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GENETIC DIVERGENCE IN CAPSICUM (*Capsicum annuum* L. var. *grossum*) BASED ON MORPHO-CHEMICAL TRAITS

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ABSTRACT

***Capsicum annuum* L. var. *grossum* is the most widely cultivated species of pepper which finds use in culinary and medicinal industry. Genetic divergence among 20 germplasm, collected from diverse locations of India, was estimated on 18 morphological, growth and chemotypic characteristics. Based on above characterization, the genotypes were grouped into 7 clusters. Cluster II had maximum number of genotypes (06), followed by Cluster I and III (4 each) and IV (3); whereas cluster V, VI and VII had only one genotype each. The highest (35.91) inter-cluster distance was observed between cluster VI and VII and lowest (12.66) between cluster I and II. Cluster IV (D^2 24.35) exhibited highest intra-cluster distance whereas cluster I had lowest intra-cluster distance (D^2 6.28). On the basis of cluster means, genotypes *viz.*, 'CITH-M-SP-12', 'UHF-1' and 'SP-461' contributed maximum towards genetic divergence for high ascorbic acid content, TSS, reducing and total sugars content. Considering diversity pattern and other horticultural performance, 'CITH-M-SP-2' from cluster VI may be considered as one of the better parents in hybridization programme for most growth and yield parameters.**

Keywords: Capsicum, cluster analysis, D^2 divergence, genotypes, selection, yield

INTRODUCTION

Capsicum is unique genus of family *Solanaceae* finding diverse uses in culinary to pharmaceutical industry. 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 colour of capsicum, while β-carotene and zeaxanthin are responsible for yellow-orange colours (Lanteri and Pickersgill, 1993). The development of promising hybrids depends largely on the selection of desirable inbred lines. To improve the quality of a variety/hybrid in all aspects, diverse parents are a pre-requisite to select better segregates for various economic traits. The choice of genetically diverse parents for hybridization under heterosis breeding programme is dependent upon the characteristics of breeding material on the basis of appropriate criteria reports (Lanteri and Pickersgill, 1993). Genetic divergence existing in a population helps in the selection of suitable parents for use in any crop breeding programme leading to the reduction in the number of crosses (Farhard *et al.,* 2010). Selection of parents depends on their performance and specific objective of a research programme. Various statistical tools are available to select suitable parents. The information on the nature and degree of genetic divergence is essential for a breeder to choose the

right parents for purposeful hybridization in heterosis breeding (Pandey and Dobhal, 1993; Indira, 1994). In order to benefit transgressive segregation, the knowledge of genetic distance between parents is necessary (Khodadabi *et al.,* 2011). The standardization of variable is also essential for the determination of genetic distance so that all variables are of similar importance in determining the distance. Various methods have been used to study genetic diversity through cluster analysis of which Tocher's method is the most popular approach. The cluster analysis is an appropriate method for determining the family relationships (Mahalanobis, 1936). Higher the genetic distance between parents, the higher heterosis in progeny may be observed (Khodadabhi *et al.,* 2011). In present study, 20 capsicum genotypes, collected from different regions of India, were cultivated following the standard package of practices at ICAR-CITH, Mukteshwar, Uttarakhand (India) and evaluated for their genetic diversity based on morpho-chemical traits so as to identify potential genotypes by cluster analysis and selection for use as varieties or parents in future breeding programme.

MATERIALS AND METHODS

A field experiment was conducted under polyhouse conditions at ICAR-CITH, Mukteshwar (India) during summer extended *rabi* season of 2014-2015. The experimental material comprised of 20 genotypes, collected from various parts of the country. Genotypes 'CITH-M-SP-1', 'CITH-M-SP-2', 'CITH-M-SP-3', 'CITH-M-SP-4', 'CITH-M-SP-6', 'CITH-M-SP-7', 'CITH-M-SP-9', 'CITH-M-SP-11', 'CITH-M-SP-12', 'CITH-M-SP-13', 'CITH-M-SP-14', 'CITH-M-SP-15', 'CITH-M-SP-16', 'CITH-M-SP-17', 'CITH-M-SP-643-3' were collected from ICAR-CITH, Mukteshwar (India), genotype 'UHF-1' from YSPUHF Solan, 'Vindale' and 'SP-461' from ICAR-CITH Srinagar (India), 'California Wonder' from IARI Katrain (HP) and 'Nishat-1' from SKUAST, Srinagar (India). Genotypes were planted in a completely randomized block design and each treatment replicated thrice. Observations were recorded on 5 randomly selected plants for 18 physicochemical traits *viz*., growth, yield and quality parameters. The vegetative growth parameters *viz*., plant height, number of primary branches plant^{-1}, number secondary branches plant^{-1}, plant spread (East-West and North-South), plant spread area cm^{-2}, total number of fruits plant^{-1}, fruit length, fruit breadth, average fruit weight, average fruit yield plant^{-1}, total fruit yield (q ha^{-1}) were recorded. The quality characteristics were analyzed following the procedures described by Ranganna (1986). The fruit juice was used to determine total soluble solids (TSS) by using a refractometer (ERMA refractometer 0-32° Brix). Titratable acidity (TA) was measured by titration of 2 mL homogenate juice with added 2 drops of 1% phenolphthalein and titrated by N/10 NaOH solution till it became light pink colour. It was calculated as under:

Titratable acidity = Standardized value malic acid (0.67)/2, the value 0.335 multiplied by titer value of juice.

Ascorbic acid content was measured by using 2, 6 dichlorophenol indophenols method, reducing and total sugars were recorded as per Ranganna (1986). Data was analyzed using ANOVA to assess the significance of treatment means. The multivariate analysis was computed using Mahalanobis D^2 statistics. Genotypes were grouped into various clusters following Tocher's method (Rao, 1952).

RESULTS AND DISCUSSION

Performance of genotypes for quantitative parameters

Significant variations were observed among the selected genotypes in terms of plant growth, yield and quality behaviour. The comparative features of growth, yield and quality of capsicum genotypes collected from different parts of the country are presented in Table 1 and 2. Significance

of mean sum of squares indicated the sufficient amount of variation among the genotypes. The average mean range of quantitative characters *viz*., plant height (27.83-94.66 cm), number of primary branches plant^{-1} (2.00-3.67), number of secondary branches plant^{-1} (4.16-5.66), total number of fruits plant^{-1} (4.8-34.3), plant spread (East-West 33.5-53.8 cm and North-South 28.0-54.3 cm and total plant spread area 106.2-2924.2 cm^2), fruit length (1.95-11.60 cm), fruit breadth (1.55-6.07 cm), fruit firmness (0.16-0.47 kg cm^{-2}), average fruit weight (36.76-90.50 g), average fruit yield plant^{-1} (313.0-2090.1 g), total fruit yield ha^{-1} (154.56-1032.14 q ha^{-1}) (Table 1) were observed in the genotypes. A total fruit yield of 387.4 q ha^{-1}) has been reported by Senthilkumar *et al*. (2018). The genotype 'CITH-M-SP-1' was the tallest (94.66 cm) whereas genotype 'UHF-1' was smallest (27.83 cm) of all. However, the numbers of primary branches plant^{-1} and secondary branches plant^{-1} were maximum in genotypes 'CITH-M-SP-2' (3.67) and 'SP-461' (5.66), respectively. The cultivar 'California Wonder' exhibited maximum plant spread in both the directions *i.e.* East-West (53.83 cm) and North-South (54.33 cm), while minimum plant spread in both directions was observed in 'CITH-M-SP-12' with 33.5 cm (East-West) and 28.0 cm (North-South).

The highest number of fruits plant^{-1} recorded in genotype 'CITH-SP-M-643-3' (34.3), fruit length (11.6 cm) in 'California Wonder', fruit breadth (6.07 cm) in 'UHF-1', fruit firmness (0.47 kg cm^{-2}) in 'CITH-M-SP-2' and average fruit weight (90.5 g) in 'Nishat-1'. However, the lowest number of fruits plant^{-1} was found in 'CITH-M-SP-7 '(4.8), fruit length in 'Nishat-1' (1.95 cm), fruit breadth in 'SP-461' (1.55 cm) and fruit firmness in 'CITH-M-SP-17' (0.156 kg cm^{-2}) and average fruit weight in 'CITH-M-SP-15' (36.76 g).

The average fruit yield plant^{-1} ranged from 313.0 to 2090.1 g with highest fruit yield in genotype 'UHF-1', followed by 'California Wonder' (1947.5 g), 'CITH-M-SP-2' (1828.0 g), 'CITH-M-SP-3' (1684.0 g) and 'CITH-M-SP-13' (1676.0 g). Sufficient variability in various growth, yield and quality parameters of sweet pepper have previously been reported (Nazir *et al.*, 2005).

Table 1: Mean performance of qualitative traits of 20 genotypes of capsicum

Variety/ Selections	Plant height (cm)	No. of primary branchs plant^{-1}	No. secondary branches plant^{-1}	Plant spread (cm)		Plant spread area (cm^2)	Number of fruits plant^{-1}	Fruit length (cm)	Fruit breadth (cm)	Average fruit weight (g)	Average fruit yield plant^{-1}	Fruit yield (q ha^{-1})
				(East-West)	(North-South)							
CITH-M-SP-1	94.66	3.16	4.83	48.16	43.00	2070.3	14.2	7.62	5.46	64.18	911.0	449.9
CITH-M-SP-2	69.33	3.67	5.16	52.00	41.50	2158.0	28.6	8.59	5.09	63.94	1828.0	902.7
CITH-M-SP-3	60.16	2.83	4.16	36.50	32.00	1168.0	32.4	7.63	4.57	52.00	1684.0	831.6
CITH-M-SP-4	60.00	2.83	4.50	39.33	34.50	1356.3	14.0	8.35	4.70	61.52	862.0	425.7
CITH-M-SP-6	57.66	2.83	5.00	39.83	33.50	1447.0	23.4	8.05	4.53	56.41	1320.0	651.8
CITH-M-SP-7	66.50	2.83	4.50	36.17	31.17	1127.1	4.8	11.25	4.93	65.04	313.0	154.6
CITH-M-SP-9	72.83	3.00	4.50	42.33	36.33	1537.3	19.2	7.57	4.93	53.62	1030.0	508.6
CITH-M-SP-11	74.50	3.66	5.16	46.83	41.33	1934.8	22.0	7.32	5.49	60.66	1335.0	659.2
CITH-M-SP-12	44.50	2.16	4.50	33.50	28.00	938.0	10.6	7.03	3.95	43.72	464.0	229.1
CITH-M-SP-13	89.16	3.16	5.50	46.16	45.50	2100.1	30.6	7.86	5.16	54.76	1676.0	827.6
CITH-M-SP-14	71.50	2.50	5.16	36.66	33.50	1228.0	12.0	8.70	4.60	62.25	748.0	369.4
CITH-M-SP-15	69.50	3.16	4.16	35.50	32.67	1159.3	20.4	5.95	3.99	36.76	750.0	370.4
CITH-M-SP-16	67.00	2.67	4.16	38.83	28.50	106.2	13.4	6.65	6.01	81.17	1087.7	537.1
CITH-M-SP-17	57.83	3.00	4.66	41.33	30.67	1267.2	9.8	6.41	4.78	43.05	421.9	208.3
CITH-M-SP-643-3	56.16	2.50	5.16	37.33	35.50	1325.1	34.3	6.29	3.83	39.52	1355.5	669.4
UHF-1	27.83	2.83	4.66	35.00	36.83	1289.0	23.8	6.61	6.07	87.93	2090.1	1032.0
Vindale	61.00	2.33	4.66	34.50	32.67	1127.0	12.7	8.36	4.22	42.81	543.7	268.5
SP-461	76.66	2.33	5.66	40.83	45.50	1857.2	6.6	2.10	1.55	73.84	489.6	241.8
California Wonder	86.00	2.33	5.00	53.83	54.33	2924.2	22.3	11.60	6.01	87.33	1947.5	961.7
Nishat-1	69.00	2.00	5.00	42.33	50.67	2144.3	9.5	1.95	2.22	90.50	862.5	425.9
Mean	66.59	2.79	4.80	40.85	37.38	1563.2	18.2	7.29	4.60	61.05	1085.5	536.3
Range	27.83-94.66	2.00-3.67	4.16-5.60	33.50-53.83	28.00-54.33	106.2-2924.2	4.80-34.3	1.95-11.60	1.55-6.07	36.76-90.50	313.0-2090.1	154.6-1032.0
$CD_{0.05}$	4.00	0.49	0.48	2.35	1.24	101.7	0.16	2.22	2.07	3.92	37.38	9.84
CV (%)	2.16	9.08	6.54	4.26	2.19	1.96	0.36	1.26	1.42	1.94	0.99	1.11

Table 2: Mean performance of qualitative traits of 20 genotypes of capsicum

Variety/Selections	Fruit firmness (kg cm^{-2})	TSS ($^{\circ}$Brix)	Acidity (%)	Ascorbic acid (mg 100 g^{-1})	Reducing sugars (%)	Total sugars (%)
CITH-M-SP-1	0.176	4.35	0.22	92.27	1.48	2.61
CITH-M-SP-2	0.471	5.76	0.29	141.44	1.97	3.46
CITH-M-SP-3	0.156	6.67	0.40	293.1	2.28	4.00
CITH-M-SP-4	0.176	6.21	0.46	225.8	2.12	3.73
CITH-M-SP-6	0.171	6.23	0.51	652.0	2.13	3.74
CITH-M-SP-7	0.168	4.94	0.25	470.13	1.69	2.96
CITH-M-SP-9	0.178	5.98	0.52	339.3	2.04	3.59
CITH-M-SP-11	0.248	6.85	0.64	440.2	2.34	4.11
CITH-M-SP-12	0.156	7.77	0.32	796.11	2.67	4.67
CITH-M-SP-13	0.193	5.78	0.44	503.00	1.97	3.47
CITH-M-SP-14	0.178	5.71	0.57	698.67	1.95	3.43
CITH-M-SP-15	0.176	4.91	0.45	465.63	1.68	2.95
CITH-M-SP-16	0.259	4.54	0.43	645.16	1.55	2.72
CITH-M-SP-17	0.156	6.52	1.64	662.17	2.23	3.91
CITH-M-SP-643-3	0.189	7.08	0.64	322.49	2.42	4.25
UHF-1	0.170	6.43	0.47	662.32	2.20	3.86
Vindale	0.160	6.22	0.39	207.00	2.12	3.73
SP-461	0.175	8.47	2.07	1770.00	2.89	5.09
California Wonder	0.179	7.00	1.56	1568.00	2.39	4.20
Nishat-1	0.228	6.68	1.26	949.22	2.28	4.01
Mean	0.198	0.198	0.68	595.13	2.12	3.72
Range	0.156-0.471	4.35-8.47	0.22-2.07	92.3-1770.0	1.48-2.89	2.61-5.09
$CD_{0.05}$	0.043	0.70	0.24	1.43	0.26	0.38
CV (%)	4.61	3.44	1.07	0.53	0.39	0.66

Plate 1: A view of fruits of different genotypes of capsicum

Performance of genotypes for quality parameters

Capsicum is considered nutritive as well as protective food due to its quality aspects. The important quality parameters *viz*., TSS, acidity, ascorbic acid, reducing sugars and total sugars were estimated from the fruits of studied germplasm material which showed that genotype 'SP-461' was having highest TSS content (8.47°B), acidity (2.07%) and ascorbic acid content (1770 mg 100 g^{-1}) while as genotype 'CITH-M-SP-1' was poorest in TSS (4.35°B), acidity (0.22%) and ascorbic acid (92.27 mg 100 g^{-1}). As far as reducing sugars and total sugars were concerned, the genotype 'SP-461' exhibited highest reducing and total sugar content i.e. 2.89 and 5.09%, respectively, but these were lowest in 'CITH-M-SP-1' i.e. 1.48 and 2.61%, respectively.

Divergence analysis

The clustering pattern of different genotypes has been presented in Table 3 and based on the degree of divergence among the genotypes, as per the Mahalanobis D^2 analysis, the genotypes were grouped into seven clusters, which revealed the presence of sufficient diversity for different traits under study. Based on these values, the genotypes within cluster had smaller D^2 value among themselves

Table 3: Distribution of capsicum genotypes in different clusters as per Mahalanobis D^2 analysis

Clusters	No. of genotypes	Varieties/ selections/genotypes
Cluster I	04	CITH-M-SP-4, CITH-M-SP-6, CITH-M-SP-9 and CITH-M-SP-13
Cluster II	06	CITH-M-SP-1, CITH-M-SP-11, CITH-M-SP-14, California Wonder, CITH-M-SP-643-3 and Vindale
Cluster III	04	CITH-M-SP-7, CITH-M-SP-15, CITH-M-SP-16 and Nishat-1
Cluster IV	03	CITH-M-SP-12, UHF-1 and SP-461
Cluster V	01	CITH-M-SP-17
Cluster VI	01	CITH-M-SP-2
Cluster VII	01	CITH-M-SP-3

themselves than those belonging to different clusters. Maximum number of genotypes (6) were accommodated in cluster II, followed by cluster I & III (4 genotypes in each) and cluster IV (3). The rest three cluster *viz*., V, VI and VII consisted of solitary individual genotype each.

Cluster analysis

The critical examination of clustering pattern indicated that there was no relationship between geographical distribution and genetic divergence. Hence, geographical diversity cannot be correlated to genetic diversity. Thus, selection of genotypes to be involved as parental lines in hybridization programme should be on genetic diversity rather than geographical distribution.

The highest intra-cluster (within the genotypes of same cluster) distance was found within the genotypes of cluster IV (24.354), followed by cluster III (24.008), cluster II (19.03) and cluster I (6.289) [Table 4]. The intra-cluster values for the rest clusters were zero. The maximum inter-cluster (between any two clusters) distance was found between cluster VI & VII (35.913), followed by cluster V & VII (34.592), cluster V & VI (33.813), cluster IV & VI (31.72) and cluster III & VII (31.12). As maximum diversity was noticed between cluster VI & VII, hence hybridization between the genotypes pertaining these clusters will produce more heterotic F_1 hybrids and superior segregants in F_2 and later segregating generations. However, sufficient inter-cluster distances existed between all inter-cluster genotypes, which provide greater chance of selection of better genotypes for hybridization and any genotypes of one cluster could be hybridized with any genotypes of other cluster of this existing clustering pattern. But, inter-crossing of genotypes of most divergence clusters would provide better chances for crossings over, which may release concealed variability reported (Occhiuto *et al*., 2014). The minimum inter-cluster distance was found between cluster I & II (12.666). Less intra-cluster distance than inter-cluster distance revealed more homogenous nature of genotypes (Pawar *et al*., 2013).

Table 4: Average intra- (bold) and inter-cluster divergence (D^2 value) and distance (D) between different clusters of capsicum genotypes

Clusters	I	II	III	IV	V	VI	VII
I (4) D^2	6.2897	12.6665	17.0851	18.2485	21.8808	22.8874	24.0620
D	2.5079	3.5590	4.1334	4.2718	4.6776	4.7840	4.9053
II (6) D^2		19.0303	21.9496	21.5764	24.0624	25.6474	28.5014
D		4.3623	4.6850	4.6450	4.9053	5.0643	5.3386
III (4) D^2			24.0086	24.1533	29.0083	28.3315	31.1227
D			4.8998	4.9146	5.3859	5.3227	5.5796
IV (3) D^2				24.3548	27.5719	31.7208	31.0875
D				4.9350	5.2508	5.6321	5.5756
V (1) D^2					00.0000	33.8130	34.5927
D					0.0000	5.8148	5.8815
VI (1) D^2						00.0000	35.9134
D						0.0000	5.9927
VII (1) D^2							00.0000
D							0.0000

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

Table 5: Cluster mean of *Capsicum annuum* L. var. *grossum* genotypes

Characters	Clusters						
	I	II	III	IV	V	VI	VII
Plant height (cm)	69.91	73.97	68.00	49.06	57.83	69.33	60.13
Primary branches plant^{-1} (No.)	02.95	02.75	02.66	2.44	3.00	3.67	2.83
Secondary branches plant^{-1} (No.)	04.87	04.99	04.45	4.95	4.66	5.16	4.16
Total fruits plant^{-1} (No.)	21.80	19.58	12.03	13.66	9.80	28.60	32.40
Plant spread (East-West) [cm]	41.91	42.88	38.20	36.44	41.33	52.00	36.50
Plant spread (North-South) [cm]	37.45	40.05	35.75	36.78	30.67	41.50	32.00
plant spread area (cm^2)	1610.2	1768.2	1134.2	1367.4	1267.2	2158.0	1168.0
Fruit length (cm)	7.96	6.56	6.23	4.99	6.41	8.59	7.63
Fruit breadth (cm)	4.83	4.28	4.29	3.86	4.78	5.09	4.57
Fruit firmness (kg cm^{-2})	0.179	0.188	0.207	0.167	0.156	0.471	0.156
Average fruit weight (g)	56.78	59.44	68.36	68.49	43.05	63.94	52.00
Average fruit yield plant^{-1} (g)	1222.0	1140.1	753.3	1014.6	421.9	1828.0	1684.0
Total fruit yield (q ha^{-1})	603.45	563.01	371.92	630.63	208.34	902.71	831.60
TSS (^{o}Brix)	06.05	06.20	05.26	7.55	6.52	5.76	6.67
Acidity (%)	00.48	00.67	00.59	0.95	1.64	0.29	0.40
Ascorbic acid (mg 100 g^{-1})	430.02	554.77	632.53	1076.14	662.17	141.44	293.10
Reducing sugars (%)	2.06	2.11	1.80	2.58	2.23	1.97	2.28
Total sugars (%)	3.63	3.72	3.16	4.54	3.91	3.46	4.00

Cluster mean analysis

The cluster means for 18 characters have been presented in Table 5. Clusters VI exhibited desirable and highest values of cluster means for the number of primary branches plant^{-1} (3.67), number of secondary branches plant^{-1} (5.16), plant spread (east-west - 52.0 cm, north-south - 41.5 cm), total plant spread (2158.0 cm^2), fruit length (8.59 cm), fruit breadth (5.09 cm), fruit firmness (0.47 kg cm^{-2}), average fruit yield plant^{-1} (1828.0 g) and total fruit yield (902.7 q ha^{-1}). Cluster V exhibited desirable means for acidity content (1.64%), while cluster IV showed desirable means or reducing sugars (2.58%) and total sugars content (4.54%). Cluster II recorded highest cluster mean values for plant height (73.97 cm). For TSS and ascorbic acid contents, cluster IV exhibited highest values of cluster means *i.e.* 7.55°B and 1076.1 mg 100 g^{-1}, respectively. Cluster mean value for the number of fruits plant^{-1} (32.4) and average fruit weight (68.36 g) recorded highest in cluster VII and III, respectively. Crossing the genotypes of cluster VI with V and IV clusters appeared to be most promising to continue the desirable traits. In present study cluster I, III and VII were more diverse and appear to have more chance of getting better segregants in F_2 and subsequent generations from the crossings of cluster I, III and VII. The significance of genetic divergence in capsicum is reported by Occhiuto *et al*. (2014). The leaf and fruit characteristics can be used for differentiation of capsicum genotypes grown under comparable conditions (Cochran, 1940).

The present study reveals that there is a considerable degree of divergence at inter-genetic, inter-cluster and intra-cluster levels in capsicum. At inter-cluster or inter-genotype level, the characteristics that played the greatest role in differentiation were fruit length, fruit breadth, fruit firmness, number of fruits plant^{-1}, plant spread, plant height, etc. It can be inferred that for the traits under study where selection is not effective, the genetic divergence could play an important role in further partitioning of the variability. The I, III and VII were found more divergent and there will be more chance of getting better segregates in F_2 and subsequent segregating generations from the crossing of genotypes of these clusters. Hybridization between the genotypes of cluster VI with cluster V and VII can be very effective for high yielding and good quality hybrid development for protected cultivation of sweet pepper.

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