



DIVERSITY STUDY IN *Capsicum* USING NUMERICAL TAXONOMY

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SUMMARY

The taxonomy of capsicum species is difficult since it displays variation especially in fruit morphology, which is a major economic component. The objective of this study was to evaluate the diversity among 60 landrace accessions of *Capsicum annuum* based on morphological traits and to determine the principal traits and their contribution to genetic variability. The accessions were analyzed for 35 qualitative and quantitative descriptors. The phenotypic divergence and relative importance of the traits were estimated by multivariate analysis. PCA analysis identified 14 morphological traits which can be used as minimum descriptors. Fruit related traits revealed highest correlation with the minimum descriptors hence can be used as principle characters for characterizing and divergence study in the *Capsicum annuum*.

Key words: Morphological diversity, genetic divergence, phylogenetic relationship, principal component analysis, minimum descriptors

Key findings: Minimum descriptors (including both qualitative and quantitative traits) were used to characterize capsicum germplasm. Fruit related traits play a major role in explaining capsicum diversity.

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INTRODUCTION

Chilli (*Capsicum annuum* L.) is a member of the *Solanaceae* family, grown for its pungent fruits, which are used both green and ripe/dry form. It is one of the most important spice crops worldwide, with a global production of 23.9 million tons for fresh chili and 2.5 million tons of dry chilli and area harvested 3.5 million ha. India contributes one fourth of world production of capsicum with an average annual production of 0.9 million tons from an area of 0.885 million hectares with a productivity of 1266 kg per hectare. (FAOSTAT, 2012). Though the plant is of Mexican origin (Pickersgill, 1979), it exhibits

extensive diversity in India, especially in the hot pepper types (Singh and Singh 1976). Chillies are grown throughout the year in India, especially *Capsicum annuum*, which has been extensively, studied (Deshpande, 1993). A large number of landraces are popular in different regions of the country besides; several advanced cultivars have been developed through selection and hybridization.

The taxonomic identity of capsicum species is difficult since it displays variations especially in fruit morphology, which is an economically major component of this species. Many researchers (Eshbaugh, 1970; Ince *et al.*, 2010; Walse *et al.*, 2001; Thul *et al.*, 2009) attempted to classify it using various

parameters ranging from morphological characters to molecular markers. Germplasm characterization is important for conservation and utilization of plant genetic resources (Thul *et al.*, 2012). Information regarding genetic diversity and genetic relationships among different genotypes is valuable in crop improvement. Analysis of genetic diversity is useful in selecting diverse parental combinations, reliable classification of accessions, and for exact identification of variety. Use of multivariate statistical algorithms is considered as an important strategy to quantify genetic similarity. Multivariate techniques permit standardization of multiple types of information of a set of characteristics (Podani and Schmera, 2006). The most widely used methods are principal component and canonical variable analysis, as well as clustering methods (Mohammadi and Prasanna, 2003; Sudre *et al.*, 2007). The principle of clustering methods is to represent genotypes in groups, so that there is uniformity within and heterogeneity among groups. These methods depend on previous estimates of dissimilarity measures derived from discrete and continuous (or categorical) variables. The most widely-used dissimilarity distance for continuous variables is the Euclidian distances. Works assessing the genetic distance in populations of plants have been successfully used to separate them within germplasm banks, as well as to identify those plants with higher potential in hybridization tests or studies of their evolution (Arunachalam, 1981).

Bio-resources are the base for several industries contributing to the national economy and human well-being. Continuous hybridization and crossing systems have narrowed the genetic variability in cultivated crop species reducing number of exploitable traits. Replacement of landraces, wild species and other primitive types of crop species due to high yielding modern cultivars is also a treat to plant genetic resources. According to Brown *et al.* (1989), preservation of loss of genetic diversity is possible when genetic or germplasm resource is realized as the most precious asset in conserving genetic diversity. This investigation was formulated to realize the values and to study the relationship among the cultivated *Capsicum annum* genotypes grown in different parts of India using numerical taxonomy. According to Mohammadi *et al.* (2010) principal component

is reduced data forms which clarify the relationship between breeding materials into interpretable fewer dimensions to form new variables. These new variables are visualized as different non correlating groups. On the basis of this an attempt has been made to identify minimum descriptors for the cultivated capsicum species.

MATERIALS AND METHODS

The experimental material consisted of 60 landraces of *capsicum annum* collected from different parts of India (Table 1). The genotypes were raised during *kharif* seasons of 2008 and 2009 at the Center for Protected Cultivation and Technology at Indian Agricultural Research Institute (IARI). The material was replicated thrice and all the agronomic practices were followed to raise a good crop.

Plant morphological traits

Morphological traits were divided in to 5 groups based on plant parts, each with 7 characters (Table 2). Ten competitive plants were randomly selected from each genotype in each replication to record the data following UPOV Test Guidelines (2006). The scores or weightage as recommended for each of the qualitative character in the capsicum descriptors and actual numerical data for the quantitative characters were used for morphological study.

Diversity analysis

A hierarchical cluster analysis, coefficient of variation on the unweighted pair group method based on arithmetic average (Sneath and Sokal, 1973) was carried out using Numerical Taxonomy and Multivariate Analysis System (NTSYS-PC) Version 2.11 statistical software (Rohlf, 2000). The principal component analysis (PCA) was performed following the method described by Sneath and Sokal (1973). The statistical computation for PCA was performed using the SPSS for MS Windows Release version 7.5 statistical software package. Selection of minimum descriptors was based on principal component analysis. The mean values from the highest and the lowest eigenvectors in the PCs were used as

thresholds for the selection of the most contributing variables, as suggested by Fundora *et al.* (1992) and trait association was studied following Manzano *et al.* (2001). The correlation between Euclidean distance

coefficients obtained from minimum descriptor traits and those obtained from all morphological traits in groups included in the study was carried out using the Mantel test (Mantel, 1967).

Table 1. List of capsicum genotypes evaluated for morphological variation.

No.	Accession number	Collection site	No.	Accession number	Collection site
1	SSTC-1	IARI, RS Katrain	31	SSTC-31	DARL, Almora
2	SSTC-2	IARI, RS Katrain	32	SSTC-32	IARI, RS Katrain
3	SSTC-3	IARI, RS Katrain	33	SSTC-33	IARI, RS Katrain
4	SSTC-4	IARI, RS Katrain	34	SSTC-34	IARI, RS Katrain
5	SSTC-5	Lam, Guntur (A P)	35	SSTC-35	IARI, RS Katrain
6	SSTC-6	Lam, Guntur (A P)	36	SSTC-36	Dr. YSPUniv. of A & F, Solan
7	SSTC-7	Lam, Guntur (A P)	37	SSTC-37	PAU Ludhiana
8	SSTC-8	UAS, Dharwad, KA	38	SSTC-38	UAS, Dharwad, Karnataka
9	SSTC-9	UAS, Dharwad, KA	39	SSTC-39	IARI, RS Katrain
10	SSTC-10	IIHR, Bangalore	40	SSTC-40	Almora
11	SSTC-11	UAS, Dharwad, KA	41	SSTC-41	IARI, RS Katrain
12	SSTC-12	IARI, RS Katrain	42	SSTC-42	Jaipur
13	SSTC-13	IARI, RS Katrain	43	SSTC-43	Jaipur
14	SSTC-14	IARI, RS Katrain	44	SSTC-44	Jaipur
15	SSTC-15	IARI, RS Katrain	45	SSTC-45	Jaipur
16	SSTC-16	IARI, RS Katrain	46	SSTC-46	Solan
17	SSTC-17	IARI, RS Katrain	47	SSTC-47	IARI, RS Katrain
18	SSTC-18	IARI, RS Katrain	48	SSTC-48	IARI, RS Katrain
19	SSTC-19	Lam, Guntur (A P)	49	SSTC-49	IARI, RS Katrain
20	SSTC-20	Lam, Guntur (A P)	50	SSTC-50	IARI, RS Katrain
21	SSTC-21	Lam, Guntur (A P)	51	SSTC-51	IARI, RS Katrain
22	SSTC-22	Lam, Guntur (A P)	52	SSTC-52	IARI, RS Katrain
23	SSTC-23	Lam, Guntur (A P)	53	SSTC-53	IARI, RS Katrain
24	SSTC-24	Panth nagar	54	SSTC-54	IARI, RS Katrain
25	SSTC-25	Jaipur	55	SSTC-55	IARI, RS Katrain
26	SSTC-26	Jaipur	56	SSTC-56	IARI, RS Katrain
27	SSTC-27	Jaipur	57	SSTC-57	IARI, RS Katrain
28	SSTC-28	IARI, RS Katrain	58	SSTC-58	IARI, RS Katrain
29	SSTC-29	CSK HP AU, Palampur	59	SSTC-59	IARI, RS Katrain
30	SSTC-30	IARI, RS Katrain	60	SSTC-60	IARI, RS Katrain

Table 2. Morphological characters under study in groups

Group I (Leaf characters)	Abbrev	Group II (plant stem characters)	Abbrev	Group III (Fruit Colour and shape)	Abbrev	Group IV (Fruit size etc)	Abbrev	Group V (Duration and others)	Abbrev
Leaf: length of blade	LPCS	Seedling: anthocyanin coloration of hypocotyls	SACH	Fruit anthocianin coloration	FAC	Fruit: length	FL	Plant habit	PHB
Leaf: width of blade	LB	Varieties with shortened internodes only:	VSI	Fruit attitude	FTS	Fruit: diameter	FD	Peduncle: attitude	PA
Leaf: undulation of margin	LUM	Plants with shortened internodes only	PSI	Fruit: color (before maturity)	FCBM	Fruit: ratio length/diameter	FRL/B	Time of beginning of flowering	TF

Table 2 (cont'd). Morphological characters under study in groups

Group I (Leaf characters)	Abbrev	Group II (Plant stem characters)	Abbrev	Group III (Fruit colour and shape)	Abbrev	Group IV (Fruit size etc)	Abbrev	Group V (Duration of maturity and others)	Abbrev
Leaf: blistering	LB	Plant: anthocyanin coloration of nodes	PACN	Fruit: texture of surface	FG	Fruit: sinuation of pericarp at basal part	FSA	Time of maturity	TM
Leaf: profile in cross	LPCS	Stem: hairiness of nodes	VSI	Fruit: color (at maturity)	FCM	Fruit sinuation of pericarp excluding basal part	FSPBP	Stem: hairiness of nodes	SHN
Leaf: glossiness	LG	Length of internodes	SIACN	Fruit: shape of apex Fruit:	FA	Fruit: glossiness Fruit	FSA	Flower: anthocyanin coloration in anther	FACA
Leaf: intensity of green color	LIGC	Stem: intensity of anthocyanin coloration of nodes	SIACN	Fruit shape in longitudinal section	FSPEXBP	Fruit: number of locules	FNL	Plant: height	PH

RESULTS

In this study the Euclidean coefficient of distance, based on 35 morphological traits among 60 capsicum accession revealed limited genetic variation among the capsicum species, with a minimum value of 2.65 (between SSTC-54 and SSTC-55) and are closet relative

and a maximum value of 15.24 (between SSTC20 and SSTC43) and are most diverse in terms of Euclidean distance value. A dendrogram based on Euclidean coefficient of distances was constructed to study the genetic relationship among the 60 genotypes (Figure 1).

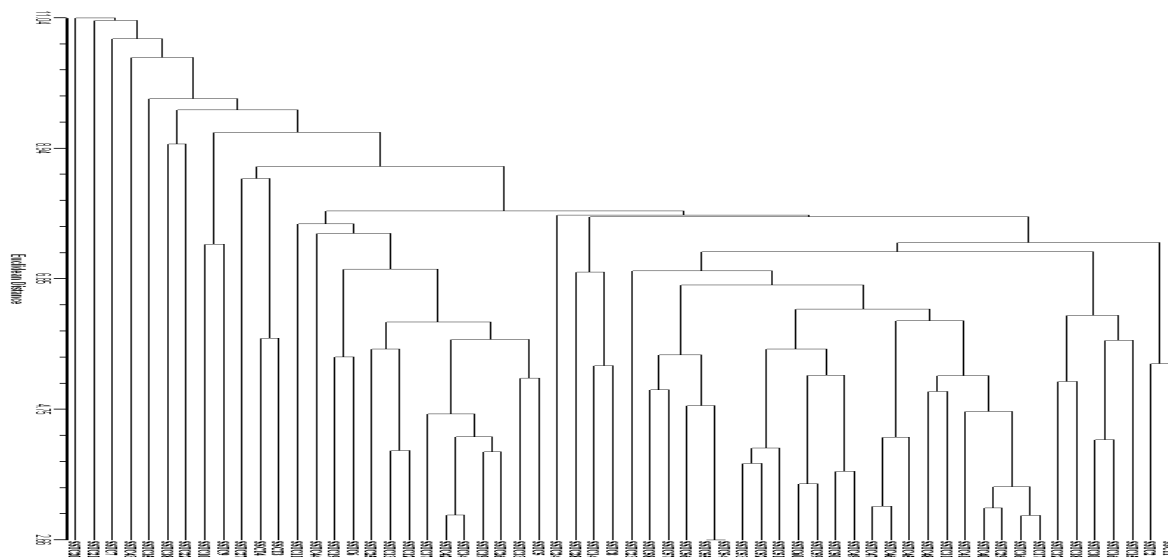


Figure 1. Dendrogram based on 35 morphological characters.

Standardized trait values were subjected to principal component analysis (PCA) to determine the traits most effective in discriminating accessions. The first component of PC contributed 16.92% of total variability and total variability contributed by first four PC was 42.53% (Table 3). The first two components showing the maximum variance were selected for the ordination analysis. To remove the redundancy and to reduce the observed variables into a smaller number that will account for most of the variance, a set of 14 characters with eigenvectors values more than 0.5 were chosen (Table 3). Identified characters are leaf-length of blade, leaf-glossiness, time of beginning of flowering, fruit-texture of surface, fruit length/diameter ratio, fruit sinuation of pericarp at basal part, fruit sinuation of pericarp excluding basal part, number of locules per fruit, fruit diameter, fruit attitude, fruit shape in longitudinal section, intensity of anthocyanin coloration of nodes in stem and fruit color (before maturity).

To determine the principle group of characters which best represent the variability and genetic relationship, Mantel test of correlation was done between the Euclidean

coefficient of distance matrices of 5 sets of traits with Euclidean coefficient distance metrics the 14 set of chosen traits using same software. The fourth group of morphological characters with fruit size related trait revealed highest correlation with 14 principal characters contributing towards total variability (Table 4). Hence were selected to study the genetic distance and relationship of the tested genotypes.

The Euclidean coefficient of distance, based on 7 fruit related traits among 60 capsicum accession ranged from of 0.026 (between SSTC-54 and SSTC-55) to of 7.94 (between SSTC20 and SSTC43) among the capsicum accession. To illustrate the evolutionary relationship a phylogenetic tree was constructed in the form of dendrogram which clearly grouped the genotypes in 2 major clusters (Figure 2). The cluster-I consist of 3 subgroups, with where cluster I-a with 7 genotypes, cluster I-b with 12 genotypes and cluster I-c with 5 genotypes. Cluster II also consist of 3 subgroups with II-b with highest number of 21 genotypes cluster II-a with 7 genotypes and cluster II-c with 8 genotypes.

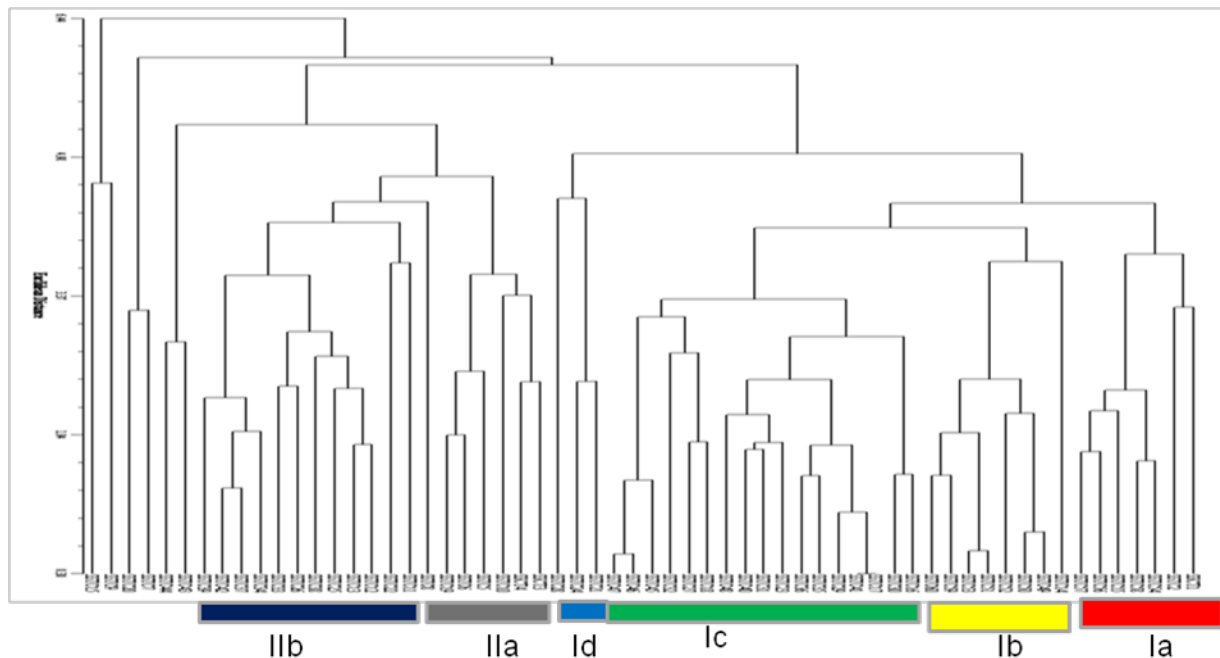


Figure 2. Dendrogram based on 14 morphological characters.

Table 3. The eigenvectors indicating the contribution of 35 characters.

Characters	C1	C2	C3	C4
SACH	0.276	0.191	0.329	0.274
PSI	-0.054	-0.288	-0.028	-0.053
VSI	0.212	0.239	0.188	0.038
PACN	-0.166	-0.100	0.192	0.214
LIGC	-0.557	-0.458	-0.090	0.492
LS	-0.246	-0.215	0.314	-0.377
PA	0.355	0.200	-0.239	0.172
FACA	0.194	0.237	0.092	-0.249
FAC	0.115	0.431	0.225	0.457
FA	-0.348	-0.429	-0.081	0.008
FCBM	-0.084	0.480	0.267	0.582
FSLs	0.768	-0.083	-0.219	0.063
FTS	-0.353	0.600	-0.441	-0.182
FCM	-0.025	0.440	-0.138	-0.113
FSA	-0.706	0.116	-0.134	-0.110
PH	-0.044	0.463	0.452	-0.138
LIN	0.125	-0.173	-0.047	0.280
SIACN	0.240	-0.256	-0.520	-0.005
SHN	0.055	0.200	0.199	0.085
PH	0.216	0.212	-0.109	0.316
LL	-0.256	-0.005	0.427	-0.261
LW	-0.284	-0.209	0.320	-0.272
LUM	0.044	-0.045	0.064	0.046
LB	-0.485	-0.294	0.438	-0.012
LPCS	-0.706	-0.016	0.305	-0.239
LG	-0.563	-0.512	-0.320	0.230
FL	0.135	-0.130	-0.059	-0.489
FD	-0.827	0.305	0.132	-0.007
FRL/B	0.695	-0.364	-0.131	-0.320
FSPBP	-0.394	0.389	-0.550	-0.101
FSPEXBP	-0.426	0.661	-0.394	-0.199
FG	-0.563	-0.512	-0.320	0.230
FNL	-0.818	0.180	-0.006	0.119
TF	-0.024	0.201	-0.608	-0.063
TM	-0.225	-0.067	-0.478	-0.006
% Contribution	16.922	10.49	9.033	6.0929
%cumulative contribution	16.9223	27.4126	36.4456	42.5385

Table 4. Mantel tests for association Euclidean distance all characters vs other group characters.

Tests for association Euclidean distance (all characters) vs Euclidean distance of groups of characters	Matrix correlation: r (= normalized Mantel statistic Z)	Approximate Mantel t -test: t	Prob. random $Z <$ obs. Z : p
Fruit size related characters	0.77570*	17.0735	1.0000
Fruit color	0.55753	8.3481	1.0000
leaf character	0.22352	4.7269	1.0000
plant stem character	0.13923	2.1566	0.9845
duration and others	0.12730	2.0528	0.9800

DISCUSSION

Assessment of diversity using morphological descriptors

Diversity in plant genetic resources provides opportunity for plant breeders to develop new

and improved cultivars with desirable characteristics, which include both farmer-preferred traits and breeders preferred traits. A comprehensive study of the genetic variation present in germplasm is useful for determining whether morphology based taxonomic classifications reveal patterns of genomic

differentiation (Thul *et al.*, 2009). In this study the dendrogram produced indicated 35 morphological traits resulted in sequential and complicated merging of clusters at different levels of genetic distances, which complicates the analysis of relationship (Figure 1).

Assessment of minimum descriptor traits

To resolve the issue and to integrate the information regarding genotypic information, genetic diversity and ancestral origin, standardized trait values were subjected to principal component analysis (PCA) to determine the traits most effective in discriminating accessions. Principal component analysis is a variable reduction procedure. It is a statistical technique used to analyze the interrelationships among a large number of variables and to explain these variables in terms of a smaller number of variables, called principal components, with a minimum loss of information. In this investigation to remove the redundancy and to reduce the observed variables into a smaller number that will account for most of the variance, a set of 14 characters with eigenvectors values more than 0.5 were chosen (Table 3) (Neto *et al.* 2003). These can be used as minimum descriptor traits to study the diversity of *capsicum annum*. This is in accordance with Rego *et al.* (2003), that when there is no replicated data, the genetic divergence and the pinpoint of characters should be calculated based on the principal component scores to avoid discarding of some important variables when determining divergence. Similar results were observed by Lefebvre *et al.* (1993) and Prince *et al.* (1992) also during diversity study with capsicum.

Principal morphological characters for genetic distance analysis

UPOV guidelines for testing of capsicum genotypes recommended 10 different characters for grouping of characteristics. Out of these 5 morphological traits are: seedling-anthocyanin coloration of hypocotyls, plant-shortened internode (in upper part), fruit: color (before maturity), fruit-shape in longitudinal section, fruit-color (at maturity) (UPOV 2006). In this study, the Mantel test of correlation revealed the fourth group of morphological characters with fruit size related trait is highly

correlated with 14 principal characters contributing towards total variability (Table 4). All 36 genotypes in the cluster II are with green fruit color before maturity, and out of 24 genotypes in cluster I, the 7 genotypes in cluster I-a exhibited green colour and rest with different fruit colours ranging from yellow, orange, red and brown. Rego *et al.* (2011) reported that similar results identifying traits which can be discarded on the basis of correlation between the variables.

There are reports on extensive diversity in fruit shape, size, wall thickness and fleshiness, colour and pungency (Andrews, 1995; IPGRI 1995), determined by genetic and environmental factors in capsicum species. Among the innumerable varieties of *C. annum*, the diversification of shapes of the fruit is striking (Wang and Bosland, 2006). Previous works showed that the selection for fruit width results in gain for fruit weight (Gopalakrishnan *et al.*, 1985; Shifriss *et al.*, 1989). These studies indicate the importance of fruit size related traits in explaining the genetic variability. Distribution of genetic diversity in a plant species depends on its evolution and breeding system, ecological and geographical factors and often on human activities. As the maximum diversity is contributed by fruit related traits. Capsicum growers have been manipulating the crop due to migration and trade, causing a steady gene flow among different geographical areas as the variability is found in fruit related characters. Therefore, on the basis the present study reveals that the most important factor affecting the current genetic structure in capsicum is human activities. However, this study was based on only 60 capsicum accessions, with a limited number of traits. Future research strategies should focus on use of large number of genotypes with more number of traits to get a clear picture of genetic relationship among the genotypes.

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