatum* is more distant to the others; however, interspecific crosses can be accomplished via an embryo rescue technique. Interspecific hybridization among these species has been shown to occur naturally; for example, 'Bhut Jolokia' the world hottest chilli was morphologically identified as *C. chinense* (Bosland and Baral, 2007), but was molecularly proven to be a natural hybrid between *C. chinense* and *C. frutescens* (Bosland and Baral, 2007; Purkayastha *et al.*, 2012). Therefore, the morphology-based species identification may not be completely accurate in all cases, which could have an impact on molecular species discrimination.

Core collection

The core collection contained 28 chilli accessions, which accounted for 12% of the entire germplasm studied. A suitable size of a core collection proposed by Frankel and Brown (1984) was 10% of the entire germplasm collection. These 28 accessions retained the 42 microsatellite alleles with an average *H* value of 0.754, which was slightly greater than the *H* value of the entire germplasm. The selection made by the PowerCore program seemed to fairly represent the entire collection (Fig. 2), although the selection did not represent all the subclusters. However, additional chilli accessions can be manually selected from the subgroups that were missed out by the PowerCore program. For example, subgroups E, H, J, K and L had no representatives, few more accessions can be manually selected from each of E and H-J-K-L. This core collection development strategy can be applied to larger chilli germplasm collections at the TVRC; thus,

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these germplasm collections can be managed more efficiently with respect to time and cost.

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