



Genetic diversity and association analysis for different morphological traits in *Capsicum annuum* L.

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Abstract

Capsicum annuum L. is the extensively cultivated species of peppers (chilies) in all over the world. Its fruits are used for spiciness (capsaicin) and color (capsanthin) in our daily foods. Pakistan is the leading chili consuming country. Genetic divergence among 25 accessions (local and exotic) collected from Ayub Agriculture Research Institute (AARI) Faisalabad, Pakistan was estimated from the data collected during the year 2014 in the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan for different morphological and growth parameters viz fruit width, fruit length, peduncle length, number of primary branches, inter nodal length, plant height, seed index, 1000 seed weight, fresh and dry fruit weight, pericarp thickness, leaf area and seeds per fruit. Based on this characterization the plants were grouped into 5 clusters and diversity among accessions was indicated by the wide range of D^2 values whereas phenotypic correlation for all the characters was found significant. Five components were selected as principle components with Eigen values > 1 . These components exhibited 77.2% of the variation. The first principal component (PC I) explained 27.2% of total variation in original data, second component (PC II) explained 18.9%, and third principal component (PC III) explained 12.5% of variation. The other principal components (PC IV and PC V explained an additional 18.6% of the variation (a total 77.2% of explained variation. Accessions with distinct identity were marked, which are likely to be quite suitable for breeding through hybridization by combining desirable traits. High estimates of broad sense heritability (90%) for all the characters except peduncle length predicted that selection could be awarding in late segregating generations and above accessions could be utilized in hybridization programme for *C. annuum* crop improvement.

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Introduction

Pepper (*Capsicum spp.*) is a complex of species originating from the inter-tropical America. The *Capsicum* genus belongs to the Solanaceae family and includes 27 recognized species (Baral and Bosland, 2002). Hot pepper are grown and used for vegetable, spice, condiment, paste, pickle and medicinal purpose. Capsicum (pepper) constitutes the most important spice is grown all over the world except in colder regions. Peppers with greater pungency are cultivated in tropical states; viz. Pakistan, India, China, Nigeria, Malaysia, Japan and Turkey. Total world green pepper production is 7 to 8 million tones and 2 to 3 million tons in dry form. It is a good source of ascorbic acid, vitamin A, E and chili oleoresin. Chilies are popular for the abundant content of vitamin C (Ascorbic acid) larger than other vegetables and fruits (Durust *et al.*, 1997; Osunagarcia *et al.*, 1998).

Pepper was familiarized to Europe by Columbus in 15th century and expanded to rest of the globe. Pepper (*Capsicum annum L.*) is the second biggest product in the international trade after black pepper (*Piper nigrum L.*) (Anonymous, 2012). The genus capsicum is often cross pollinated crop and natural cross pollination may go up to 50% depending upon the extent of style exertion, time of dehiscence of anthers, wind direction and insect population (Murthy and Murthy, 1962 and Hosmani, 1993). The pungency of chilies is due to capsaicin which is an alkaloid present in placenta of chili fruit (Reddy and Lokesh, 1992; Kogurea *et al.*, 2002; Bhattacharya *et al.*, 2010).

Capsicum annum L. is broadly cultivated species due to variable in shape, size, color, pungency and texture (Russo and Biles, 2004). In Pakistan the area under chilies has reduced from 84.5 to 74.8 thousand hectares but production has increased from 174.6 to 188.9 thousand tones (Govt. of Pakistan, 2013). Sindh is the major pepper growing province with mean area of 55.3 thousand hectares and production of 122.9 thousand tons (Khokhar, 2010). Chilies are substantial vegetable crop of tropical, subtropical and temperate climate of the world (Hazra *et al.*, 2011).

A wide range of variability has been reported in the pepper crop (Sreelathakumary and Rajamony, 2004). Genetic difference is basic requirement for real selection within population and population arising out of hybridization. Genetic resources play an important role for desirable traits improvement. Genetic differences help to select the better parents for breeding program (Singh and Choudry, 1985). The importance of genetic diversity in the crop improvement has been studied in both self and cross pollinated crops (Griffing and Lindstrom, 1954; Murthy and Anand, 1966; Gaur *et al.*, 1978).

Genetic diversity is an importance tool to know about desirable genes within the germplasm (Tomooka, 1991). For most cultivated species, the loss of genetic variability started as soon as the domestication process, and may have been worsened with migration events from original to secondary diversification sites depending on the history of plant species exploitation (Tang *et al.*, 2010). Multivariate analysis is used for the assessment of degree of variability and contribution of various characters to total variability in self-pollinated crops (Das and Gupta, 1984; Natarajan *et al.*, 1988; Golakia and Maken, 1992; Sindhu *et al.*, 1992). Yatung *et al.* (2014) assessed the genetic variability of thirty hot pepper entries observed abundant diversity for different morphological characters. Cluster analysis grouped thirty accessions into six clusters, highest number of genotypes were present in cluster three (14) and lowest in cluster four and five. Inter cluster distance between two and four clusters was maximum (459.8) and minimum in one and four cluster (36.04). Maximum intra cluster was found in cluster three ($D^2=67.66$) and minimum in cluster two ($D^2=11.19$). Chattopadhyay *et al.* (2011) observed extreme variability present among the cultivars/landraces for shape, size, yield, quality, and other characters existing in India germplasm and concluded 'Chaitali Pointed' and 'BC CH Sel-4' most promising genotypes with respect to green fruit yield (272.79 g, 221.10 g per plant) and dry fruit yield (54.56 g, 44.44 g per plant). Phenotypic and genotypic Coefficient of Variation values for green fruit weight (119.95%,

111.26%), green fruit girth (89.76%, 48.93%), weight of red ripe fruit (112.02%, 111.93%), weight of dry fruit (111.63%, 110.97%) and number of fruits per plant (86.05%, 85.02%). Dutonde *et al.* (2008) studied the genetic diversity of forty accessions of hot pepper through cluster analysis. The genotypes were grouped into seven clusters, each cluster containing different accessions of hot pepper. Cluster four and seven showed highest inter cluster distance ($D^2=104.98$). Cluster two, four and seven was used to develop high yielding varieties with desirable characters through crossing among genotypes belonging to these clusters. Del *et al.* (2007) estimated the morphological and phenotypic variation of both vegetative and reproductive features of hot pepper (*Capsicum annum* L.) by following Principles Components Analysis (PCA) and analyzed hierarchical conglomeration (AHC). Ample variability was found in entries for length of limb, diameter of the limb, shape and color of leaf, fruit calyx and days to flowering and fructification. Manju and Sreelathakumary (2004) grouped 32 accessions of hot pepper into 6 clusters showing maximum intra cluster distance in clusters 1 because 21 genotypes were present in this cluster. Karad *et al.* (2002) grouped 40 genotype of pepper (indigenous and exotic) into 8 cluster and their D^2 values ranged 0.1032 to 8.7702. The maximum inter cluster distance between cluster II and V was (7.45) and minimum inter cluster distance was between cluster III and VII (1.15). The objective the present study was to evaluate the existing chili germplasm for genetic diversity and correlation among the plant growth traits because capsicum is known to have relatively low levels of genetic diversity. A better understanding of variation and relationships among possible sources of novel genes would be valuable because it provides valuable information for future breeding programs to develop high yielding varieties.

Materials and methods

Genetic divergence among twenty-five genotypes (local and exotic) of chilies (*Capsicum annum* L.) was found. The germplasm was collected from Ayub Agriculture Research Institute (AARI) Faisalabad,

Pakistan. Seeds were sown in peat trays to establish nursery and at four true leaf stage seedlings were transplanted in the field keeping 50 cm row-to-row and 30 cm plant-to-plant distance. Experiment was performed following randomized complete block design (RCBD) with two replications. All the plant protection measures, fertilizers and other management practices were applied as per package. At maturity data was collected for different morphological and yield contributing traits during the year 2014 in *Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan*. Five plants were selected in each replication that were tagged and the mean data were used for statistical analysis. Analysis of variance, cluster analysis based on Tocher's method using squared Euclidean distance (Kumar *et al.*, 2012) was performed using the statistical software Indo-stat and statistical package for agricultural research (SPAR) version 2.0 programme. The genetic divergence was calculated according to Mahalanobis D^2 statistics (1936).

Table 1. Name of genotype and code.

Genotypes code	Genotypes name
HP-1	Main levara
HP-2	Main C-9-2012
HP-3	Main UK-1- 2012
HP-4	Main 37-1-2012
HP-5	32-1-2012
HP-6	9-1-2012
HP-7	N-2012
HP-8	I-9 Dolinward
HP-9	C-217
HP-10	S-32-2012
HP-11	Golden prize
HP-12	32-2-2012
HP-13	C-9 -2012
HP-14	C-9 -2012
HP-15	S-5 2012
HP-16	2-29 Ikram1
HP-17	Main 1-26
HP-18	39-1-2012
HP-19	S-120
HP-20	8-35 Ikram 2
HP-21	6-33 Ikram 3
HP-22	4-31 Ikram 4
HP-23	30-3 Ikram -5
HP-24	9-46 Ikram 6
HP-25	5-64-2012

Statistical Analysis

Five plants per plot were selected and the mean data points were used for statistical analysis. Analysis of variance (Steel *et al.*, 1997), cluster analysis based on Tocher's method using squared Euclidean distance (Kumar *et al.*, 2012) was performed using the statistical software Indo-stat and statistical package for agricultural research (SPAR) version 2.0 programme. The genetic divergence was calculated according to Mahalanobis D^2 statistics (1936).

Results and discussion

Phenotypic and genotypic variances observed in all the pepper genotypes for all the characters were significantly different and all the genotypes showed similar behavior towards genotypic and phenotypic coefficient of variation. Heritability estimates of all the characters observed were high (90%) coupled with high values of genetic advance indicating the possibility of improvement through heterosis breeding. Similar findings were observed in the previous studies (Mini and Khader, 2004; Nandadevi and Hosamani, 2003; Das and Maurya, 2004; Rajamony, 2004).

Table 2. Genetic variability, heritability and genetic advance.

Variable	V_G	V_P	h^2_{bs} (%)	GCV	PCV	GA	GA%
FW	17.4881	17.5151	99.85	0.350	0.351	8.608	72.100
FL	3.8196	3.8430	99.39	0.234	0.235	4.014	48.084
PL	0.0982	0.1068	91.92	0.171	0.178	0.619	33.705
PB	1.8472	2.3270	79.38	0.243	0.272	2.494	44.523
INL	0.9101	0.9182	99.11	0.148	0.149	1.956	30.394
PH	200.7022	201.7315	99.49	0.212	0.212	29.109	43.511
SI	1.9471	1.9544	99.63	0.316	0.316	2.869	64.918
FRFW	4.9512	4.9531	99.96	0.600	0.600	4.583	123.570
DRFW	0.1273	0.1328	95.87	0.289	0.295	0.720	58.322
PT	0.1679	0.1693	99.19	0.322	0.323	0.841	65.993
LA	84.5479	84.7151	99.80	0.333	0.333	18.922	68.428
SPF	280.9289	282.1204	99.58	0.218	0.219	34.455	44.825

Five components were selected as principle components with Eigen values > 1. These components exhibited 77.2% of the variation. The first principal component (PC 1) explained 27.2% of total variation in original data, second component (PC 2) explained 18.9%, and third principal component (PC 3) explained 12.5% of variation. The other principal components (PC 4 and PC 12) explained an additional

18.6% of the variation (a total 77.2% of explained variation, Table 2). The %age of variance explained by the 5 components and the correlation between the PC and the original morphological characteristics of the pepper genotypes are shown in Table 2. The 77.2% total variability obtained in PCA showed a moderate percentage of variation (Pla, 1986).

Table 3. Eigen values and proportion of variance explained by the 5 principal components with respect to 25 pepper genotypes.

	PC Axis				
	PC 1	PC 2	PC 3	PC 4	PC 5
Eigen value	3.5396	2.4557	1.6268	1.3316	1.0773
Explained proportion of variation (%)	0.272	0.189	0.125	0.102	0.083
Cumulative proportion of variation (%)	0.272	0.461	0.586	0.689	0.772

** PC (Principal component).

The PC 1 contributed by positive loading of FW, INL, PH, FRFW, DRFW, PT, and Y, followed by negative and minor loading of PL, SI and FL, PB, LA and SPF (Table 2). The PC 2 contributed by positive loading of FW. The PC 3 contributed by positive loading for SI and DRFW followed by negative and minor loading of FW, PL, PB, PT and Y (Table 2). FCSC and FL have been previously reported as significant contributors to the main principal component in morphological characterization of *C. annuum* for the PC 1 (Latournerie *et al.*, 2002; Castanon-Najera *et al.*, 2008). It showed that fruit morphological variables are the principal contributors of the variation in shape of fruit of *C. annuum* L. (Pardey *et al.*, 2006; Moscone *et al.*, 2007; Castanon-Najera *et al.*, 2010).

Data regarding cluster analysis is presented in fig.1. Based on the D² value 25 genotypes were grouped into five clusters. The results showed that Cluster I comprised of 7 genotypes i.e HP-1, HP-15, HP-8, HP-24, HP-18, HP-7 and HP-5. Cluster II also comprised

of 7 genotypes i.e. HP-13, HP-23, HP-22, HP-20, HP-19, HP-21 and HP-25. Cluster III comprised of 3 genotypes i.e. HP-2, HP-9 and HP-14. Cluster IV comprised of 5 genotypes which are HP-3, HP-12, HP-16, HP-11 and HP-17. Cluster V comprised of 3 genotypes i.e. HP-4, HP-6 and HP-10. The clustering pattern showed that genotypes were associated with each other and geographical distribution for the formation of cluster in genetic divergence. Similar findings were also reported by Sreelathakumary (2004) and Indra *et al.* (2000).

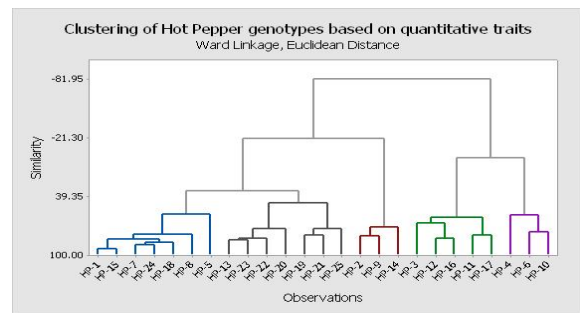


Fig. 1. Clusters of pepper genotypes.

Table 4. Average inter and intra cluster distances of 25 pepper genotypes.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	0.0000	25.3614	26.3753	38.3771	16.0230
Cluster II	25.3614	0.0000	38.4115	59.2612	39.5281
Cluster III	26.3753	38.4115	0.0000	36.0652	36.0656
Cluster IV	38.3771	59.2612	36.0652	0.0000	36.4439
Cluster V	16.0230	39.5281	36.0656	36.4439	0.0000

The data on intra and inter cluster distance have been presented in table 3. Among the different clusters maximum inter Cluster distance (59.2612) was observed in between Cluster II and Cluster IV followed by 39.5281 in Cluster V and Cluster II. Minimum inter cluster distance (16.0230) was observed in Cluster V and Cluster I. Clusters with large inter cluster distance showed that the genotypes included in these clusters have the greater diversity and hence the crossings between the genotypes in these clusters are expected to give desirable combinations. The level of variation found in present genotypes shows high potential for developing pepper genotypes for different desired morphological traits. The results are in line with those of Zewedie and

Zeven (1997) who reported variation among hot pepper accessions.

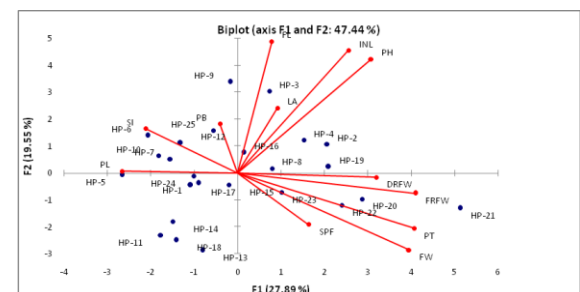


Fig. 2. Bi-plot distribution of traits.

Biplot results showed that 25 genotypes of chili are scattered in four quadrants. Each quadrant showed association of genotypes with different characters.

Quadrant 1 showed that HP-12 has strong association with no. of primary branches and HP-6 correlates with seed index, HP-10 and HP-7 are strongly correlated with peduncle length and seed index. In quadrant 2, HP-3 correlates with fruit length and leaf area index whereas HP-16, HP-4, HP-2, HP-8 and HP-19 are correlated with plant height and internodal distance. Similarly in quadrant 3, HP-15 and HP-23

are strongly correlated with seed per fruit and fruit weight. Genotypes HP-22 and HP-20 have good characters like pericarp thickness, dry and fresh fruit weights whereas genotypes HP-21, HP-24, HP-1, HP-17, HP-14, HP-18, HP-11 and HP-5 can be excluded as these have no significance favorable characteristics for crop improvement program.

Table. 5. Correlation Matrix among 12 Traits of Hot Pepper.

	DRFW	FL	FRFW	FW	INL	LA	PB	PH	PL	PT	SI
FL	0.0665*										
FRFW	0.7499**	-0.0277									
FW	0.3865*	-0.227*	0.6092**								
INL	0.1699	0.6353**	0.2232	0.124							
LA	0.0283	0.0376*	0.1524	-0.0848*	0.2294						
PB	-0.0488	-0.0091	-0.0956	-0.1545	-0.0887*	0.1082					
PH	0.1909	0.5435**	0.2982	0.138	0.7551**	0.3764	0.2243				
PL	-0.3262	-0.131	-0.2748	-0.202	0.1097*	-0.027	0.2062	-0.2495			
PT	0.2802	-0.1861	0.4614	0.7358**	0.2058	0.0547	-0.0671	0.2521	-0.4106		
SI	0.1148	0.0664	-0.165	-0.4429*	-0.0054	0.0683	-0.2902	-0.1205	0.1192	-0.5246**	
SPF	0.0139	-0.0879	0.1299	0.3227	-0.0497	-0.0594	-0.3653	0.1759	-0.0584	0.2516	-0.1801

1. FW = Fruit width

2. FL = Fruit Length

3. PL = Peduncle Length

4. PH = Plant height

5. PB = No. of primary branches

6. INL = Inter nodal length

7. SI = Seed Index

8. FRFW = Fresh red fruit weight

9. DRFW = Dry red fruit weight

10. PT = Pericarp thickness

11. LA = Leaf area

12. SPF = Seeds per fruit

Fruit width has significant positive correlation with dry red fruit weight and highly significant positive correlation with fresh red fruit weight. Highly significant correlation was found between fruit length and dry red fruit weight. Peduncle length has significant correlation with inter nodal length similar results were shown in the previous studies (Kumar *et al.*, 2012 and Sreelathakumary *et al.*, 2002). Number of primary branches has significant negative correlation with intermodal length also found by Prabhakaran *et al.*, 2004. Inter nodal length have positive and significant correlation with fruit length (Choudhury *et al.*, 2004). Plant height has significant positive correlation with fruit length and inter nodal length as found by Bhagyalakshmi *et al.* 1990. Seed index have positive significant correlation with fruit width and negative significant correlation with pericarp thickness (Rathod *et al.*, 2002).

Fresh red fruit weight has highly significant correlation with dry red fruit weight also found in previous research (Acharyya *et al.*, 2003). Pericarp thickness has significant positive correlation with fresh red fruit weight and fruit width (Devi *et al.*, 1999). Leaf area has significant correlation with fruit length and fruit width as concluded by Munshi *et al.* 2000 and Acharyya *et al.* 2003. Seed per fruit have significant negative correlation with PB similar to Chattopadhyay *et al.*, 2011 findings.

Conclusion

It can be concluded that there is abundant variation among the genotypes for most of the characters studied. So, these germplasm may be utilized for future breeding program. Moreover, selection may be effective and rewarding based on those characters having high heritability along with higher genetic advance indicating additive gene effect.

This study may be helpful in selecting superior genotypes for development of varieties with favorable traits.

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