

Genetic Variability in Germplasm Accessions of *Capsicum annuum* L

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ABSTRACT

Capsicum annuum is the most widely cultivated species of peppers (chilies) in the world. For culinary purposes, its fruits are used for pungency (capsaicin) and also color (capsanthin). Capsaicin is also used for medicinal purposes particularly in anti-inflammatory formulations. Genetic divergence among 38 accessions collected from diverse locations in India (28 from Uttar Pradesh, 5 from Assam, 3 from Maharashtra and 2 from Uttaranchal), was estimated from the data pooled over 3 consecutive years for 15 morphological, growth and chemotypic characters that included days to first and second flowering, fruit onset, plant height, primary, secondary and tertiary branches, leaf surface area, fruit length and diameter, fruit surface area, fresh and dry fruit weight, capsaicin and capsanthin content. Based on this characterization the plants could be grouped into 7 clusters wherein substantial diversity among accessions was indicated by the wide range of \bar{D}^2 values (752.901 - 1918683.00). Accessions with distinct identity were marked, which are likely to be quite suitable for breeding through hybridization combining desirable traits. The accessions labeled number 38, 27, 26, 14 and 24 to high capsaicin content (%); 35, 23, 3, 16, 29 and 11 for high capsanthin content (%) and 26 and 27 for dual purpose had characteristics desirable. Above accessions could be utilized in hybridization programme for *C. annuum* crop improvement.

Keywords: Genetic Diversity, *Capsicum Annuum*, Capsaicin and Capsanthin Content, Recombination Breeding, Geographical Distribution

1. Introduction

Capsicum is the unique genus of the family Solanaceae finding diverse uses from nutritional and culinary to pharmaceutical uses. In this genus, native to the Americas, more than 30 species have been described, but only five of them, *Capsicum annuum* var. *annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* var. *pendulum*, and *C. pubescens* are considered to be domesticated (Pandey, Pozzobon *et al.*, 2006; Moscone *et al.*, 2007) [1-3]. Pungent peppers commonly known as chilies in India belong to the species *C. annuum* L. which is the most widely cultivated species in the world. Both hot and sweet peppers have been reported to have originated from *C. annuum* [4].

The pungent chemical principle of *Capsicum* is capsaicin, which is synthesized and accumulated specifically in fruits. The capsaicin has found use in a wide range of pain problems, including post-mastectomy syndrome, urticaria, psoriasis, diabetic neuropathy, arthritis, pruritis, contact allergy, post-surgical neuromas etc. [5]. The sweet

pepper on the other hand is one of the richest sources of carotenoids. The ketoxanthophylls, capsanthin and capsorubin are the major pigments contributing to the red color of *Capsicum*, while β -carotene and zeaxanthin are responsible for the yellow-orange colors [6].

The characterization and the evaluation of the *Capsicum* domesticated species are particularly interesting for gene bank curators, since a wide variability, not yet fully known and exploited, is available in these species (Guzmán *et al.* [7], 2005; Sudré *et al.* [8], 2006; Ince *et al.* [9], 2009). In this study, genetic variability for diverse traits in available germplasm collections assembled from different places in India were evaluated as a prelude to crop improvement.

2. Materials and Methods

Genetic divergence among 38 indigenous accessions of *Capsicum annuum* collected from various geographical locations (**Table 1**) in India was studied. The plants were grown in randomized block design at the research farm

Table 1. Accessions/collections of *Capsicum annuum* and their geographic origin.

Accession No.	Place of collection/acquisition
1	Lucknow, U.P.
2	"
3	"
4	"
5	"
6	"
7	"
8	"
9	"
10	"
11	"
12	"
13	"
14	"
15	"
16	"
17	"
18	Ramnagar, Uttranchal.
19	Pantnagar, Uttranchal.
20	Lucknow, U.P.
21	"
22	"
23	Nagpur, Maharashtra.
24	Assam.
25	Assam.
26	Assam.
27	Assam.
28	Assam.
29	Lucknow, U.P.
30	"
31	"
32	"
33	"
34	"
35	"
36	"
37	Pune, Maharashtra.
38	Pune Maharashtra.

of CIMAP, Lucknow, India during 1998, 1999 and 2000 with two replicates in each year. Each treatment consisted of single row 45 cm long and 60 cm apart. The

plant received normal intercultural operations, irrigations and fertilizer applications (20 kg N, 30 kg P₂O₅ and 30 kg K₂O per hectare).

Observations were recorded for the 15 characters for days to first flowering, days to second flowering, days to fruit initiation, plant height, primary branches/plant, secondary branches/primary branch, tertiary branches/secondary branch, leaf surface area, fruit length, fruit diameter, fruit surface area, fruit weight fresh and dry, capsaicin and capsanthin content. Mean values for all the characters (pooled over three years) were subjected to D² and canonical analysis of genetic divergence based on the procedures outlined by Mahalanobis [10] and Rao [4].

3. Results

Highly significant differences ($P < 0.01$) among the collection of *C. annuum* for all the studied 15 characters indicated the presence of considerable genetic diversity. Tremendous variation in shape and size of fruits were observed (Table 2, for the mean, ranges and C.D.). Hence D² values for 703 pairs of genotypes were developed. Based on these values, all 38 accessions/genotypes could be grouped into seven clusters such that the genotype within the clusters had smaller \bar{D}^2 -values among themselves than those belonging to different clusters (Table 3). Divergence for the pooled characters (in terms of D² values) ranged from 752.901 to 1918683 and cluster mean (for 164 to 241 days—first flowering, 000 - 243 days to second flowering, 259 - 336 days of fruit initiation, 50 - 106 cm plant height, 5 - 8 primary branches, 7 - 10 secondary branches, 5 - 9 tertiary branches, 20.06 - 45.39 cm² leaf surface area, 2.332 - 9.960 cm fruit length, 0.802 - 1.830 cm fruit diameter, 4.312 - 27.400 cm² fruit surface area, 0.76 - 4.17 mg fruit weight fresh, 0.20 - 0.75 mg fruit weight dry, 0.000% - 0.0869% capsaicin content and 0.063% - 0.517% capsanthin content). It is evident from the results, Table 2 and Figure 1 (in Figure 1, $D = \sqrt{\bar{D}^2}$), That the mean inter-cluster \bar{D}^2 - values and the cluster mean indicated the highest divergence between clusters designated III and VII ($\bar{D}^2 = 1665293.0$), corroborating the proposed variation of the germplasm into different gene complexes based on cytogenetic aspects and botanical characters. Divergence was also noted between clusters designated I and VII ($\bar{D}^2 = 1661982.0$), II and VII ($\bar{D}^2 = 1167484.0$) and between IV and VII ($\bar{D}^2 = 1119514.0$). The lowest inter-cluster distance was noticed in between cluster I and cluster III ($\bar{D}^2 = 76941.01$) (Table 3). Considerable differences in economic traits, growth and other parameters were noted (Tables 2 and 4). The comparative characteristic features of *Capsicum annuum* plant collected from various places

Table 2. Mean performance (X), ranges and critical difference of 38 accessions of *Capsicum annuum*.

Accession No.	Days to first flowering	Days to second flowering	Days to fruit initiation	Plant height (cm)	No. of primary branches/plant	No. of secondary branches/primary branch	No. of tertiary branches/secondary branch	Leaf surface area (cm ²)	Fruit length (cm)	Fruit diameter (cm)	Fruit surface area (cm ²)	Fruit weight fresh (g)	Fruit weight dry (g)	Capsaicin (%)	Capsanthin (%)
1	173	233	271	100	4	9	5	29.98	5.98	0.66	6.65	0.81	0.23	0.018	0.036
2	180	243	275	103	7	7	6	34.66	3.01	1.09	5.18	2.81	0.50	0.036	0.052
3	156	000	207	53	7	6	5	11.41	2.23	0.65	2.64	0.48	0.16	0.090	0.307
4	184	237	267	63	8	7	6	19.68	5.18	0.81	7.52	0.93	0.32	0.102	0.046
5	177	242	272	106	5	8	6	24.00	2.62	1.84	10.94	3.81	0.88	0.002	0.015
6	177	241	253	91	8	9	6	18.26	2.16	0.68	3.23	0.54	0.23	0.049	0.008
7	178	237	253	79	5	9	8	40.19	3.90	1.35	10.56	4.44	0.96	0.002	0.140
8	144	000	174	41	8	8	6	10.62	1.61	1.08	3.58	0.72	0.24	0.004	0.186
9	174	237	275	91	9	9	6	16.59	4.38	0.68	5.35	0.74	0.17	0.075	0.183
10	178	238	267	96	8	8	7	23.04	4.19	0.67	4.67	1.26	0.23	0.043	0.071
11	180	244	261	82	8	8	6	14.880	2.56	0.71	3.04	0.36	0.14	0.011	0.204
12	168	221	251	98	8	7	4	16.63	4.18	0.77	4.69	0.53	0.23	0.014	0.062
13	175	232	255	92	7	8	6	14.19	3.11	0.78	3.32	0.45	0.15	0.086	0.106
14	181	237	256	90	10	7	7	24.79	4.74	0.76	5.52	1.21	0.25	0.140	0.042
15	189	240	281	92	11	6	8	38.75	3.06	0.79	4.28	0.53	0.20	0.100	0.079
16	178	241	267	84	9	10	6	13.10	3.75	0.74	5.21	0.85	0.25	0.089	0.291
17	179	240	266	81	11	10	7	8.53	3.96	0.97	6.32	0.52	0.19	0.049	0.067
18	152	000	179	58	8	10	8	8.83	4.52	0.82	6.60	1.00	0.25	0.045	0.035
19	168	000	207	50	6	7	5	14.18	3.50	0.81	4.07	0.61	0.21	0.060	0.078
20	166	237	279	85	8	8	6	37.37	3.24	1.51	8.42	2.26	0.49	0.036	0.149
21	175	242	250	98	8	6	6	27.69	4.06	1.31	9.75	2.05	0.44	0.082	0.101
22	171	248	310	91	9	10	6	19.55	3.95	1.21	8.31	1.83	0.42	0.083	0.145
23	176	240	297	79	8	7	7	25.27	3.72	1.21	9.52	2.55	0.54	0.044	0.334
24	183	237	303	110	10	10	5	22.50	1.62	1.35	4.98	1.21	0.20	0.128	0.043
25	161	241	268	96	8	11	6	9.40	2.13	1.03	4.01	2.56	0.51	0.082	0.017
26	179	237	302	111	11	10	5	43.28	1.45	1.35	4.97	1.31	0.32	0.144	0.191
27	183	240	265	72	7	7	5	6.62	2.65	0.74	3.84	0.48	0.20	0.145	0.145
28	180	237	305	42	9	9	5	44.01	2.78	0.76	4.13	0.77	0.22	0.131	0.045
29	157	000	187	59	8	10	6	17.66	3.80	0.76	4.24	0.69	0.23	0.011	0.214
30	177	232	266	50	5	7	5	23.23	5.84	1.43	14.98	2.01	0.49	0.001	0.051
31	179	244	278	74	8	7	5	29.07	3.32	0.74	4.38	0.80	0.22	0.001	0.111
32	159	224	252	83	7	9	5	20.77	2.97	1.02	4.28	0.65	0.25	0.068	0.180

33	180	225	271	78	8	7	5	24.45	2.38	0.99	2.91	0.47	0.16	0.096	0.014
34	181	226	270	84	6	9	6	25.45	3.38	0.74	4.47	1.30	0.28	0.026	0.193
35	176	243	271	106	6	8	6	45.39	3.61	1.82	14.68	2.75	0.70	0.000	0.478
36	218	000	294	92	8	10	8	44.42	9.96	1.59	27.40	3.18	0.82	0.104	0.086
37	178	245	254	102	10	11	6	28.03	4.65	0.70	5.54	1.27	0.24	0.085	0.053
38	160	000	187	41	6	7	5	9.45	4.30	1.09	8.60	1.05	0.31	0.219	0.100
Mean (X)	174	237	258	81	7	8	5	23.31	3.64	1.00	6.64	1.36	0.34	0.065	0.123
Range	144 - 218	221 - 248	174 - 310	41 - 111	4 - 11	6 - 11	4 - 8	6.62 - 45.39	1.45 - 9.96	0.65 - 1.84	2.64 - 27.40	0.36 - 4.44	0.14 - 0.96	0.00 - 0.22	0.008 - 0.478
CD (1%)	5.133	4.228	4.559	2.608	1.740	1.591	1.363	0.567	4.508	4.159	0.084	0.036	3.069	0.004	0.003

Table 3. Average intra- (bold) and inter cluster divergence (\bar{D}^2 value) in *Capsicum annuum* accessions.

Clusters	I	II	III	IV	V	VI	VII	\bar{D}^2	Accession included in clusters
I (21)	43480.89	91363.24	76941.01	219655.20	303668.90	298298.30	1661982.00	2695389.50	3, 6, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 26, 27, 28, 29, 31, 32, 33, 34, 37
II (7)		54333.34	118249.60	141860.60	166808.30	126699.80	1167484.00	1866798.80	1, 4, 18, 21, 22, 23, 38
III (5)			60444.35	133822.00	257733.40	331515.70	1665293.00	2643999.00	2, 8, 20, 24, 25
IV (2)				32932.59	84986.18	195033.90	1119514.00	1927804.40	5, 7
V (1)					00000.00	90935.49	758072.30	1662204.50	35
VI (1)						00000.00	604546.80	1647029.90	30
VII (1)							00000.00	6976892.10	36

\bar{D}^2 = Average D^2 value.

Table 4. Cluster means and other allied genetic parameters in *Capsicum annuum* accessions.

Clusters	Days to 1st Flower Ing	Days to 1ind Flowering	Days of Fruit Initiation	Plant Height (cm)	Primary Branches /Plant	Sec. Br./Pri. Br.	Teri. Br./Sec. Br. (Sq.cm)	Leaf Surface Area (Sq.cm)	Fruit Length (cm)	Fruit Diameter (cm)	Fruit Surface Area (Sq.cm)	Fruit Wt. (Fresh) (Mgs)	Fruit Wt. (Dry) (Mgs)	Capsaicin Content (%)	Capsanthin Content (%)
I	175	231	262	80	8	8	5	21.59	3.25	0.80	4.31	0.76	0.22	0.0869	0.156
II	164	171	267	76	7	8	6	20.06	4.53	1.01	8.14	1.46	0.33	0.074	0.120
III	167	239	259	87	8	8	6	22.90	4.53	1.01	8.14	1.46	0.33	0.074	0.120
IV	177	240	262	92	5	8	7	32.09	3.26	1.59	10.75	4.17	0.75	0.000	0.111
V	176	243	336	106	6	8	6	45.39	3.62	1.83	14.68	2.75	0.71	0.000	0.517
VI	177	232	265	50	5	7	5	23.23	5.85	1.43	14.99	2.01	0.50	0.004	0.019
VII	241	000	293	91	8	10	9	44.42	9.96	1.59	27.40	3.19	0.71	0.039	0.148
Z 1 vector	1.7709	-0.1702	-4.1256	4.8078	7.4035	-6.4503	-2.9924	0.2495	0.3307	0.1573	0.7911	0.1059	0.3597	-5.3054	1.1607
Ranks	III	XI	XIII	II	I	VX	XII	VIII	VII	IX	V	X	VI	IVX	IV
Z 2 vector	-0.0115	-9.8341	-1.6872	0.1167	5.6000	0.1705	-0.2291	0.5147	-0.4022	0.3234	-8.6604	0.5715	-0.1568	-2.7722	-3.7760
Rank	VII	VX	XI	VI	I	V	IX	III	X	IV	IVX	II	VIII	XII	XIII

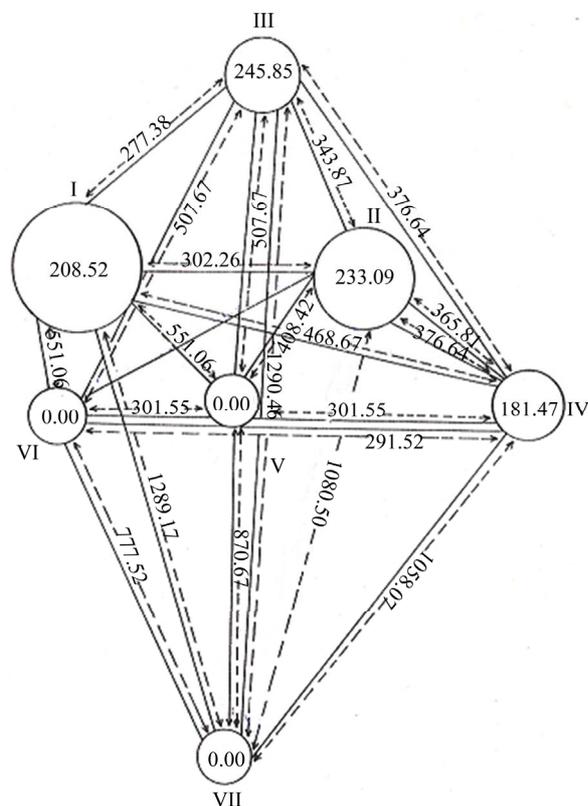


Figure 1. Genetic diversity ($D = \sqrt{\bar{D}^2}$) among 38 accessions of *C. annuum*.

of India are presented in **Table 1**. Days to first flowering varies from 174 - 175, days to 2nd flowering 237 - 247, days to fruit initiation 265 - 267, plant height 81 - 82 cm, primary branches/plant 7 - 8, secondary branches/primary branch 7 - 8, tertiary branches/ secondary branch 5 - 6, leaves surface area 23.11 - 23.30 per sq.cm., fruit length 3.6 - 3.63 cm, fruit diameter 0.998 - 1.00 cm fruit surface area 6.642 - 6.65/sq.cm, fruit weight fresh 1.37 - 1.38 mg, fruit weight dry 0.309 - 0.311 mg, capsaicin content 0.072% - 0.077%, capsanthin content 0.140 - 0.142% (**Table 5**). Other morphological characters were also exhibited tremendous variation such as color of leaf flower and fruits, plant type, shape and morphology of fruit etc. (**Table 5**).

4. Discussion

In the *Capsicum annuum* accessions, 55.263% of the accessions could be grouped within one cluster I (21 accessions); in cluster II, 18.421% (7 accessions); cluster III 13.158% (5 accessions), followed by cluster IV 5.263% (2 accessions). The cluster V, VI and VII were found unique included only one accession in each namely, 35, 30 and 36, which were in 7.896% of the total accessions (**Table 3**). An analysis of cluster composition revealed

that largest cluster grouping was comprised of accessions collected from Lucknow (Uttar Pradesh); Pantnagar and Ramnagar (Uttaranchal); Assam and Pune (Maharashtra) followed by cluster II comprised of accessions originated from Uttar Pradesh, Uttaranchal and Maharashtra, and cluster III comprised the accessions collected from Uttar Pradesh and Assam states of India.

Notwithstanding the fact that, based on \bar{D}^2 -values, all 38 accessions/genotypes could be grouped into seven clusters such that the genotype within the clusters had smaller \bar{D}^2 -values among themselves than those belonging to different clusters (**Table 3**). It is evident from the results, **Table 3** and **Figure 1**, that the mean inter-cluster \bar{D}^2 -values and the cluster mean indicated the highest divergence between clusters designated III and VII ($\bar{D}^2 = 1665293.0$), corroborating the proposed variation of the germplasm into different gene complexes by Moscone *et al.* (2007) [3] in investigation based on cytogenetic aspects and botanical characters. Divergence was also noted between clusters designated I and VII ($\bar{D}^2 = 1661982.0$). II and VII ($\bar{D}^2 = 1167484.0$) and between IV and VII ($\bar{D}^2 = 1119514.0$). The lowest inter-cluster distance was noticed in between cluster I and cluster III ($\bar{D}^2 = 76941.01$) (**Table 3**). The highest intra cluster \bar{D}^2 values depicted by cluster III ($\bar{D}^2 = 60444.35$) having 5 accessions followed by cluster II ($\bar{D}^2 = 55333.340$ having 7 and cluster I ($\bar{D}^2 = 43480.89$) having 21 accessions. The 0.00 intra-cluster value depicted by clusters V to VII having unique individual and highly diverse accession (**Figure 1** in terms of $D = \sqrt{\bar{D}^2}$).

Accession number 5, 7, 30, 35 and 36 from Lucknow (Uttar Pradesh) appeared highly divergent being represented within four clusters. The clusters V to VII were highly divergent and unique including only one accession each (35-V, 30-VI and 36-VII cluster). All belonging from Lucknow (Uttar Pradesh) represented enormous variation with respect to their morphology, growth behavior, ecological requirement and genetic diversity. The primary branches per plant (7.4035, rank I) followed by plant height (4.8078, rank II) and days to first flowering (1.7709, rank III) at the primary axis (Z1) and the primary branches per plant (5.6000, rank I) followed by fruit weight fresh (0.5715, rank II) and leaf surface area (0.5147, rank III) at the secondary axis (Z2), respectively were the largest contributor to genetic divergence. The least contributor to genetic divergence were secondary branches per primary branch (-6.4503) followed by capsaicin content (-5.3054) and days of fruit initiation (-4.1256) at primary axis, respectively. Least contributing traits fruit surface area (-8.660), capsanthin content (-3.776) and capsaicin content (-2.7722) at the secondary axis, respectively (**Table 4**). The distribution pattern

Table 5. Average intra and inter cluster D value in *Capsicum annuum* accessions.

Clusters	I	II	III	IV	V	VI	VII
I (21)	208.52	302.26	277.38	468.67	551.06	546.16	1289.17
II (7)		283.09	343.87	376.64	408.42	355.94	1080.50
III (5)			245.85	365.81	507.67	575.77	1290.46
IV (2)				181.47	291.52	441.62	1058.07
V (1)					000.00	301.55	870.67
VI (1)						000.00	777.52
VII (1)							000.00

Where $D = \sqrt{\bar{D}^2}$.

of accessions of diverse origin in the clusters indicates that genetic diversity observed within *C. annuum* was not related to geographic origin. Noted difference in plant characters probably occurred over time with free movement of plant material from location to location, mutation, hybridization, gene recombination and selection. Our findings were also in consonance of results of Pandey and Dobhal [1] and Varalakshmi and Haribabu [11] in *C. annuum* crop. Selection of promising accessions based on genetic divergence and capsaicin and capsanthin content quality are useful in chili crop improvement. The accessions labeled number 38, 27, 26, 14 and 24 to high capsaicin content (%); 35, 23, 3, 16, 29 and 11 for high capsanthin content (%) and 26 and 27 for dual purpose had characteristics desirable. Among the above, the accession no. 35 (0.000% capsaicin content) should be utilized for the study of genetics of capsaicin content, making hybrids between the parents 0.000 X high capsaicin content.

Mainly due to the versatility of application, *C. annuum* plants have become important from an economic point of view in several countries since their fruits can be used for different purposes, such as in cooking, in industry (e.g., production of “pepper spray”) and for medicinal and ornamental purposes (Pickersgill, [12] 1971; [13] Moscone *et al.* [3], 2007). In Brazil this vegetable is of great importance, ranking second in the generation of foreign currency among exported vegetables (Embrapa, [6,14] 2009). Therefore, the above listed accessions of the *C. annuum* could be utilized, as parents for hybridization in recombination breeding programme followed by repeated selection to obtain maximum gain.

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