

## GENETIC VARIABILITY FOR MORPHOLOGICAL ATTRIBUTES AND SEED PROTEIN PROFILING IN CHILI (*CAPSICUM ANNUUM* L.)

FAIZA MUSHTAQ, SHAKEEL AHMAD JATOI\*, SYEDA SANA AAMIR AND SADAR UDDIN SIDDIQUI

Plant Genetic Resources Institute, National Agricultural Research Center, Park Road, Islamabad, Pakistan

\*Corresponding author's email: sajatoi@gmail.com

### Abstract

Twenty three chili accessions originating from nine countries including Pakistan were investigated for genetic variability for morphological attributes and total seed protein during the cropping seasons of 2011 and 2012 at National Agricultural Research Centre (NARC), Islamabad, Pakistan. Traits diversity was analyzed by employing statistical tools that revealed not only considerable variation but also grouping on similarities and associations that can accelerate their utilization in research. Considerable differences noted for different morpho-agronomic traits revealed substantial variation for leaf length, plant height, number of locules, fruit width, seed diameter and fruit bearing period. However, pedicel length, fruit wall thickness and number of seeds/fruit displayed low variation in both years. Association among various quantitative parameters based on correlation coefficients was significant. Fruit weight and fruit wall thickness showed significant association with number of fruits, fruit yield per plant, total yield and number of locules. The first three PCs cumulatively contributed 61.44% and 85.88% during 2011 and 2012, respectively. Clustering pattern observed was regardless of their origin. Contrary to variability observed in field evaluation, seed-protein profiling through SDS-PAGE depicted low variation in chili accessions. The genetic variability among chili accessions observed in this study would be a good base line to devise a better crop improvement program in chili.

**Key words:** Capsicum, Genetic diversity, Principal components, SDS-PAGE, Phenotypic variability, Biochemical evaluation.

### Introduction

Chili (*Capsicum annum* L.) is adaptable and valuable cash crop (vegetable, condiment as well as spice crop) grown all over the world (Toquica *et al.*, 2003). They are the only source of an alkaloid capsaicin, which is an important daily diet ingredient helpful in digestion. Carotenoid present in fruit is the key pigment which gives colour to the fruit. Unique aroma is the characteristic of several *C. annum* genotypes. Its flavour, texture, nutrition and colour are the basics of food industry around the globe (Bosland & Vatava, 2000). Differences in environments do not affect its aroma (Abu *et al.*, 2013). During 2014, in Pakistan the area under chili crop was 62.7 thousand hectares with the production of 146.3 thousand tonnes (Anon., 2013-14). Kunri region in the Sindh province is main chili growing area of Pakistan.

Characterization is a powerful tool in developing varieties, identifying traits in germplasm and utilizing it in research and development work (Nisar *et al.*, 2007; Ince *et al.*, 2009). Species can be evaluated by using hybridization, cytology, molecular markers, isozymes and morphology, for diversity assessment, selection, clarifying taxonomic relations, cultivar discrimination and breeding for desirable traits (Akond *et al.*, 2012; Shah *et al.*, 2015; Aamir *et al.*, 2016).

Identification of *Capsicum* species is traditionally done by using morphological descriptors. Morphological characters particularly flower and inflorescence colour of flower and calyx constriction is used as the taxonomic descriptors. Agro-morphological evaluation though considered time consuming and prone to environmental influence, is a more practical way of progressing in the germplasm evaluation process and its worth is still acknowledged (Hussain *et al.*, 2004; Jatoi & Watanabe, 2013).

Identification owing to morphological characters is difficult as most of the characters are influenced by

environment and plant development stage. Moreover, number of morphological descriptors is also limited accession assignment (Ince *et al.*, 2009). For all these reasons concern has been shifted to biochemical methods for germplasm characterization as this method is more sensitive and eliminates or reduces environmental effects (Odeigah & Osanyinpeju, 1996). Various biochemical techniques are being used worldwide for estimating genetic diversity. Sodium-dodecyl-sulphate-polyacrylamide-gel-electrophoresis (SDS-PAGE) is one of the economical and extensively used biochemical techniques to study seed protein diversity (Fufa *et al.*, 2005, Iqbal *et al.*, 2005). Total seed protein profiling in *Capsicum* has also been used for phylogenetic relationship (Panda *et al.*, 1986).

Current study has been undertaken to reveal the extent of genetic variation for different agromorphological traits, total seed protein profile using SDS-PAGE and investigating association in chili germplasm.

### Materials and Methods

The study was carried out at Plant Genetic Resources Institute, NARC, Islamabad, Pakistan, during the cropping season of 2011 and 2012. The germplasm comprised of twenty three chili accessions obtained from National Genebank of Pakistan (local and acquired), NARC, Islamabad. Passport information of chili accessions is given in Table 1. The quantitative traits were selected from *Capsicum* descriptors developed by Biodiversity International and these included plant height(cm), leaf length (cm), leaf width(cm), number of days to flowering, number of flowers per axil, number of days to fruiting, fruit bearing period, single fruit weight (g), fruit length (cm), fruit diameter (cm), fruit pedicel length (cm), fruit wall thickness (mm), number of locules per fruit, seed diameter

(mm), 100-seed weight (g), number of seeds per fruit, number of fruits per plant and fruit yield per plant (g). Various qualitative traits viz. life cycle, plant growth habit, tillering, leaf color, leaf shape, fruit shape, fruit color at mature stage and seed color were observed.

Healthy seeds were used for SDS-PAGE to analyze diversity for total seed protein in chili accessions. Electrophoresis procedure as outlined by Odeigah and Osanyinpeju, 1996 was followed using slab type SDS-PAGE model. For protein extraction 0.5ml Tris-base with 6.8 pH, 0.2% Sodium Dodecylsulphate and 5M urea was used. 400µl of the protein extraction buffer was taken in a 1.5ml micro tube, seed mixed well, centrifuged for 15 minutes followed by electrophoresis until complete bands separation. The gel was stained overnight and it was followed by gel drying at 60°C for 1 hour 30 minutes. The band scoring data was recorded on dried gel.

The quantitative data was subjected to multivariate analysis (Sneath & Sokal, 1973) by using the softwares SPSS version 16 and STATISTICA version 5.0. Euclidean distances among the accessions were calculated according to Ward's method, which was then applied to compose clusters in the form of phenogram. Correlation matrix based on quantitative characters was used to analyze principal components. Inter cluster variation for quantitative parameter was determined by computing mean and standard deviation for each cluster.

## Results and Discussion

**Variability in morphological traits:** The descriptive statistics of the traits studied demonstrated considerable variability among 23 chili accessions (Table 2). The accessions investigated showed a high variations during both years (2011-12) in fruit yield, number of fruits, mature leaf length, leaf width, fruit length, fruit width,

total fruit yield, fruit wall thickness, seed diameter, number of seed per fruit and fruit size (Table 2). Rest of the traits viz., seed diameter, plant height, displayed low to moderate variation in chili germplasm. In 2011, high variation was observed in fruit yield per plant and it was followed by number of fruits per plant, seeds/plant, fruit bearing period, plant height and days to flowering, almost same pattern was observed in 2012, displaying a consistent trend in both years that can be exploited more precisely in future chili breeding.

The variability pattern in days to flowering and fruiting offered opportunity to categorize germplasm into distinct groups that can be tested for adaptability into diverse ecologies particularly in areas where summer season is short. Similar could be the case with plant height. Small stature plants could be suitable for wind blowing areas as compared to tall plants that cannot withstand high winds. These traits may also be included in the future breeding in chili for developing climate resilient superior genotypes. Although the germplasm investigated is limited but showed diverse genetic base, probably due to originating from different countries.

The success in the breeding program rely mainly on the availability of substantial knowledge on variability, genetic diversity, relationship among traits and the extent of contribution of each character to desired trait e.g. fruit yield. This can be achieved only when we evaluate the germplasm for variability and divergence at different levels that provide the basis for selection. The genetic diversity available within the various domesticated *Capsicum* species has been very little exploited and prospects are good for the further improvement of all the cultivated species of *Capsicum* through breeding (Pickersgill, 1997). Substantial genetic variability in any crop plant is a key to ensure high productivity.

**Table 1. Passport information of 23 chili accessions investigated.**

Codes	Genus	Accessions	Plant ID	Origin
C1	<i>Capsicum annum</i>	Grif 14045	Grif 14046	India, Assam
C2	<i>Capsicum annum</i>	Grif 14046	Grif 14047	India, Assam
C3	<i>Capsicum annum</i>	PI 123469 01	2428	India
C4	<i>Capsicum annum</i>	PI 18440	Mircha	India
C5	<i>Capsicum annum</i>	PI 273415	Longhot	Italy
C6	<i>Capsicum annum</i>	PI 288301	39	India
C8	<i>Capsicum annum</i>	PI 371940	Habanero	Mexico
C10	<i>Capsicum annum</i>	PI 639647	S034	Syria
C12	<i>Capsicum annum</i>	PI 640473	India#1	India
C13	<i>Capsicum annum</i>	PI 640508	Calcutta Round #1	India
C14	<i>Capsicum annum</i>	PI 640547	Hisarvijay	India
C16	<i>Capsicum annum</i>	PI 640605	NP46A	India
C17	<i>Capsicum annum</i>	PI 640635	PusaSadabahar	India
C18	<i>Capsicum annum</i>	PI 640818	Hot Shot	Philippines
C19	<i>Capsicum annum</i>	PI 645487	Grif 14044	Panama
C20	<i>Capsicum annum</i>	PI 645517	Tiwari II	India
C21	<i>Capsicum annum</i>	PI 653639	PS-2	India
C22	<i>Capsicum annum</i>	PI 659098	Wenk's Yellow Hots	United State
C29	<i>Capsicum annum</i>	001780	PAK0010468	Pakistan
C35	<i>Capsicum annum</i>	03425-B	--	Pakistan
C37	<i>Capsicum annum</i>	BGV0000534	--	Spain
C39	<i>Capsicum annum</i>	027896	CH-3	Pakistan
C42	<i>Capsicum annum</i>	027897	Um Pai	Thailand

**Table 2. Descriptive statistics showing variability profile of various plant traits in chili.**

Traits	Mean $\pm$ SE	S.Dev	Variance	Range
<b>2011</b>				
Plant height (cm)	52.11 $\pm$ 2.03	14.03	196.87	20-87
Leaf length (cm)	7.54 $\pm$ 0.33	1.6	2.55	4.33-11.6
Leaf width (cm)	3.63 $\pm$ 0.34	1.62	2.63	1.41-4.47
Days to flowering	66.34 $\pm$ 1.54	7.38	54.44	48-81
Flowers/axil	1.92.19 $\pm$ 0.19	0.9	0.82	1-4
Days to fruiting	78.44 $\pm$ 2.28	10.69	114.3	54.22-98
Fruit bearing period	98.31 $\pm$ 3.19	15.3	234.12	62.5-115.33
Fruit length(cm)	5.44 $\pm$ 0.38	1.82	3.3	2.3-9.25
Fruit width(cm)	1.82 $\pm$ 0.21	1.03	1.06	0.61-4.48
Single fruit wt (g)	4.65 $\pm$ 1.37	6.58	43.3	0.51-31.98
Fruit pedicel length (cm)	2.55 $\pm$ 0.14	0.67	0.44	1.4-3.8
Fruit wall thickness (mm)	1.49 $\pm$ 0.15	0.72	0.52	0.5-3.27
Number of locules	2.41 $\pm$ 0.07	0.36	0.13	2-3.33
Seed diameter (mm)	3.22 $\pm$ 0.11	0.53	0.28	2.12-4.25
100-seed wt (g)	0.63 $\pm$ 0.03	0.15	0.02	0.37-0.93
Seeds/ fruit	39.31 $\pm$ 3.92	18.82	354.03	7-78
Fruit/plant	188.33 $\pm$ 30.68	147.14	21651.62	5-476
Fruit yield/ plant (g)	425.95 $\pm$ 58.82	282.11	79585.44	16.7-1134
<b>2012</b>				
Plant height (cm)	41.98 $\pm$ 2.40	11.5	132.31	22-63.4
Leaf length (cm)	4.12 $\pm$ 0.23	1.09	1.2	2.03-7.77
Leaf width (cm)	1.51 $\pm$ 0.11	0.53	0.28	0.38-2.63
Days to flowering	65.7 $\pm$ 2.06	9.86	97.13	49-83
Flowers/axil	1.23 $\pm$ 0.10	0.5	0.25	1-3
Days to fruiting	80.17 $\pm$ 2.28	10.71	114.68	62-102.25
Fruit bearing period	110.51 $\pm$ 4.64	22.23	494.3	65-144.5
Fruit length(cm)	4.31 $\pm$ 0.22	1.04	1.07	2.49-6.22
Fruit width(cm)	1.41 $\pm$ 0.12	0.56	0.32	0.59-2.84
Single fruit wt (g)	2.77 $\pm$ 0.46	2.21	4.89	0.77-11.9
Fruit pedicel length (cm)	1.92 $\pm$ 0.10	0.46	0.21	1.36-3.7
Fruit wall thickness (mm)	1.41 $\pm$ 0.11	0.51	0.26	0.79-2.9
Number of locules	2.33 $\pm$ 0.05	0.22	0.05	2.04-2.8
Seed diameter (mm)	3.51 $\pm$ 0.07	0.32	0.1	2.98-4.23
100-seed wt (g)	0.55 $\pm$ 0.03	0.15	0.02	0.23-0.8
Seeds/ fruit	41.62 $\pm$ 3.57	17.12	292.94	7.8-77.6
Fruit/plant	99.29 $\pm$ 18.09	86.75	7525.12	5.9-344
Fruit yield/ plant (g)	58.54 $\pm$ 12.14	58.23	3390.83	1.2-236

**Relationship among chili accessions:** Multivariate analysis based on Cluster analysis grouped twenty three chili accessions into three clusters in 2011 (Fig. 1). Cluster I contained two genotypes C1 and C2 that displayed close resemblances with each other and divergence from rest of the accessions. Cluster 2 contained eighteen genotypes that were grouped into various small sub-clusters that were originated from 7 countries. Cluster III comprised two accessions viz. C19 (Panama) and C42 (Thailand) that made a diverse cluster, however, one accession from Syria (C10) formed a separate taxonomic unit. Compared mean values of both clusters showed the differences between clusters for specific traits. Plant height showed highest mean value in cluster I while low in cluster II (Table 3). Number of fruits showed high mean value in cluster I while low in cluster II. Average fruit yield per plant had high mean value in cluster I than in cluster II. Seed diameter had low mean value in cluster I and high in cluster II. Three Pakistani accessions C29, C35 and C39 were present in same cluster but in different sub-clusters in both years, this showed their same origin and closeness but somehow different characters e.g. C35.

Grouping pattern in three clusters was almost similar to clustering arrangement in 2011. In 2012, chili germplasm grouped into 3 clusters in which small cluster I and III comprised 2 and 3 genotypes, respectively. Cluster II further split into 2 sub-clusters that contained rest of the chili accessions. However, within cluster II, arrangement of genotypes was different as compared to 2011. It was noted that two genotypes from India (C1 and C2) and one each from Panama (C19) and Thailand (C42) showed divergence from rest of the accessions and grouped separately into two different clusters in both the years wherein C13 and C10 were influenced by the environmental variation over the year. Cluster analysis grouped both exotic and local chili accessions together which displayed high genetic similarity regardless of their origin. The genetic variability among these clusters facilitates various breeding techniques like selection and hybridization for further improvements in chili. Clustering pattern demonstrates relatedness among genotypes which could be due to common ancestral background (Aras *et al.*, 2005) and low genetic divergence can be depicted as narrow genetic base (Akond *et al.*, 2012).

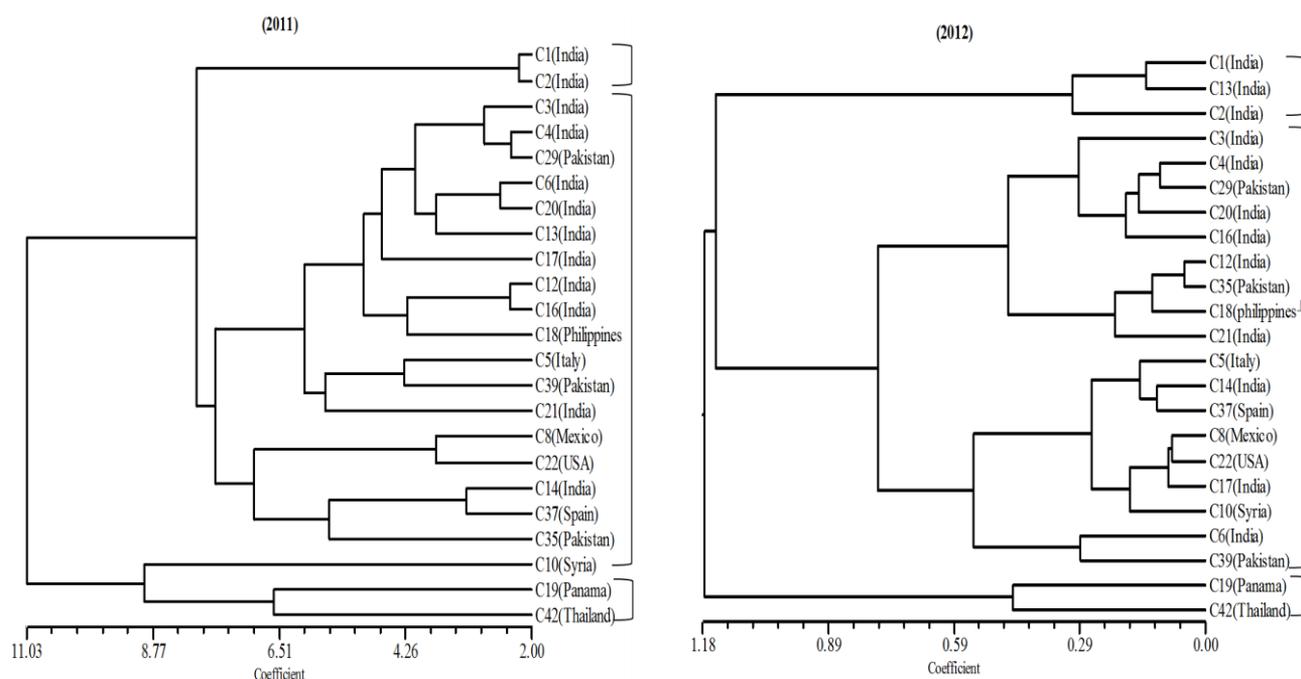


Fig. 1. Cluster analysis showing grouping pattern of various chili accessions (2011 and 2012). Accession codes correspond to list given in Table 1.

**Table 3. Inter cluster variation observed in chili accessions in 2011 and 2012.**

	2011			2012		
	Group I	Group II	Group III	Group I	Group II	Group III
PH	33 ± 18.38	54.48 ± 12.69	42.5 ± 0	40 ± 24.04	44.53 ± 11.42	53.3 ± 14.28
LL	10.25 ± 1.92	7.28 ± 1.38	7.2 ± 0	6.12 ± 2.33	3.89 ± 0.69	4.29 ± 1.82
LW	5.47 ± 0.38	3.15 ± 0.80	9.51 ± 0	4.05 ± 0.78	2.45 ± 1.55	2.11 ± 1.68
DF	78.75 ± 0.35	65.32 ± 6.69	62 ± 0	76.5 ± 6.36	64.81 ± 9.21	63.4 ± 18.10
F/A	2.5 ± 2.12	1.85 ± 0.82	2 ± 0	2.50 ± 0.71	1.26 ± 0.55	1 ± 0
DFr	100.25 ± 6.72	77.56 ± 10.51	79 ± 0	97 ± 7.07	80.04 ± 10.16	75.5 ± 19.09
FBP	75.75 ± 18.74	99.91 ± 13.68	112 ± 0	75 ± 14.14	112.08 ± 19.85	130.8 ± 13.85
FL	4.32 ± 1.25	5.36 ± 1.68	9.25 ± 0	4.67 ± 1.37	4.66 ± 1.23	3.16 ± 0.95
FW	2.82 ± 0.40	1.44 ± 0.67	4.48 ± 0	2.82 ± 0.03	1.31 ± 0.57	1.11 ± 0.37
SFW	7.34 ± 4.28	3.02 ± 2.51	31.97 ± 0	7.91 ± 5.67	3.43 ± 5.12	1.41 ± 0.62
FPL	3.63 ± 0.24	2.43 ± 0.60	2.9 ± 0	2.87 ± 1.17	1.81 ± 0.24	2.11 ± 0.21
FWT	2.93 ± 0.47	1.29 ± 0.51	2.64 ± 0	2.65 ± 0.36	1.27 ± 0.33	1.21 ± 0.48
NL	3.02 ± 0.45	2.31 ± 0.24	3.21 ± 0	2.60 ± 0.28	2.30 ± 0.21	2.32 ± 0.56
SD	3.17 ± 0.47	3.18 ± 0.51	4.25 ± 0	3.82 ± 0.59	3.49 ± 0.27	3.39 ± 0.58
100SW	0.56 ± 0.14	0.64 ± 0.16	0.71 ± 0	0.60 ± 0.28	0.55 ± 0.13	0.51 ± 0.35
S/F	39.5 ± 45.96	43.15 ± 15.28	33 ± 0	38.9 ± 3.98	45.59 ± 14.63	41.58 ± 3.48
F/P	15.5 ± 3.54	231.85 ± 129.03	19 ± 0	11 ± 1.41	185.36 ± 112.48	362.25 ± 25.79
FY/P	55.41 ± 0.88	467.24 ± 257.65	603.07 ± 0	51.95 ± 0.07	454.95 ± 231.25	226.1 ± 14.28

PH= Plant height (cm), LL= Leaf length (cm), LW= Leaf width (cm), DF= Days to flowering, F/A= Number of flower/axil, DFr= Days to fruiting, FBP= Fruit bearing period, FL= Fruit length (cm), FW= Fruit width (cm), SFW= Single fruit weight (g), FPL= Fruit pedicel length (cm), FWT= Fruit wall thickness (mm), NL= Number of locules, SD= Seed diameter (mm), 100-SW= 100-seed weight(g), S/F= Number of seeds/fruit, F/P= Total number of fruits/ plant, FY/P= Fruit yield per plant (g)

**Association in various morphological traits:** The correlation coefficient computed among 18 quantitative traits in chili displayed significant relationship among various parameters (Table 4). Leaf length showed a positive and significant relationship with days to fruiting, fruit width and fruit wall thickness in both years. Leaf width was significantly correlated with fruit width, single fruit weight, fruit wall thickness, number of locules and seed diameter. Number of days to flowering displayed positive association with days to fruiting, whereas, it negatively correlated with fruit bearing period. Future studies on the interaction of these contrasting traits will reveal insight on gene interaction of leaf length and thickness. Fruit wall thickness was also revealed to have negative association with fruit bearing period. Single fruit weight and fruit length had a highly significant positive correlation with each other in 2011 and 2012. Fruit width was strongly associated with single fruit weight, fruit wall thickness, number of locules and seed diameter; whereas, it showed negative but highly significant relationship with number of fruits per plant. Similar pattern was also observed for single fruit weight with these parameters. Number of fruits per plant displayed negative and highly significant correlation with fruit wall thickness; whereas, it was highly significant and positively correlated with number of locules. Correlation analysis provided a tool to select combination of desirable traits and these parameters can be prioritized and exploited for improving fruit yield in chili. The correlation among different traits is an important feature for any breeding program on account of the reason that it offers the probabilities for genotype selection having desirable traits (Aamir *et al.*, 2016). Shafiq *et al.*, (2006) illustrated negative and positive correlations between all the morphological traits. It was observed that genotypes with large fruit size had a fewer number of fruits per plant as compared to the genotypes with small sized fruits that were abundant. Flowers tend to abort facilitating other larger fruits to reach maturity than small fruiting cultivars (Rodriguez *et al.*, 2008). The same pattern has also been observed by Nsabiya *et al.*, (2013). Such trend observed in present study has also been strongly supported by the significant and negative correlation between single fruit weight and number of fruits per plant during both the years; challenging breeders to attain large fruits in high numbers through conventional breeding.

**Grouping pattern among chili accessions:** Field evaluation data of various traits of chili was subjected to principal component analysis that provided a useful distribution pattern on scatter plot for both years (Fig. 2). The contribution of the first three PCs towards total variation was 61.44% and 85.88% for 2011 and 2012, respectively showing considerable variability in chili germplasm under investigation (Table 5). Grouping pattern of chili revealed by PCA complemented the clustering arrangement reflected by cluster analysis. C1 & C2 and C19 & C20 remained distinctive on the scatter plot reflecting genetic divergence. The large fraction of accessions, though present in each quarter, spotted in the central region of the scatter plot. Three representative accessions from Pakistan (C29, C35 and C39) grouped apart from each other displaying genetic divergence. Similarly one accession from each Thailand and Panama maintained their distinctiveness from rest of the chili germplasm assayed through both methods of multivariate analyses.

The accessions were scattered in all quarters revealing high variability (Fig. 2). In PC1 accession C1, C2, C10, C19, C42 showed high variation in both years. Variability among these accessions is associated with differences in morpho-agronomic traits. This distinctiveness was confirmed by bi-plotting the principal components. The distribution of the chili accessions on scatter plot depicted diverse base of chili germplasm. This variability can be exploited for developing new varieties. Including highly diverse genotypes in the breeding programme in chili could be suitable choice (Nsabiya *et al.*, 2013, Bibi *et al.*, 2017) since yield is a complex trait controlled by various components, it is thus imperative to know the inter-relationship between yield and its components to arrive at an optimal selection index for improvement. Individual fruit weight and number of fruits per plant are considered major yield contributing traits that needs to be focused in crop improvement programme (Bozokalfa & Kilic, 2010). In this study we found PCA useful in fragmenting the magnitude of the genetic diversity in the germplasm as reported by Brown-Guedira, 2000. The plot separated the germplasm of chili according to morphological traits into different groups nearly confirming the preceding results of cluster analysis.

**Qualitative traits:** Qualitative descriptors are the valuable tool to identify *Capsicum* species and can potentially be used in various production sectors (Sudré *et al.*, 2010). The growth habit of major fraction of chili genotypes was erect type (46.8% & 38.1%) followed by intermediate (36.1% & 28.5%) and prostrate (17.0% & 33.3%) in 2011 and 2012, respectively (Table 6). In 2011-2012 tillering in plants was intermediate in 46.8% and 27.0% genotypes, dense tillering (17.0% & 12.5%) and remaining genotypes showed sparse tillering (36.1% and 60.4%). Chili genotypes at intermediate stage with dark green leaf colour were 57.83% & 61.4%, light green 17.0% & 7.29%, light purple 25.17% & 30.2% and variegated color 0% & 1.0% in 2011 and 2012, respectively. Leaf shape recorded at intermediate stage was 42.5% & 26.0% lanceolate shaped while 23.4% & 43.7% deltoid and 34.0% & 30.2% ovate shaped found in 2011 and 2012, respectively.

Similarly for fruit colour at mature stage 59.5% & 66.83% plants showed red color and 31.9% & 24.17% had dark red coloration and 8.5% and 9% were light red in color during 2011 and 2012, respectively. In 2011 fruit shape at mature stage was elongated in 57.4%, triangle shape in 25.3%, round shaped in 12.77%, companulate in 4.2%, whereas in 2012 for the same categories elongated were 59.3%, triangular 35.42%, round 4.1% found almost round and companulate 1.0%. Fruit shape and fruit color (intermediate and ripen) are the potential morphological markers that can discriminate among different genotypes particularly varieties (Peeraulle and Ranghoo-Sanmukhiya, 2013). The seed color recorded after maturity displayed 55.32% & 63.75% accessions having straw color, whereas 27.66% & 23.5 accessions were white, 10.6% & 6.25% brown and 6.38% & 4.5% were black in color during 2011 and 2012, respectively (Table 6). The yearly deviations in characters might be attributed to environmental conditions affecting the quality of chili plants as some characters may also species specific (Riaz *et al.*, 2011).

Table 4. Trait associations among various traits in chili based on simple correlation coefficients. The lower diagonal shows 2011 and upper diagonal shows 2012.

	PH	LL	LW	DF	F/A	Dfr	FBP	FL	FW	SFW	FPL	FWT	NL	SD	100SW	S/F	F/P	FY/P
PH	0.05	-0.24	0.01	0.09	0.02	0.11	0.11	-0.17	-0.29	-0.33	-0.18	-0.18	-0.51*	-0.06	-0.23	0.36	0.43*	-0.07
LL	-0.03	0.24	0.55**	0.29	0.46*	-0.31	-0.31	0.19	0.54**	0.39	0.28	0.56**	0.13	0.67**	0.18	0.29	-0.39	-0.26
LW	-0.21	0.50*	0.23	0.47*	0.27	-0.38	-0.38	0.34	0.79**	0.80**	0.13	0.59**	0.55**	0.58**	0.47*	-0.22	-0.42	0.18
DF	-0.09	0.63**	0.29	0.36	0.92**	-0.44*	-0.44*	-0.09	0.35	0.20	0.18	0.44*	-0.15	0.36	0.34	-0.04	-0.44*	-0.18
F/A	-0.15	0.21	0.31	0.33	0.47*	-0.50*	-0.50*	0.06	0.50*	0.43*	0.10	0.43*	0.26	0.28	0.10	0.03	-0.16	-0.10
Dfr	-0.27	0.56**	0.30	0.79**	0.01	-0.53**	-0.53**	-0.20	0.33	0.16	0.20	0.43*	-0.05	0.31	0.25	-0.03	-0.46*	-0.07
FBP	0.16	-0.30	-0.16	0.54**	-0.25	-0.28	-0.28	0.03	-0.40	-0.20	-0.33	-0.50*	-0.17	-0.33	-0.23	0.09	0.35	0.01
FL	0.001	0.22	0.37	-0.13	-0.29	0.12	0.12	0.12	0.33	0.56**	-0.04	0.14	-0.03	0.34	0.00	0.03	-0.33	0.11
FW	-0.16	0.43*	0.84**	0.08	0.24	0.03	-0.26	0.23	0.81**	0.34	0.34	0.88**	0.57**	0.64*	0.53**	-0.13	-0.67**	-0.14
SFW	-0.18	0.21	0.85**	-0.05	0.05	0.06	0.06	0.53**	0.83**	-0.06	-0.06	0.49*	0.47*	0.59**	0.34	-0.09	-0.48*	0.03
FPL	-0.16	0.45*	0.34	0.46*	-0.15	0.51*	-0.39	0.38	0.19	0.25	0.54**	0.20	-0.15	-0.15	0.29	-0.34	-0.23	-0.44*
FWT	-0.34	0.45*	0.69**	0.28	0.35	0.12	-0.58**	-0.04	0.84**	0.52*	0.32	0.47*	0.60**	0.54**	0.54**	-0.15	-0.65**	-0.22
NL	-0.37	0.44*	0.72**	0.33	0.34	0.33	-0.26	-0.05	0.63**	0.48*	0.19	0.72**	0.31	0.17	0.17	-0.19	-0.27	0.11
SD	-0.20	0.24	0.46*	0.22	-0.03	0.18	-0.07	0.43*	0.46*	0.52*	0.19	0.38	0.22	0.10	0.31	0.25	-0.49*	0.18
100SW	-0.16	-0.39	-0.06	-0.34	-0.05	-0.30	-0.30	0.11	0.08	0.07	-0.06	0.19	-0.24	0.13	-0.14	-0.43*	-0.09	-0.09
S/F	0.24	0.44*	-0.51*	0.06	-0.05	0.01	0.15	0.08	0.21	0.06	-0.08	-0.03	-0.06	0.10	-0.51*	0.18	0.18	0.30
F/P	0.25	-0.33	-0.51	-0.04	-0.06	-0.12	0.40	-0.26	-0.65**	-0.54**	-0.49*	-0.66**	-0.39	-0.30	-0.21	-0.05	-0.43*	0.15
FY/P	0.29	0.07	0.05	-0.13	0.07	-0.19	0.15	0.35	0.10	0.14	-0.40	-0.17	-0.10	0.07	-0.21	0.47*	0.24	0.24

PH= Plant height (cm), LL=Leaf length (cm), LW=leaf width (cm), DF= days to flowering, F/A=number of flower/axil, Dfr=days to fruiting, FBP=fruit bearing period, FL=fruit length (cm), FW=fruit width (cm), SFW= single fruit weight (g), FPL=fruit pedicel length (cm), FWT= fruit wall thickness (mm), NL=number of locules, SD=seed diameter (mm), 100-SW=100-seed weight(g), S/F=number of seeds/fruit, F/P=total number of fruits/ plant, FY/P=fruit yield per plant (g)

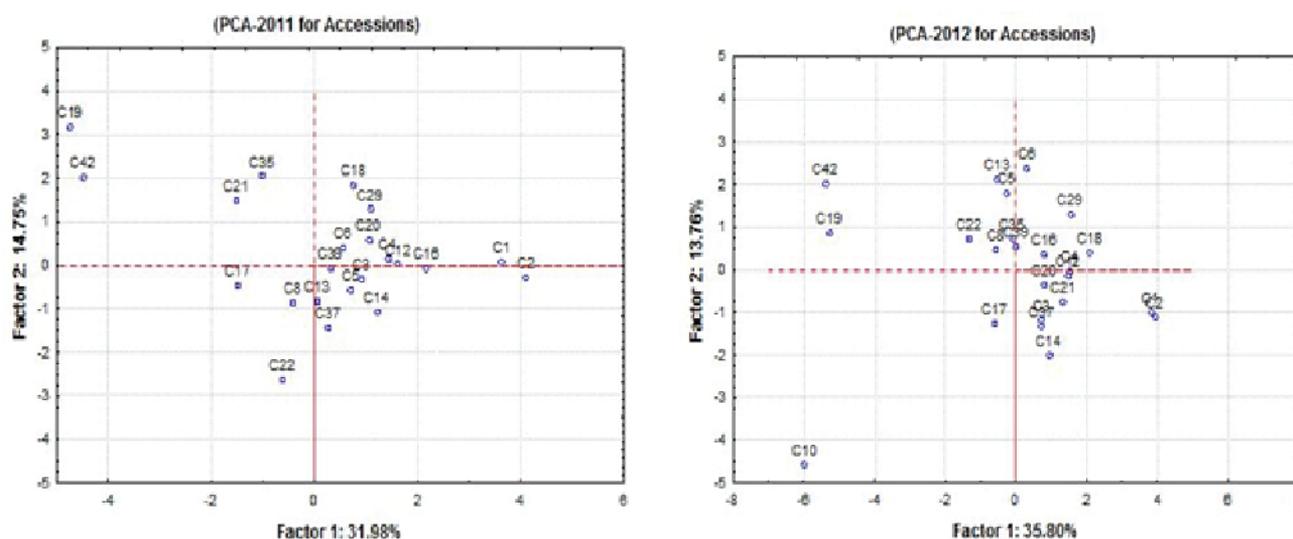


Fig. 2. Two dimensional scatter plot displaying genetic relationship among 23 chili accessions in 2011 and 2012. Accession codes correspond to list given in Table 1.

**Table 5. Eigenvectors, Eigenvalues, total variance, and cumulative variance for 18 morpho-agronomic traits of chili genotypes (2011-12).**

Traits	2011			2012		
	PC1	PC2	PC3	PC1	PC2	PC3
Total variance (%)	35.80	13.76	11.88	68.66	10.70	6.52
Cumulative variance (%)	35.8	49.56	61.44	68.66	79.36	85.88
<b>Accessions</b>						
Total variance (%)	31.98	14.78	11.35	35.80	13.76	11.88
Cumulative variance (%)	31.98	46.76	58.11	35.80	49.56	61.44

**Table 6. Qualitative characters observed in the chili germplasm in 2011 and 2012.**

Trait/Parameter	2011	2012
	% Age	% Age
<b>Plant growth habit</b>		
Prostrate	17.02	33.33
Intermediate	36.17	28.54
Erect	46.81	38.13
<b>Tillering</b>		
Sparse	36.17	60.42
Intermediate	46.81	27.08
Dense	17.02	12.50
<b>Fruit colour at mature stage</b>		
Light red	8.51	9
Red	59.57	66.83
Dark red	31.91	24.17
<b>Fruit shape</b>		
Elongate	57.45	59.38
Almost round	12.77	4.17
Triangle	25.53	35.42
Companulate	4.26	1.04
<b>Leaf colour</b>		
Light green	17	7.29
Dark green	57.83	61.46
Light purple	25.17	30.21
Variegate	0	1.04
<b>Leaf shape</b>		
Deltoid	23.40	43.75
Ovate	34.04	30.21
Lanceolate	42.55	26.04
<b>Seed colour</b>		
Straw	55.32	63.75
Brown	10.64	6.25
Black	6.38	4.5
White	27.66	23.5

**Biochemical evaluation:** Low variation in terms of molecular weight was observed in the distribution pattern of seed-protein in chili accessions. Chili varieties were possible to separate into prominent groups by the differences in staining intensities and the absence or presence of bands (Odeigah and Osanyinpeju, 1996). The polypeptide bands were major, intermediate and minor, based on staining intensities. However, these accessions were grouped into different clusters regardless of their origin. Robert *et al.*, (1985) have reported the conservation of peptide sequences among the storage proteins from several legumes and cereals. These observations imply that the storage proteins are coded by ancient genes which have been conserved during evolution (Hari & Okita, 1986). It is suggested that the protein profile of seed storage proteins in chilies are also well conserved and show little variation among different accessions, or this may be particular to accessions selected in this study.

Chili germplasm assayed reveal moderate to high variation for various agro-morphological traits however, low variability for seed storage proteins was observed in this study. Trait association through simple correlation coefficients, either positive or negative, provided deep insight that could be useful for devising further targeted and meaningful studies for crop improvement in chili. Multivariate analyses helped to understand the grouping pattern of the chili germplasm into various clusters and sub-clusters. The study suggests strategy for the germplasm with high variation in characters like plant

height, fruit bearing period, number seeds/fruit, fruit yield/plant and number of fruit/plant, to be incorporated in the chili breeding program and traits with low genetic variability should be collected and acquired from diverse ecologies to broaden the genetic base.

## References

- Aamir, S.S., M.M.Q. Baig, A. Ghafoor, I.A. Hafiz, T. Ahmad, N.A. Abbasi, I. Ali and M. Yaseen. 2016. Molecular and morphological characterization of Rose mutants produced via *In vitro* mutagenesis. *Phill. Agric. Scientist*, 99(1): 90-98.
- Abu, N.E., M.I. Uguru and I.U. Obi. 2013. Genetic stability and correlation among quantitative characters in genotypes of aromatic pepper grown over years. *Academic J.*, 12(20): 2792-2801.
- Akond, M., S. Jin and X. Wang. 2012. Molecular characterization of selected wild species and miniature roses based on SSR markers. *Scientia Horticulturae*, 147: 89-97.
- Anonymous. 2013-14. Agriculture Statistics of Pakistan, (GOP). Ministry of National Food Security and Research (Economic Wing), Islamabad, Pakistan.
- Aras, S., J.B. Polat, D. Cansaran and G. Soylemezoglu. 2005. RAPD analysis of genetic relations between BUZGULU grape cultivar (*Vitisvinifera*) grown in different parts of Turkey. *Acta Biol. Cracov. Ser. Bot.*, 47: 77-82.
- Bibi, K., Inamullah, H. Ahmad, A. Bibi, R. Masood, H. Khan and Aziz-ud-Din. 2017. Genetic diversity assessment of indigenous wheat germplasm from different areas of Pakistan using agro-morphometric traits. *Pak. J. Bot.*, 49(SI): 221-227
- Bosland, P.W. and E.J. Votava. 2000. Peppers: Vegetable and spice *Capsicums*. CABI publishing Wallingford. pp. 204.
- Bozokalfa, M.K. and M. Kilic. 2010. Mathematical modeling in the estimation of pepper (*Capsicum annum* L.) fruit volume. *Chilean J. Agri. Res.*, 70: 626-632.
- Brown-Guedira, G.L., J.A. Thompson, R.L. Nelson and M.L. Warburton. 2000. Evaluation of genetic diversity of soybean introductions and North American ancestors using RAPD and SSR markers. *Crop Sci.*, 40(3): 815-823.
- Fufa, H., P.S. Baenziger, B.S. Beecher, L. Dweikat, R.A. Graybosch and K.M. Eskridge. 2005. Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. *Euphytica*, 145(2): 133-146.
- Hari, B.K. and T.W. Okita. 1986. Structural relationship among the rice glutelin polypeptides. *Plant Physi.*, 81: 748-753.
- Hussain, A. and M.A. Khan. 2007. Effect of growth regulators on stem cutting of *Rosa bourboniana* and *Rosa gruss-an-teplitz*. *J. Agri. Biol.*, 6(5): 931-932.
- Ince, A.G., M. Karaca and A.N. Onus. 2009. Development and utilization of diagnostic DAMD-PCR markers for *Capsicum* accessions. *Genet Resour Crop Evol.*, 56: 211-221.
- Iqbal, S.H., A. Ghafoor and N. Ayub. 2005. Relationship between SDS-PAGE markers and Ascochyta blight in chickpea. *Pak. J. Bot.*, 37(1): 87-95.
- Jatoi, S.A. and K.N. Watanabe. 2013. Diversity analysis and relationships among ginger landraces. *Pak. J. Bot.*, 45(4): 1203-1214.
- Nisar, M., A. Ghafoor, M.R. Khan, H. Ahmad, A.S. Qureshi and H. Ali. 2007. Genetic diversity and geographic relationship among local and exotic chickpea germplasm. *Pak. J. Bot.*, 39(5): 1575-1581.
- Nsabiyeera, V., M. Logose, M. Ochwo-Ssemakula, P. Sseruwagi, P. Gibson and C. Ojiewo. 2013. Morphological characterization of local and exotic hot pepper (*Capsicum annum* L.) collections in Uganda. *Bioremediation, Biodiversity and Bioavailability*, 7: 22-32.
- Odeigah, P.G.C. and A.O. Osanyinpeju. 1996. Seed protein electrophoretic characterization of cowpea (*Vignaunguiculata*) germplasm from IITA gene bank. *Genet Res Crop Evol.*, 43: 485-491.
- Panda, R.C., O.A. Kumar and K.R. Rao. 1986. The use of seed protein electrophoresis in the study of phylogenetic relationships in chili pepper (*Capsicum annum* L.). *Theoretical and Applied Genetics*, 72(5): 665-670.
- Peeraullee, N. and V.M. Ranghoo-Sanmukhiya. 2013. Assessment of genetic diversity in local chili (*Capsicum annum*) varieties in Mauritius. *Int. J. Agric. Biol.*, 15(5): 891-896.
- Pickersgill, B. 1997. Genetic resources and breeding of *Capsicum* spp. *Euphytica*, 96: 129-133.
- Riaz, A., M. Hameed, A.I. Khan, A. Younis and F.S. Awan. 2011. Assessment of biodiversity based on morphological characteristics and RAPD markers among genotypes of wild rose species. *Afri. J. Biotech.*, 10 (59): 12520-12526.
- Robert L.S., K. Adeli and I. Altosaar. 1985. Homology among 3S and 7S globulins from cereals and pea. *Plant Physiol.*, 78: 812-816.
- Rodriguez, Y., T. Depestre and O. Gomez. 2008. Efficiency of selection in pepper lines (*Capsicum annum*), from four sub-populations, in characters of productive interest. *CienciaeInvestigacionAgraria*, 35: 29-40.
- Shafiq, M., A. Younas, M.A. Khan, A.A. Khan and A. Riaz. 2006. Correlation studies in Rosa species under Faisalabad (Pakistan) conditions. *J. Agric. Soc. Sci.*, 2(1): 58-59.
- Shah, S.M., K. Aslam, G. Shabir, A.R. Khan, B.H. Abbasi, Z.K. Shinwari and M. Arif. 2015. Population structure and diversity of the AA genome of rice based on simple sequence repeats variation in organelle genome. *Pak. J. Bot.*, 47(5): 1773-1782.
- Sneath, P.H.A. and R.R. Sokal. 1973. Numerical Taxonomy: The Principles and Practice of Numerical Classification. Freeman, San Francisco, CA. 573 pp.
- Sudré, C.P., L.S.A. Gonçalves, R. Rodrigues, A.T. do AmaralJúnior, E.M. Riva-Souza and C. dos S. Bento. 2010. Genetic variability in domesticated *Capsicum* spp as assessed by morphological and agronomic data in mixed statistical analysis. *Genetics and Molecular Research*, 9(1): 283-294.
- Toquica, S.P., F. Rodriguez, E. Martinez, M.C. Duque and J. Tohme. 2003. Molecular characterization by AFLPs of *Capsicum* germplasm from the Amazon department in Colombia, characterization by AFLPs of *Capsicum*. *Genet Resour. Crop Evol.*, 50: 639-647.