Research Article

Evaluation of Morphological and Molecular Diversity among South Asian Germplasms of *Cucumis sativus* and *Cucumis melo*

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Cucumber, *Cucumis sativus* ($2n = 2x = 14$), and melon, *Cucumis melo* ($2n = 2x = 24$), are two common vegetable crops worldwide. The present study evaluated eighteen *Cucumis* accessions (nine *C. sativus* and nine *C. melo*) that were collected from three South Asian countries that have the most diversity of *Cucumis*. Nine quantitative and twenty-three qualitative characteristics were measured. The values of fruit weight displayed the biggest divergence among the nine quantitative traits and much variation was displayed in twenty-three qualitative traits among eighteen accessions. For eight morphological quantitative traits other than fruit weight, eighteen accessions were divided into three groups by using Principle Component Analysis and K-means cluster analysis. Also, two chloroplast genes *rbcL* and *matK* of eighteen accessions were sequenced. Combined sequences were subjected to construct phylogenetic trees by Neighbor-Joining and Maximum Likelihood methods. Topologies of nine melon accessions were same in these two methods and nine cucumber accessions showed difference. The genetic distances among each of *C. sativus* and *C. melo* accessions were not high. We conclude that the genetic relationship among the eighteen accessions used in this study is not distant although they display significant morphological variations. The information on novel *Cucumis* germplasms provided here would contribute to breeding program as well as evolutionary study in *Cucumis*.

1. Introduction

Cucumber, *Cucumis sativus* ($2n = 2x = 14$), and melon, *Cucumis melo* ($2n = 2x = 24$), are two common vegetable crops worldwide, which are both within the 20 most important vegetables [1]. Within the genus *Cucumis*, two groups (African group and Asian Group) are accepted. They are defined by geographic origin and chromosome number. Geographically, chromosome number of the African group is $2n = 2x = 24$. Africa, which contains the most numerous wild species, was assumed to be the place of origin of *C. melo* [2]. *C. sativus* is indigenous to South Asia and estimated to be domesticated about 300 years ago and was soon cultivated in the south and east of the Himalayas. Diversity in *C. sativus*, the Asian group, is not abundant as that in *C. melo*, the African group. Cucumber, *C. sativus*, is thought to be

the representative of Asian group with the chromosome number $2n = 14$ [3, 4]. On the other hand, a recent study proposed a different opinion about the origin of *C. melo* and *C. sativus*. Sebastian et al. proposed that melon and cucumber were both of Asian origin and had numerous relatives in Australia and around the Indian Ocean which were overlooked previously [5].

It was known that there are thirty subspecies in *C. melo*, but only two varieties in *C. sativus* (*C. sativus* var. *sativus* and *C. sativus* var. *hardwickii*). *C. sativus* var. *hardwickii* ($2n = 14$) is a wild, sympatric botanical variety of *C. sativus* var. *sativus*, which grows in the foothills of Himalayas mountains. This botanical variety has the multiple fruiting and branching habits which are not present in *C. sativus* var. *sativus*. *C. sativus* var. *hardwickii* is cross-compatible with *C. sativus* var. *sativus*. Therefore, *C. sativus* var. *hardwickii*

has a potential for increasing the genetic diversity available for breeding commercial cucumber [6].

C. melo is thought to contain the most diverse varieties in the genus *Cucumis* [7]. Significant morphological variations exist in fruit traits such as color, size, shape, texture, and taste. In 2005, 67 Japanese melon cultivars were evaluated by RAPD and SSR markers. These cultivars were clustered to three horticulture variety groups: Group Cantalupensis, Group Inodorus, and Group Conomon [8]. In China, the important secondary center of diversity, the genetic diversity of *C. melo* was firstly assessed by Luan et al. [9]. Sixty-eight Chinese *C. melo* cultivars were evaluated by RAPD markers, and the results indicated that the Chinese accessions were seem to be rich resources of genetic diversity for plant improvement. Furthermore, to show the genetic diversity of Chinese Hami melon, 120 melon accessions, including 24 accessions of Hami melon, were analyzed by RAPD markers and the accessions of Hami melon were classified into the large-seed type [10]. In *C. sativus*, 16 selected cucumber cultivars were divided into four groups by evaluating the traits related to fruit flavor such as taste, aroma, and dry matter, and the correlations among these traits were confirmed [11]. Recently, a high density (97.5% of chromosomes were covered) genetic map of cucumber was constructed, which was composed of 1,369 markers, including 1,152 SSR, 192 SRAP, 21 SCAR, and one STS locus [12]. Genetic diversity assessments and linkage map construction can increase the effectiveness of breeding programs [13].

Elucidating the phylogenetic relationships in the genus *Cucumis* is of great importance, because the closest relatives and natural composition can provide valuable information to improve melon and cucumber breeding [14]. By analyzing combined chloroplast sequences for 123 of the 130 genera of Cucurbitaceae, it was concluded that *C. sativus* evolved within an Australian/Asian clade and was phylogenetically far from *C. melo* than its relationship considered based on the current morphological classification [15]. Recently, by using DNA sequences from plastid and nuclear markers for 100 accessions from Africa, Australia, and Asia, it was shown that melon and cucumber are both of Asian origin and have some species-level relatives in Australia which were overlooked previously and further investigation of wild species should be concentrated in Asia and Australia [5].

Here, we evaluate genetic diversity in eighteen accessions of *C. sativus* and *C. melo* collected from South Asia. Also, the phylogenetic relationships are proposed based on the molecular data. Importance of genetic diversity for the purpose of crop improvement and evolutionary relationships are discussed.

2. Materials and Methods

2.1. Plant Materials. The materials used in this study comprised of eighteen genotypes of cucumber and melon collected from south Asian countries, most of which are from India (Table 1). The field work was conducted at College Orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India. All of the eighteen accessions were planted with two replications inside

TABLE 1: Cucumber and melon accessions analyzed in this study.

Acc. No.	Germplasm	Spp.	Source	Country
1	AAUC 2	<i>C. sativus</i>	IIVR	India
2	Andaman	<i>C. melo</i>	TNAU	India
3	CM 1	<i>C. melo</i>	TNAU	India
4	Cus 125/71	<i>C. sativus</i>	CGN	China
5	IC 4079	<i>C. sativus</i>	CGN	India
6	JL 3 Dhillon	<i>C. sativus</i>	CGN	India
7	Long Green	<i>C. sativus</i>	CGN	India
8	Mayilam	<i>C. melo</i>	TNAU	India
9	Muskmelon Orange	<i>C. melo</i>	TNAU	India
10	Mysore	<i>C. melo</i>	TNAU	India
11	N 78	<i>C. melo</i>	NBPGR	India
12	Nanhai 2 Aouri	<i>C. sativus</i>	CGN	China
13	Nepal Local	<i>C. sativus</i>	CGN	Nepal
14	Olakkur	<i>C. melo</i>	TNAU	India
15	Perundurai	<i>C. sativus</i>	TNAU	India
16	Pudukkottai	<i>C. melo</i>	TNAU	India
17	Shantung Thorny	<i>C. sativus</i>	CGN	China
18	Theni	<i>C. melo</i>	TNAU	India

IIVR: India Institute of Vegetable Research

TNAU: Tamil Nadu Agriculture University

CGN: The Centre for Genetic Resources, the Netherlands

NBPGR: National Bureau of Plant Genetic Resources.

the shade net house and each replication had eight plants. All the recommended crop production and protection measures were adopted for raising a healthy crop.

2.2. Evaluation of Quantitative and Qualitative Characters. Morphological characters were recorded using five plants in each replication according to combined standards as described by National Bureau of Plant Genetic Resources (NBPGR). In total, 32 traits including nine quantitative and 23 qualitative characters were recorded, which are shown in Table 2 with their abbreviations.

2.3. Extraction of DNA. Genomic DNA was extracted from fresh leaves. Seeds were sown on filter paper in petri dish and grown in room temperature. A week-old seedlings were ground by using mortar and pestle in liquid nitrogen to fine powder and genomic DNA was extracted by using the CTAB method [16]. DNA samples were diluted to 5 ng/ μ L for polymerase chain reaction (PCR) amplification.

2.4. *rbcl* and *matK* Gene Sequencing. PCR amplification was performed in a 10 μ L mixture containing 5 ng genomic DNA, 0.01 μ mol primer, 0.4 mmol dNTP, 0.05 U Ex Taq (Takara, Japan), 1x PCR Buffer 2 mM MgCl₂ (Takara, Japan). PCR reaction was performed in Takara PCR thermal cycler (Takara, Japan) and the cycle can be described as an initial denaturing step at 95°C for 5 min, then 30 cycles of at 95°C for 30 sec, 55°C for 30 sec, and 72°C for 25 sec. The final extension step was at 72°C for 10 min. The PCR products were subjected to sequencing by using ABI 3130 Genetic Analyzer. Primers used in PCR and sequencing are shown

TABLE 2: Characters observed and their abbreviations and standards.

Character	Abb.	Standards
Fruit weight (g)	FW	The weight of fruits at fully ripe stage
Peduncle length (cm)	PL	The length from the base to fruit intersects peduncle
Fruit length (cm)	FL	The length from stalk end to the tip of the fruit
Fruit girth (cm)	FG	The girth at the middle portion
Leaf length (cm)	LL	The length from the tip to the leaf intersects petiole
No. of primary branches	NPB	The number of branches borne on the main stem
Leaf width	LW	The width at the broadest place of the leaf
Internodal length (cm)	IL	The length between two subsequent nodes
Leaf Petiole length (cm)	LPL	The length from the base of to the intersects petiole
Plant growth	PG	Determinate, semideterminate, and indeterminate
Stem shape	SS	Round, angular, and others
Stem color	SC	Yellow, light green, green, dark green, and others
Stem pubescence density	SPD	Sparse, medium, and dense
Stem pubescence type	SPT	Soft, intermediate, and hard
Leaf shape	LS	Orbicular, ovate, elliptical, reniform, cordate, triangular, entire, trilobate, pentalobate, and others
Leaf margin	LM	Crenate, denticulate, entire, lobate, serrate, serrulate, spiny, undulate, and others
Leaf color	LC	Light green, green, dark green, and others
Leaf glossiness	LG	Dull, intermediate, and glossy
Leaf pubescence density	LPD	Sparse, medium, and dense
Leaf pubescence type	LPT	Soft, intermediate, and hard
Flower color	FC	White, cream, yellow, orange, and others
Fruit shape	FS	Globular, flattened, oblate, elliptical, pyriform, ovate, acorn, elongated, and others
Stem end fruit shape	SFS	Depressed, flattened, rounded, and pointed
Blossom end fruit shape	BEFS	Depressed, flattened, rounded, and pointed
Fruit skin luster	FSL	Matt, intermediate, glossy, and others
Fruit skin texture	FST	Smooth, rough, spiny, and others
Fruit skin hardness	FSH	Soft, intermediate, hard, and others
Predominant fruit skin color	PFSC	White, yellow, cream, pale green, green, dark green, orange, brown, grey, and others
Secondary fruit skin color	SFSC	Absent, white, yellow, cream, pale green, green, dark green, orange, brown, grey, and others
Secondary skin color pattern	SSCP	Absent, spotted, striped, netted, short streaked, long Streaked, and others
Fruit mesocarp color	FMC	White, cream, green, orange, and others
Seed color	SC	White, cream white, yellow, light brown, and others

TABLE 3: Primers used in PCR and sequencing.

Primer	Sequence
<i>matK</i> (Forward)	5'-CTATATCCACTTATCTTTCAGGAGT-3'
<i>matK</i> (Reverse)	5'-AAAGTTCTAGCACAAGAAAGTCGA-3'
<i>rbcl</i> (Forward)	5'-ATGTCACCACAAACAGAACTAAAGC-3'
<i>rbcl</i> (Reverse)	5'-CTACGTCTGGAAGACCTGCGAATCC-3'

in Table 3. Consensus sequences were assembled and edited by using the program GENETYX-MAC version 14 (Genetyx, Japan) followed by manual adjustments.

2.5. Data Analysis. Principal Component Analysis (PCA) and k-means cluster analysis was performed by using R version 2.15.0 with bpca package for statistical computing. Phylogenetic analysis was performed under neighbor-joining (NJ) and maximum likelihood (ML) methods. Sequences of *rbcl* and *matK* were aligned with “clustalw2”

on the website: <http://www.ebi.ac.uk/Tools/msa/clustalw2/>. The resulting aligned data matrix was prepared in MEGA 5.05. Bootstrap method is taken for the test of phylogeny. The number of bootstrap replications was 1000. The model of NJ tree was maximum composite likelihood, and the model of ML tree was Tamura-Nei model.

3. Results and Discussion

3.1. Quantitative and Qualitative Characterization. Fruit appearances of eighteen accessions are shown in Figures 1 and 2, and their morphological performances are shown in Tables 4 and 5. For the nine quantitative characters, there was a large divergence among the values of fruit weight. *C. melo* “N78” and *C. melo* “Andaman” had the biggest fruit weights, 1,390 g and 1,350 g, respectively. These two values exceeded all the other accessions ranging from 210 g of *C. sativus* “Long Green” to 850 g *C. melo* “Theni.” *C. melo* cultivars had bigger fruit weights than those of *C. sativus*

FIGURE 1: Photographs of fruits of *Cucumis sativus* accessions used in the present study. Scale: 5 cm.

FIGURE 2: Photographs of fruits of *Cucumis melo* accessions used in the present study. Scale: 5 cm.

TABLE 4: The values on 9 quantitative characters of 18 accessions.

Accession	Species	FW (g)	PL (cm)	FL (cm)	FG (cm)	LL (cm)	LW (cm)	NPB	IL (cm)	LPL (cm)
Cus 125/71	CS	330	2.9	21.5	14.9	12.7	16.9	10.5	7.5	11.9
Long Green	CS	210	6.1	17.3	16.9	12.5	17.1	6.5	4.9	17.3
JL-3 Dhillon	CS	500	1.6	36.5	15.5	14.8	16.1	8.5	8.7	10.4
Nanhai 2 Aouri	CS	230	3.6	16.9	15.3	13.9	15.9	6.5	9.3	9.7
Nepal Local	CS	590	2.9	22.5	20.4	15.5	16.1	9.5	8.2	9.9
Shantung Thorny	CS	360	1.3	35.9	14.8	15.5	18.3	9.5	12.1	10.3
AAUC-2	CS	790	3.3	24.3	18.7	15.7	20.1	8.5	14.5	15.3
Perundurai	CS	400	2.5	23.9	18.3	12.7	17.5	12.5	8.5	12.5
IC-4079	CS	390	2.5	26.9	21.1	14.3	16.5	5.5	9.9	9.9
M		422.22	2.96	25.07	17.32	14.17	17.16	8.61	9.28	11.91
SD		182.12	1.38	7.06	2.41	1.29	1.34	2.21	2.73	2.71
CV %		43%	47%	28%	14%	9%	8%	26%	29%	23%
N-78	CM	1390	2.9	32.9	21.3	14.9	13.3	10.5	9.3	12.7
Mysore	CM	290	3.3	21.3	24.2	8.2	12.7	5.5	5.6	7.5
CM-1	CM	820	7.1	33.7	39.3	14.3	16.3	8.5	12.2	8.6
Olakkur	CM	640	3.9	22.7	26.3	13.5	13.3	5.5	8.5	12.3
Mayilam	CM	770	5.6	30.3	27.9	13.7	15.5	6.5	7.7	11.9
Muskmelon Orange	CM	520	3.1	14	35.3	11.9	14.5	9.5	9.1	13.6
Pudukkottai	CM	795	5.3	46.3	18.9	12.1	14.4	6.5	7.9	11.7
Theni	CM	850	2.6	18.9	39.3	14.9	17.7	8.5	6.5	14.3
Andaman	CM	1350	3.2	40.7	29.9	14.6	17.9	12.5	10.6	14.7
M		825	4.11	28.97	29.15	13.12	15.06	8.16	8.6	825
SD		355.82	1.53	10.61	7.46	2.15	1.91	2.39	2.01	355.82
CV %		43%	37%	37%	26%	16%	13%	29%	23%	43%

(Note) M: Mean, SD: Standard Deviation, CV: Coefficient of Variance, CS: *C. sativus*, CM: *C. melo*.

cultivars. *C. melo* accessions were almost above 500 g and the mean was 825 g. On the other hand, *C. sativus* accessions were 420 g on average and 400 g less than *C. melo*. The exceptions were *C. melo* “Mysore” and *C. sativus* “AAUC-2”, being the lightest *C. melo* and the heaviest *C. sativus*, with the weights of 290 g and 790 g, respectively (Table 4). López-Sesé et al. reported that the mean fruit weight for a collection of 125 Spanish accessions was 1.5 kg, while the mean fruit weight of Villaconejos landraces also from Spain was 2.08 kg [17]. It was also reported that the variability in fruit weight among Spanish varieties was 0.8 to 4.95 kg [18]. The range of fruit weight in the present study was 0.29 to 1.39 kg, not as heavy as the Spanish group. The difference might be due to the different collection region, as well as the different breeding process in the long history of cultivation between the Mediterranean region and south Asian region.

The other eight quantitative traits did not show significant difference as fruit weight. We divided these traits into two groups, namely, fruit-related traits and stem, and leaf-related traits. The first group included fruit length, fruit girth, and peduncle length. According to the results of fruit girth and length, it was shown that the fruit size of *C. melo* was bigger than *C. sativus* except one cucumber accession “Shantung Thorny,” which was bigger

than *C. melo* “Mysore”. “CM-1” and “Andaman” were the biggest ones in melon accessions. The second group were the traits including leaf width, leaf length, number of primary branches, internodal length, and leaf petiole length, which showed smaller variance than fruit-related characters.

By Principal Component Analysis (PCA) and k-means cluster analysis, eight quantitative values were covered by three principle components (PC1, PC2, and PC3). Contribution of variables in each principle components was calculated (Table 6). Fruit length and fruit girth dominated in PC1 and PC2, respectively. Five characters, number of primary branches, internodal length, leaf petiole length, leaf length, and leaf width, had the similar contribution to PC3. Based on the result of principle component analysis, eighteen accessions were classified into three groups (Figure 3). Group 1 contained nine accessions (seven *C. sativus* accessions: AAUC-2, Perundurai, Nepal Local, IC-4079, Cus125/71, Long Green, and Nanhai2 Aouri and two *C. melo* accessions: Olakkur and Mysore) and Group2 contained six accessions (two *C. sativus* accessions: Shantung Thorny and JL-3 Dhillon and four *C. melo* accessions: Andaman, N-78, Pudukkottai, and Mayilam). Group 3 contained three *C. melo* accessions (CM-1, Theni, and Muskmelon Orange). The relative dispersion among accessions within Group2 was

TABLE 5: Characteristics of the 18 accessions on 23 qualitative traits.

Accession	Species	PL	SS	SC	SPD	SPT	LS	LM	LC
Cus 125/71	CS	Indeterminate	Angular	Dark green	Medium	Hard	Entire	SPINY	Dark green
Long Green	CS	Indeterminate	Angular	Light green	Medium	Hard	Entire	SPINY	Green
JL-3 Dhillon	CS	Indeterminate	Angular	Dark green	Dense	Intermediate	Entire	SPINY	Dark green
Nanhai 2 Aouri	CS	Indeterminate	Angular	Dark green	Medium	Hard	Entire	SPINY	Dark green
Nepal Local	CS	Indeterminate	Angular	Dark green	Dense	Intermediate	Cordate	OTHERS	Dark green
Shantung Thorny	CS	Indeterminate	Angular	Dark green	Medium	Hard	Entire	SPINY	Dark green
AAUC-2	CS	Indeterminate	Angular	Green	Sparse	Intermediate	Entire	SPINY	Dark green
Perundurai	CS	Indeterminate	Angular	Dark green	Dense	Intermediate	Entire	SPINY	Green
IC-4079	CS	Indeterminate	Angular	Dark green	Dense	Intermediate	Entire	SPINY	Dark green
N-78	CM	Indeterminate	Angular	Green	Medium	Hard	Entire	SPINY	Light green
Mysore	CM	Indeterminate	Angular	Green	Medium	Hard	Triangular	SPINY	Light green
CM-1	CM	Indeterminate	Angular	Dark green	Medium	Hard	Entire	SPINY	Green
Olakkur	CM	Indeterminate	Angular	Green	Medium	Hard	Reniform	UNDULATE	Light green
Mayilam	CM	Indeterminate	Angular	Green	Medium	Hard	Pentalobate	LOBATE	Light green
Muskmelon Orange	CM	Indeterminate	Angular	Green	Medium	Hard	Pentalobate	LOBATE	Light green
Pudukkottai	CM	Indeterminate	Angular	Dark green	Medium	Hard	Reniform	ENTIRE	Light green
Theni	CM	Indeterminate	Angular	Green	Medium	Hard	Reniform	ENTIRE	Light green
Andaman	CM	Indeterminate	Angular	Green	Medium	Intermediate	Cordate	SPINY	Light green
Accession	Species	LG	LPD	LPT	FC	FS	SFS	BEFS	
Cus 125/71	CS	Intermediate	Dense	Soft	Yellow	Elongated	Rounded	Pointed	
Long Green	CS	Intermediate	Sparse	Soft	Yellow	Elongated	Rounded	Rounded	
JL-3 Dhillon	CS	Intermediate	Dense	Soft	Yellow	Elongated	Pointed	Pointed	
Nanhai 2 Aouri	CS	Glossy	Dense	Soft	Yellow	Elongated	Rounded	Rounded	
Nepal Local	CS	Glossy	Medium	Hard	Yellow	Elongated	Rounded	Rounded	
Shantung Thorny	CS	Glossy	Dense	Soft	Yellow	Elongated	Pointed	Pointed	
AAUC-2	CS	Glossy	Dense	Soft	Yellow	Others	Flattened	Flattened	
Perundurai	CS	Dull	Dense	Soft	Yellow	Elongated	Flattened	Rounded	
IC-4079	CS	Intermediate	Dense	Soft	Yellow	Elongated	Rounded	Pointed	
N-78	CM	Intermediate	Medium	Hard	Yellow	Elongated	Pointed	Rounded	
Mysore	CM	Intermediate	Dense	Soft	Yellow	Elliptical	Pointed	Rounded	
CM-1	CM	Intermediate	Dense	Soft	Yellow	Pyriiform	Rounded	Rounded	
Olakkur	CM	Intermediate	Medium	Intermediate	Yellow	Others	Flattened	Flattened	
Mayilam	CM	Intermediate	Dense	Soft	Yellow	Pyriiform	Pointed	Depressed	
Muskmelon Orange	CM	Dull	Dense	Soft	Yellow	Globular	Flattened	Flattened	
Pudukkottai	CM	Intermediate	Dense	Soft	Yellow	Elongated	Rounded	Rounded	
Theni	CM	Intermediate	Sparse	Intermediate	Yellow	Globular	Flattened	Depressed	
Andaman	CM	Intermediate	Medium	Hard	Yellow	Elongated	Pointed	Rounded	
Accession	Species	FSL	FST	FSH	PFSC	SFSC	SSCP	FMC	SC
Cus 125/71	CS	Glossy	Spiny	Hard	Dark green	Pale green	Cream	Spotted	White
Long Green	CS	Intermediate	Rough	Hard	Brown	White	Green	Striped	Green
JL-3 Dhillon	CS	Intermediate	Spiny	Hard	Pale green	Yellow	Cream	Striped	White
Nanhai 2 Aouri	CS	Matt	Spiny	Hard	Pale green	Yellow	Cream	Striped	White
Nepal Local	CS	Glossy	Spiny	Intermediate	Brown	Absent	Absent	Striped	White
Shantung Thorny	CS	Intermediate	Spiny	Hard	Yellow	Absent	Absent	Spotted	White
AAUC-2	CS	Matt	Spiny	Hard	Brown	Orange	Cream	Netted	White
Perundurai	CS	Intermediate	Spiny	Hard	Pale green	Green	White	Netted	Cream
IC-4079	CS	Matt	Rough	Hard	Brown	Yellow	Others	Spotted	White
N-78	CM	Glossy	Smooth	Intermediate	Green	Yellow	Cream	Spotted	White
Mysore	CM	Matt	Smooth	Intermediate	Yellow	Absent	Absent	Spotted	White
CM-1	CM	Glossy	Smooth	Hard	Dark green	Pale green	Cream	Spotted	White
Olakkur	CM	Intermediate	Smooth	Soft	Pale green	Yellow	Cream	Spotted	Cream

TABLE 5: Continued.

Accession	Species	PL	SS	SC	SPD	SPT	LS	LM	LC
Mayilam	CM	Intermediate	Smooth	Hard	Yellow	Dark green	White	Netted	Cream
Muskmelon Orange	CM	Glossy	Others	Hard	Yellow	Dark green	Cream	Short streaked	White
Pudukkottai	CM	Glossy	Smooth	Hard	Yellow	Pale green	Orange	Netted	White
Theni	CM	Intermediate	Smooth	Soft	Yellow	Dark green	Cream	Short streaked	White
Andaman	CM	Intermediate	Smooth	Soft	Yellow	Pale green	Orange	Spotted	White

Note. CS: *C. sativus*, CM: *C. melo*.

TABLE 6: Eight quantitative characters contribution to three principle components.

Quantitative character	PC1	PC2	PC3
No. of primary branches	0.041	0.027	0.457
Internodal length	0.086	0.046	0.422
Leaf petiole length	-0.047	-0.001	0.452
Leaf length	0.053	0.016	0.385
Leaf width	0.017	-0.029	0.487
Peduncle length	-0.008	0.068	-0.135
Fruit length	0.935	0.329	-0.065
Fruit girth	-0.332	0.939	0.007

greater than the other two. Group 3 contained the least number of accessions, that is, *C. melo* cultivars only. Group 1 was mainly constituted of *C. sativus* accessions, while Group 2 and Group 3 contained most of *C. melo* accessions. The dispersion among *C. melo* accessions was greater than that among *C. sativus* accessions.

Characteristics on twenty-three qualitative traits of eighteen accessions are shown in Table 5. Traits observed were varied from stem characters, leaf characters, fruit characters and seed characters. Same growth patterns were shown in all the plants observed and all of them were indeterminate. For stem characters, all of the accessions had angular stems with different degrees of green color. *C. sativus* “Long Green” was the only one with light green stem, which was lighter than the others. Besides, *C. sativus* “AAUC-2” displayed sparse stem pubescence and *C. sativus* “JL-3 Dhillon”, *C. sativus* “Nepal Local”, “Perundurai,” and “IC-4079” showed dense stem pubescence. Other accessions were between them. For leaf characters, five types of leaf shape were recorded, namely, entire, cordate, triangular, reniform, and pentalobate. Among them, the entire shape was common, including ten accessions showing entire leaves with spiny margin. All accessions had green leaves with different density, and *C. sativus* “Perundurai” was the only one without leaf glossiness. Pubescence was also observed on leaves as on stems in all accessions, and the leaf pubescence was softer than those on stem. The pubescence trait is closely related to plant defense system. It is under investigation for map-based cloning by SSR markers. The biggest diversity appeared on the fruit-related characteristics, as shown with the results of quantitative characteristics. Except *C. sativus* “AAUC-2”, the fruit shapes of all the *C. sativus* accessions were elongated. For *C. melo* accessions, fruit shapes of *C. melo* “Mayilam”

and “CM-1” were pyriform. *C. melo* “Mysore” was only elliptical and *C. melo* “Muskmelon Orange” and “Theni” were globular. Fruit characters were further recorded on fruit skin luster, texture, and hardness, which are related to the appearance and taste. *C. sativus* “Nanhai 2 Aouri”, “AAUC-2”, “IC-4079”, and *C. melo* “Mysore” did not have fruit skin luster. Fruit skin textures of all the *C. melo* accessions were smooth and those of *C. sativus* accessions were rough or spiny. Three *C. melo* accessions, “Olakkur”, “Theni”, and “Andaman,” had soft fruit skins. *C. sativus* “Nepal local”, *C. melo* “N-78”, and “Mysore” had harder fruit skin than *C. melo* “Olakkur”, “Theni”, and “Andaman”, but softer than the others. Besides, all of accessions had yellow flowers and white or cream seeds, expect that the seeds of “Long Green” were green.

3.2. Phylogenetic Analysis. The lengths of *matK* and *rbcl* genes ranged from 750 to 1067 bp, sequences were submitted to DNA Database of Japan (DDBJ). The aligned data matrix combined with *matK* and *rbcl* gene was 1,728 bp long. Based on the aligned matrix, phylogenetic trees were made by Neighbor-joining and maximum likelihood method (Figures 4 and 5).

On the topology of *C. sativus*, “JL-3 Dhillon” showed divergence from other accessions. Fruit morphologies of “JL-3 Dhillon”, the longest fruit length and yellow fruit skin, differed from other *C. sativus* (Tables 4 and 5). In a previous phylogenetic study of *Cucumis*, some special morphological traits were circumscribed within some certain clades [5]. Species of the Asian/Australian clade had more of less ellipsoid fruits that stay green or turn yellow-orange at maturity. *C. ritchei*/*C. maderaspatanus* clade was characterized by smooth and round fruits that turn red at maturity [5]. Also here in the present study, this correlation between morphological traits and clade divergence at molecular level was shown in the case of “JL-3 Dhillon”.

CGN23965 “I.C 4079” has the scientific name *C. sativus* group *Gherkin*, while CGN24494 “Nepal local” has the scientific name *C. sativus* var. *sikkimensis* (TANU and CGN). In the phylogenetic tree, “I.C 4079” and “Nepal local” did not show big divergence with other accessions, both of them were nested in the same clade. Two Chinese *C. sativus* accessions “Cus 125/71” and “Nanhai 2 Aouri” were nested in the same clade as well as the accessions from different areas, India and Nepal. One of reasons for narrow molecular divergence could be due to the accessions derived from narrow areas of India and Himalaya.

FIGURE 3: (a) Distribution of eighteen accessions on the first and second principle components; (b) distribution of eighteen accessions on the first and third principle components.

FIGURE 4: Neighbor-joining tree for 18 accessions in this study based on combined sequence of *rbcL* and *matK* genes. The tree is rooted on *Cucumis hirsutus*. Bootstrap values based on 1000 replicates above branches.

FIGURE 5: Maximum likelihood tree for 18 accessions in this study based on combined sequence of *rbcL* and *matK* genes. The tree is rooted on *Cucumis hirsutus*. Bootstrap values based on 1000 replicates above branches.

According to the previous works and present study, comparing with *C. sativus*, genetic diversity in *C. melo* seems to be much bigger. African melon group has 30 species that is divided into six subgroups [2], and ancient melon varieties were distributed throughout South Asia, which could be the first genetic diversity center [7]. *C. melo* accessions studied in the present study were mainly introduced from three institutions, namely, India Institute of Vegetable Research (IIVR), Tamil Nadu Agricultural University (TNAU), and National Bureau of Plant Genetic Resources (NBPGR), USA. They distributed all around India, but in the phylogenetic tree, the accessions from different places nested in the same clade with each other. A big divergent node was found with *C. melo* “CM1”, branching with the other accessions. Special morphological traits were also found in accession “CM1”, such as the longest intermodal length and unique fruit color (Figure 2). “Muskmelon Orange” branch was close to “CM1”, and they were grouped into Cluster 2 in the cluster analysis (Figure 3).

The fruit shapes of *C. melo* “Muskmelon Orange” and “Theni” were globular, being distinguished from the other

C. melo accessions. This discrepancy was not supported by the phylogenetic results. Besides, *C. melo* “Mayilam” branch was the biggest divergent node. It shared the same group with “N-78” in cluster analysis in spite of that they were far from each other in phylogenetic trees. So in *C. melo*, correlations between morphological traits and phylogenetic tree were not as supportive as those in *Cucumis sativus*. It could be concluded that *C. melo* holds bigger diversity in morphology than *C. sativus*, which has caused more complex factors for analyzing phylogenetic relationships.

4. Conclusion

Eighteen *Cucumis* accessions derived from south Asia, which represent the most diverse genetic resources, were evaluated morphologically and at the molecular level. It was shown that, although large morphological diversity was found among *C. melo* and *C. sativus* accessions, genetic diversity based on the molecular analysis showed larger diversity in *C. melo* than in *C. sativus*. Thus, it would be necessary to investigate phylogenetic relationships at the molecular

level among *Cucumis* species using accessions collected more widely in Asia, Africa, and Australia continents. Also, the accessions studied here are new germplasms and could be alternative candidates for breeding programs in this area.

Authors' Contribution

C. Zhang and A. S. Pratap contributed equally to this work.

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References

- [1] FAOSTAT, "Statistical database (online) of Food and Agriculture Organization of the United Nations," <http://faostat.fao.org/>, 2008.
- [2] J. H. Kirkbride Jr., *Biosystematic Monograph of the Genus Cucumis (Cucurbitaceae)*, Parkway, Boone, NC, USA, 1993.
- [3] C. Jeffrey, "A review of the Cucurbitaceae," *Botanical Journal of the Linnean Society*, vol. 81, pp. 233–247, 1980.
- [4] T. W. Whitaker and G. N. Davis, *Cucurbits: Botany, Cultivation, and Utilization*, Interscience, New York, NY, USA, 1962.
- [5] P. Sebastian, H. Schaefer, I. R. H. Telford, and S. S. Renner, "Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 32, pp. 14269–14273, 2010.
- [6] J. E. Staub, F. C. Serquen, and J. D. McCreight, "Genetic diversity in cucumber (*Cucumis sativus* L.): III. An evaluation of indian germplasm," *Genetic Resources and Crop Evolution*, vol. 44, no. 4, pp. 315–326, 1997.
- [7] R. W. Robinson and D. S. Decker-Walters, *Cucurbits*, CAB International, New York, NY, USA, 1997.
- [8] E. Nakata, J. E. Staub, A. I. López-Sesé, and N. Katzir, "Genetic diversity of Japanese melon cultivars (*Cucumis melo* L.) as assessed by random amplified polymorphic DNA and simple sequence repeat markers," *Genetic Resources and Crop Evolution*, vol. 52, no. 4, pp. 405–419, 2005.
- [9] F. S. Luan, I. Delannay, and J. E. Staub, "Chinese melon (*Cucumis melo* L.) diversity analyses provide strategies for germplasm curation, genetic improvement, and evidentiary support of domestication patterns," *Euphytica*, vol. 164, no. 2, pp. 445–461, 2008.
- [10] Y. Aierken, Y. Akashi, P. T. P. Nhi et al., "Molecular analysis of the genetic diversity of Chinese Hami melon and Its Relationship to the melon germplasm from Central and South Asia," *Journal of the Japanese Society for Horticultural Science*, vol. 80, no. 1, pp. 52–65, 2011.
- [11] T. K. Behera, A. K. Singh, and J. E. Staub, "Comparative analysis of genetic diversity in Indian bitter melon (*Momordica charantia* L.) using RAPD and ISSR markers for developing crop improvement strategies," *Scientia Horticulturae*, vol. 115, no. 3, pp. 209–217, 2008.
- [12] W. W. Zhang, H. L. He, Y. Guan et al., "Identification and mapping of molecular markers linked to the tuberculate fruit gene in the cucumber (*Cucumis sativus* L.)," *Theoretical and Applied Genetics*, vol. 120, no. 3, pp. 645–654, 2010.
- [13] Z. Fan, M. D. Robbins, and J. E. Staub, "Population development by phenotypic selection with subsequent marker-assisted selection for line extraction in cucumber (*Cucumis sativus* L.)," *Theoretical and Applied Genetics*, vol. 112, no. 5, pp. 843–855, 2006.
- [14] S. M. Chung, J. E. Staub, and J. F. Chen, "Molecular phylogeny of *Cucumis* species as revealed by consensus chloroplast SSR marker length and sequence variation," *Genome*, vol. 49, no. 3, pp. 219–229, 2006.
- [15] S. S. Renner, H. Schaefer, and A. Kocyan, "Phylogenetics of *Cucumis* (Cucurbitaceae): Cucumber (*C. sativus*) belongs in an Asian/Australian clade far from melon (*C. melo*)," *BMC Evolutionary Biology*, vol. 7, p. 58, 2007.
- [16] J. J. Doyle and J. L. Doyle, "A rapid DNA isolation procedure for small quantities of fresh leaf tissue," *Phytochemical Bulletin*, vol. 19, pp. 11–15, 1987.
- [17] A. I. López-Sesé, J. E. Staub, and M. L. Gomez-Guillamon, "Genetic analysis of Spanish melon (*Cucumis melo* L.) germplasm using a standardized molecular-marker array and geographically diverse reference accessions," *Theoretical and Applied Genetics*, vol. 108, no. 1, pp. 41–52, 2003.
- [18] S. Escribano and A. Lázaro, "Agro-morphological diversity of Spanish traditional melons (*Cucumis melo* L.) of the Madrid provenance," *Genetic Resources and Crop Evolution*, vol. 56, no. 4, pp. 481–497, 2009.