Current Botany 2022, 13: 76-80
doi: 10.25081/cb.2022.v13.7468
https://updatepublishing.com/journal/index.php/cb

Research Article

ISSN: 2220-4822

Intra and interspecific morphological variations of three species of *Capsicum* (Solanaceae)

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ABSTRACT

The widely cultivated pepper, *Capsicum spp*., important as a vegetable and spice crop worldwide, is one of the most diverse crops. Considerable morphological variation, especially in fruit shape, color, and size exist in the genus. The present study analysed the intra and interspecific morphological variations of three species of *Capsicum* (Solanaceae) using thirty nine morphological characters. Morphological variations with respect to vegetative, floral and fruit characteristics were observed among the members of the same species. Morphological data was used to obtain UPGMA derived dendrogram and the cluster analysis indicated genetic divergence among the three species. Present observations imply a great potential for chilli breeding through a hybridization programme or direct use of the varieties for the successful production of promising cultivars.

Received: January 10, 2022
Revised: October 16, 2022
Accepted: October 17, 2022
Published: November 01, 2022

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KEYWORDS: *Capsicum*, Intra-specific, Morphology, Solanaceae

INTRODUCTION

The genus *Capsicum* belongs to the family Solanaceae (Nightshade) and is one of the most commonly used spices, condiments and vegetables across the world. It is also an important cash crop for small and marginal farmers in many developing countries across Asia (China, India, Pakistan, Bangladesh, Thailand and Indonesia) and Africa (Egypt, Ethiopia, Nigeria and Ghana) (Rai *et al*., 2013). India is the largest producer of dry chilli fruits, accounting for more than 43 % of the world's total dry chilli production (FAOSTAT, 2011).

Currently, 38 species of Capsicum are reported. Of these, five *(C. annuum, C. frutescens, C. chinense, C. pubescens, and C. baccatum)* are thought to have been domesticated through at least five independent events. These domesticates are believed to be derived from three distinct genetic lineages, with *C. pubescens* and *C. baccatum* each representing independent lineages while the domesticated taxa *C. annuum, C. frutescens* and *C. chinense* are considered members of a species complex that were each independently derived from wild progenitors that may or may not be independent species (Kothari *et al*., 2010; Hill *et al*., 2013). *Capsicum* species are diploids with most of them having 24 chromosomes (n=x=12), but species with a genome sizes of 2n=2x=32 and 2n=2x=48 also have been reported (Wang & Bosland, 2006; Dafadar *et al*., 2012). It is a self-pollinated dicot plant. However, the occurrence of cross pollination leads to the formation of variants within the species. It is probably introduced by the Portuguese into Southern parts of India and cultivation spread out throughout India by the end of 19th century. Due to the long history of cultivation, selection and popularity of crops sufficient genetic variability has been generated. A rich diversity of *Capsicum* exists due to the varied geoclimatic regions of the Indian continent. Rich variability in morphological traits in hot pepper occurs throughout India, particularly in southern peninsular region, North Eastern foot hills of the Himalayas and Gangetic plains (Basu & De, 2003; Pradheep & Veeraragavathatham, 2006). The Indian germplasm is mainly represented by two species, *C. annuum* and *Capsicum frutescens*, with a number of varieties cultivated throughout the country and cultivated mainly in southern states of India nearer to the tropics where the climate is very favorable for *C. annuum*.

Collection and maintenance of the genetic diversity in *Capsicum* are important to avoid genetic erosion. Besides the identification of species, the characterization and evaluation of genotypes maintained in gene banks are of fundamental importance (Sudre *et al*., 2010). Genetic cataloguing based on standard descriptors helps to easily describe the morphological features of a genotype and thus helps the exchange of information about new genotypes.

Characterization and evaluation of germplasm are prerequisite for the utilization of the available diversity in the chilli improvement programme. Desirable parental combinations provide the basis for selection in the follow up hybrid breeding process for the exploitation of heterosis (Thul *et al*., 2009). The desirable parental combination can be identified on the basis of cluster analysis. To initiate any breeding work, it is necessary to assess the genetic variability present in the indigenous genotypes (Datta & Das, 2013). Genetic resources play a pivotal role in its economical utilization and desirable traits improvements. *Capsicum* species are traditionally identified by morphological descriptors or related traits. Morphological descriptors are considered essential for a more accurate germplasm characterization, such as the ones indicated by the IPGRI *et al.* (1995). Characterization and evaluation of the domesticated species of *Capsicum* are particularly interesting for gene bank curators, since a wide variability, not yet fully known and exploited, is available in these species. Genetic diversity studies of chilli accessions from different countries have been reported earlier (Rai *et al*., 2013; Hill *et al*., 2013). Previous reports are available on the genetic variability of *Capsicum* from different regions of India including Kerala (Manju & Sreelathakumary, 2002; Sreelathakumary & Rajamony, 2004). Fruit morphological features in five varieties of *C. annuum* in order to evaluate the reliability of these characters and their relevance to the taxonomic consideration of the *C. annuum* varieties were reported earlier (Zhigila *et al*., 2014). Hence the present study was conducted with the objective to contribute additional information on the genetic variability of *Capsicum* landraces from the Thiruvananthapuram district of Kerala to help the ongoing crop improvement efforts.

MATERIALS AND METHODS

The plant materials used for the present study include ten *Capsicum* varieties *viz*., *C. annuum*, *C. annuum (Guder)*, *C. chinense*, *C. chinense (round)*, *C. chinense (blue)*, *C. chinense (Small)*, *C. frutescens*, *C. frutescens (white)*, *C. frutescens (small) and C. frutescens (large)*. The experiment was conducted in January-May 2013. Healthy seeds of ten varieties were collected from different localities of Thiruvananthapuram district, Kerala, India and were sowed in plastic bags of 20L containing sand soil and cow dung (1:1) as ten seeds per bag. Three replicas were maintained for each variety. Healthy seedlings were transferred to pots after two weeks and maintained well. Morphological data based on twenty six qualitative (Table 1) and thirteen quantitative traits (Table 2) were taken from ten plants of each variety. Quantitative traits *viz*., Number of Seeds, Internodal length, Leaf length, Petiole length, Leaf width and Pedicel length were measured using a centimeter scale and the results were expressed as mean ± Standard error. Vegetative characters were evaluated a hundred days after planting, and reproductive characters were evaluated after the flowering and fruiting stage. A total of thirty nine traits were assessed based on different descriptor states (IPGRI *et al*., 1995) and each descriptor state was numerically coded and the coded data were tabulated and compiled. The coded morphological data were used to assess intra and interspecific variations among the species by means of cluster analysis. Cluster analysis was performed on the basis of UPGMA derived dendrogram based on Euclidean distance measures using the software MVSP.

Table 1: List of qualitative characters used for the morphological characterization of ten *Capsicum* varieties

Sl. No.	Descriptor name	Descriptor state
1	*Habit* Plant growth habit	1- Erect 2- Intermediate 3-Erect branched
2	*Stem* Stem colour	1-light green 2-green 3- Dark green 4-Yellow
3	Stem Shape	1-Quadrangular 2-Round
4	Nodes	1-Short 2- Long
5	Pubescence	0- Absent 1- Present
6	*Leaf* Leaf colour	1- light green 2- green 3-Dark green 4-bluish green
7	Leaf shape	1-Cordate 2- Long 3- Obtuse
8	Leaf surface	1-Coriaceous
9	Pubescence	0- Absent 1-Present
10	*Inflorescence* Number of flower axil	1-One 2-Two 3- Three or more 4-Many flower in branches but each in individual axis
11	Pedicel position at anthesis	1-Axillary 2-Terminal
12	*Flower* Calyx colour	1-Green 2-Greenish blue
13	Corolla colour	1-White 2-Yellow white 3- yellow 4- greenish yellow 5- blue
14	Corolla throat colour	1-Yellow 2- Yellow Green 3- Green 4-Greenish blue
15	Corolla shape	1-Companulate
16	*Androecium* Filament colour	1-Blue 2-Green 3-Yellow
17	Anther colour	1-Blue 2-Green 3-Bluish yellow 4-Blue green 5-Yellow
18	*Gynoecium* Shape of stigma	1-Capitate 2-Simple 3-Bifid
19	*Fruit* Fruit colour at immature stage	1-Light green 2-Dark green 3-Green brown 4-Blue green 5-Yellow
20	Fruit colour at mature stage	1-Dark green 2-Blue 3-Red 4-Brown
21	Fruit shape	1-Triangular 2-Elongate 3-Round
22	Fruit surface	1-Rough 2-Smooth
23	Fruit shape at pedicel attachment	1- Pulvinate 2-Lobate 3-Obtuse
24	Fruit position	1-Axillary 2-Nodes 3-Terminal 4-Terminal/Axillary
25	*Seeds* Seed colour	1-White 2-Yellow
26	Seed shape	1-Round 2-Half circle

RESULTS AND DISCUSSION

Capsicum varieties analysed in the present study showed considerable variations in both vegetative and reproductive characters (Tables 3, 4, 5 & 6). Qualitative characters showed variation between species however quantitative characters differed among members of the same species. Erect plant habit was observed in most of the varieties studied. The stem was green in colour, quadrangular and short in most with a few exceptions. *Capsicum chinense* (blue) possessed a pubescent stem, bluish green leaves and bluish green sepals. Internodal length showed considerable variation from 2.92 ± 1.2545

(*C. frutescens (large)* to 9.79 ± 2.1849 (*C. chinense (Small)*. The leaf surface was coriaceous in all varieties. Leaf shape was long, cordate or obtuse. Colour of corolla ranged from white to blue. Corolla throat colour also showed variations with respect to different varieties. The shape of corolla was campanulate in most. Anther colour ranged from yellow to blue in varieties under study. Fruit colour at immature and mature stages, shape, fruit surface and fruit length showed variation among members of the same species. The shape at pedicel attachment was also different among members of *C. chinense*. Seed colour was white except in *C. chinense* (blue) in which bluish white seeds were observed. The seed shape was round except in *C. annuum* in which it was half circle. The number of seeds ranged from 12.4±0.7266 (*C. frutescens (large)* to 88.4 ± 0.3765 (*Capsicum chinense* (blue).

Table 2: List of quanitative characters used for the morphological characterization of ten *Capsicum* varieties

Sl. No.	Descriptor name	Descriptor state
1	Plant height	< 25
		25-45
		46-65
		66 – 85
2	Internodal length (cm)	4 – 6
		6-8
		8 – 10
3	Length of petiole	< 2.5
		2.5 – 5.5
		5.5 – 7.5
		7.5 – 8.5
4	Leaf length (cm)	<6
		6.1-12>12
5	Leaf width (cm)	1-5
		5.1-10
6	*Flower*	0.5-2
	Length of pedicel (cm)	2-4
7	Number of Sepals	1. 5
8	Corolla length (cm)	1. 3
		2. 5
8	Number of filaments	1. 5
9	Anther length(mm)	1. 2
10	Fruit length (cm)	1. <2.9
		2. 3.0– 5.9
		3. 6 – 8.9
11	Fruit width (cm)	1. <2.5
		2. 2.5-5
		3. 5 – 7.5
		4. 7.5 – 8.5
12	Number of Locules	1. 2
		2. 3
		3. 4
13	Number of seeds	1. 10 – 25
		2. 25-50
		3. 50-75
		4. 75-100

UPGMA derived dendrogram grouped the samples into two major clusters (Figure 1). Cluster I grouped *C. chinense* (round) *C. chinense* (blue) together. All the other samples were grouped in Cluster II. The second major cluster was further divided into two main clusters Cluster II A and Cluster II B. Cluster II A grouped all the *frutescence* samples together while Cluster II B grouped *C. annuum* and the rest of the *C. chinense* samples separately.

The *Capsicum* varieties analysed in the present study were highly variable with respect to morphological characters as reported earlier. Flower morphology, such as flower color, calyx constriction, and number of flowers per node, is among the mostly used taxonomic descriptors in identification studies of *Capsicum*. White-flowered species include the domesticated species *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., and *C. frutescens* L. Purple-flowered species include one domesticated (*C. pubescens* R. and P.) and three undomesticated species (*C. eximium* A.T. Hunz, *C. cardenasii* Heiser & Smith, and *C. tovarii* Eshbaugh, Smith & Nickrent) (Thul *et al*., 2011). In the present study, *C. frutescens* corolla was yellow coloured and blue in *C. chinense* (blue). A clear difference was observed for different species in relation to the flower color in the earlier reports also (Thul *et al*., 2009).

In the present study, fruit characters seem to be more discriminating among accessions. The genus *Capsicum* (sweet and hot pepper) showed intra- and inter-specific diversity in fruit type, colour and shape as in the previous studies (Zhigila *et al*., 2014). Fruit shapes can be used for the classification of the varieties and cultivars of *Capsicum annuum* as reported earlier (Zhigila *et al*., 2014). Fruits form as the result of the development and differentiation of the gynoecium after fertilization. They are therefore the product of late morphological and structural modifications in the carpels. A considerable degree of divergence was reported at intergenetic stock (between genotypes), intercluster (between clusters), and intracluster (within cluster) levels of diversification in *C. annuum* and other related species (Thul *et al*., 2011). Fruit diameter, number of fruits per plant, and leaf diameter played the greatest role in differentiation at intercluster and intergenotype level (Yatung *et al*., 2014).

Table 3: Morphological characterization of three *Capsicum* species based on qualitative traits (Stem, Leaf)

Species	Plant habit	Height (cm)	Stem				Leaf			
			Colour	Shape	Nodes	Pubescence	Colour	Shape	Surface	Pubescence
C. annuum	Erect	40-60	Light green	Quadrangular	Long	-	Dark green	Long	Coriaceous	-
C. annuum (Guder)	Erect	50 – 60	Green	Quadrangular	Long	-	Green	Cordate	Coriaceous	-
C. chinense	Erect	45 – 60	Light green	Quadrangular	Long	-	Light green	Cordate	Coriaceous	-
C. chinense (blue)	Erect	50 – 60	Yelow	Round	Short	Pubescent	Bluish green	Obtuse	Coriaceous	Pubescent
C. chinense (round)	Intermediate	20-40	Dark green	Round	Short	-	Dark green	Long	Coriaceous	Pubescent
C. chinens e (Small)	Erect	45 – 60	Light green	Quadrangular	Long	-	Light green	Cordate	Coriaceous	-
C. frutescens	Erect branched	45 – 55	Green	Quadrangular	Short	-	Dark green	Cordate	Coriaceous	-
C. frutescens (white)	Erect	40 – 50	Green	Quadrangular	Short	-	Dark green	Long	Coriaceous	-
C. frutescens (small)	Erect	20 – 30	Green	Quadrangular	Short	-	Dark green	Cordate	Coriaceous	-
C. frutescens (large)	Erect	20 – 50	Green	Quadrangular	Short	-	Dark green	Cordate	Coriaceous	-

Table 4: Morphological characterization of three *Capsicum* species based on qualitative traits (Inflorescence, Calyx, Corolla, Androcieum, Gynocieum)

Species	Inflorescence		Calyx		Corolla				Androcieum			Gynocieum
	No. of flower per axil	Pedicel positon at anthesis	Colour	No. of sepals	Colour	Throat colour	Shape	Length (cm)	No. of filaments	Colour	Length (cm)	Shape of stigma
C. annuum	1 – 2	Axillary	Green	5	White	Green	Campanulate	5	5	Green	2	Capitate
C. annuum (Guder)	2-3	Axillary	Green	5	Yelow	Green	Campanulate	3	5	Green	2	Capitate
C. chinense	2 – 3	Terminal	Greenish blue	5	Yellow white	Green	Campanulate	5	5	Bule green	2	Simple
C. chinense (blue)	1 – 2	Terminal	Green	5	Blue	Greenish blue	Campanulate		5	Dark green	2	Simple
C. chinense (round)	3 – 5	Axillary	Green	5	Green yellow	Yellow green	Campanulate	5	5	Yellow	2	Simple
C. chinense (Small)	2-3	Terminal	Green	5	Yelow white	Green	Campanulate	5	5	Blue green	2.1	Simple
C. frutescens	3	Axillary	Green	5	Yelow	Yellow	Campanulate	3	5	Blue	2	Bifid
C. frutescens (white)	2-3	Terminal/ Axillary	Green	5	Yelow	Green	Campanulate	3	5	Bluish yellow	2	Bifid
C. frutescens (small)	2 – 3	Axillary	Green	5	Yelow	Yellow	Campanulate	3	5	Blue	2	Bifid
C. frutescens (large)	1-3	Axillary	Green	5	Yelow	Yellow	Campanulate	3	5	Blue	2	Bifid

Table 5: Morphological characterization of three *Capsicum* species based on qualitative traits (Fruit, Seed)

Species	Fruit								Seed	
	Colour at immature stage	Colour at mature stage	Shape	Length (cm)	Surface	Shape at pedicel attachement	No. of locules	Position	Colour	Shape
C. annuum	Light green	Dark green	Elongate	8.5	Rough	Pulvinate	3	Nodes	White	Half circle
C. annuum (Guder)	Yellow	Yellow	Triangular	8.5	Smooth	Pulvinate	2	Axillary	White	Round
C. chinense	Light green	Dark green	Triangular	4.5	Rough	Lobate	2	Terminal	White	Round
C. chinense (blue)	Blue	Blue green	Triangular	2.5	Rough	Obtuse	2	Terminal	Whitish blue	Round
C. chinense (round)	Green yellow	Dark green	Round	1.4	Smooth	Pulvinate	4	Terminal	White	Round
C. chinense (Small)	Light grreen	Dark green	Triangular	4.5	Rough	Obtuse	2	Terminal	White	Round
C. frutescens	Light green	Dark green	Triangular	1.5	Rough	Pulvinate	2	Axillary	White	Round
C. frutescens (white)	Yellow	Red	Triangular	5.5	Rough	Pulvinate	2	Terminal/ Axillary	White	Round
C. frutescens (small)	Light green	Dark green	Short	6	Smooth	Pulvinate	2	Axillary	White	Round
C. frutescens (large)	Light grreen	Dark green	Triangular	1.6	Rough	Pulvinate	2	Nodes	White	Round

Table 6: Morphological observations on quantitative traits of three *Capsicum* species

Name of variety	Number of Seeds (Mean ± SE)	Length of internodes (cm, Mean ± SE)	Length of leaves (cm, Mean ± SE)	Length of petiole (cm, Mean ± SE)	Leaf width (cm, Mean ± SE)	Length of pedicel (cm, Mean ± SE)
C. annuum	18 ± 0.7483	4.86 ± 0.3014	9.04 ± 0.6029	2.26 ± 0.0963	7.08 ± 0.7845	1.08 ± 0.8492
C. annuum (Guder)	36.6 ± 0.5899	5.64 ± 1.7889	8.86 ± 0.2325	3.12 ± 0.2528	4.08 ± 0.1559	1.46 ± 0.3418
C. chinense	20.4 ± 0.4117	3.88 ± 1.6063	7.46 ± 0.6703	2.32 ± 0.9020	7.02 ± 0.7317	1.2 ± 0.0489
C. chinense (blue)	88.4 ± 0.3765	5.68 ± 0.6851	5.32 ± 0.2551	1.2 ± 0.0489	2.94 ± 0.2342	2.5 ± 0.1232
C. chinense (round)	32.8 ± 0.6588	7.26 ± 2.0744	11.0 ± 0.6212	2.6 ± 0.1469	4.66 ± 0.1991	3.18 ± 0.5715
C. chinense (Small)	31.4 ± 0.5366	9.79 ± 2.1849	14.68 ± 0.4951	1.16 ± 0.1436	5.24 ± 0.1661	2.12 ± 0.9717
C. frutescens	33 ± 0.7975	9.78 ± 2.2454	14.7 ± 0.0628	1.16 ± 0.2296	4.94 ± 0.0829	2.12 ± 0.6343
C. frutescens (white)	39.2 ± 0.1099	7.84 ± 0.8851	8.78 ± 0.2440	3.04 ± 0.1522	5.9 ± 0.1938	2.12 ± 0.4037
C. frutescens (small)	33.2 ± 0.5214	3.42 ± 2.5020	9 ± 0.1370	8.2 ± 0.3346	3.92 ± 0.1579	1.2 ± 0.0489
C. frutescens (large)	12.4 ± 0.7266	2.92 ± 1.2545	5.72 ± 0.1774	1.36 ± 0.1332	6.22 ± 0.2249	1.08 ± 0.8989

In the present study, accessions of *C. annuum* and *C. frutescence* showed a clustering pattern indicating a single lineage for each. *C. chinense* accessions were grouped into two subclusters. In the previous reports, also all the accessions of *C. annuum* were grouped together indicating a single lineage and all the other accessions of different species were formed separate clusters apart from the major *C. annuum* cluster, and the accessions of *C. frutescens* and *C. chinense* formed another subcluster (Thul *et al*., 2011).

Crop improvement is made by generating variability in desired traits followed by selection. Continued success in crop improvement can only be realized when new substantial variability is found and used in a population. The divergence between any two parents expresses the allelic differences between them (Yatung *et al*., 2014). The genotypes grouped into the same cluster presumably diverge very little from one another. Crossing of genotypes belonging to the same cluster is not expected to yield desirable segregants. Consequently, a

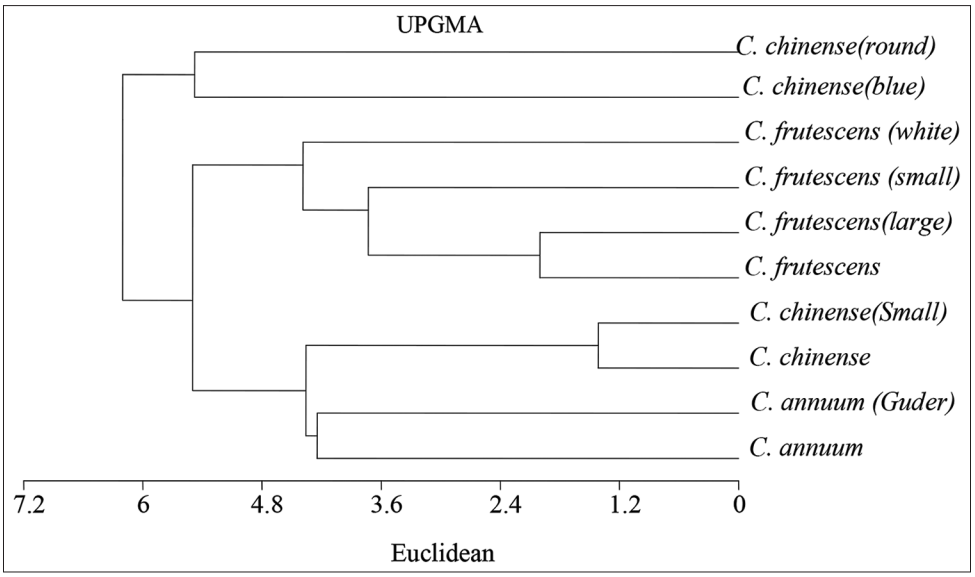

Figure 1: UPGMA derived dendrogram

crossing programme should be conducted with putative parents belonging to different characters. Therefore, crosses between the members of clusters separated by inter-cluster distances are likely to be beneficial for further improvement (Yatung *et al*., 2014).

CONCLUSIONS

From this study, it may be concluded that a wide range of variations for almost all morphological traits is present in this crop. This implies a great potential for breeding through a hybridization programme or direct use as a variety for successful chilli production. Further, one or two promising genotypes from different clusters may be chosen for further genetic studies either by way of diallel or line x tester analysis.

Significance Statement

The present study observed considerable morphological variations among the members of the same species in the genus *Capsicum* which reveals greater potential for the varieties under study to be used directly in crop improvement efforts.

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