

## Genetic Diversity, Phylogenetic Tree and Principal Component Analysis Based on Morpho-Metric Traits of Assam Chilli

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### Abstract

We evaluated a set of 37 chilli genotypes collected and maintained at Assam Agricultural University, Jorhat for 27 different traits related to plant habit (5), leaf (6), flower (2), fruit (13) and biotic stress (1). The variation in fruit yield among the genotypes could be attributed to high coefficients of variability for component traits viz., number of fruits per plant (91.7%), plant height (80.8%), leaf breadth (55.9%), fruit weight (49.7%), leaf length (45.4%), fruit length (35.8%), fruit breadth (35.5%) and number of branches per plant (22.2%). Maximum phenotypic variants were observed for fruit traits followed by leaf characteristics. Phylogenetic analysis revealed Euclidean distances varying from a minimum of 2.065 and a maximum of 13.311 indicating the diverse nature of the genotypes. UPGMA clustering grouped the genotypes into 5 distinct clusters. The largest one, cluster I, had 26 genotypes belonging to *Capsicum annuum* var. *acuminatum*. Cluster II consisted of *Capsicum annuum* var. *conooides* with cone-shaped fruits. Cluster III included Moni Jolokia, a perennial shrub with cone-shaped globose erect fruits which clustered in between the other local *C. annuum* sp. Bireek and Mem Jolokia. The fourth cluster (IV) included the local chilli genotypes - Mem Jolokia, Bhokuri Jolokia and Haitha Jolokia which were perennial, with green stem and leaves. Cluster V included the *C. chinense* genotypes consisting of Manipuri Bhut, Bor Bhut and Lota Bhut. The first principal component explained 34.93% of the total variation contributed by mostly leaf and fruit characteristics. The fruit characters in this component showed significant positive correlation with leaf length, breadth and plant height indicating their importance in the morphological characterization of the chilli genotypes.

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## Introduction

Chilli (*Capsicum annum* L.) ( $2n = 24$ ) of the *Solanaceae* family is a dicotyledonous flowering plant grown worldwide [1] and is a very important spice and vegetable crop valued for its aroma, taste, flavour and pungency. India is the leading chilli producing country in terms of area and production. The origin of this spice and vegetable crop can be traced to South and Central America [2]. A few cultivars of hot pepper are cultivated outside their region of origin and nearly all those grown in tropics are local selections. *C. annum* was domesticated in highlands of Mexico and includes Mexican, Asian and African hot chilli. There are taxonomic controversies regarding cultivated and wild forms of chillies but the most desirable forms are under *C. annum* (both hot and sweet pepper) which are widely cultivated. *Capsicum* sp. are grouped under three species complexes- the *C. annum*, *C. baccatum* and *C. pubescence* complexes. Among the five cultivated species of chilli, *C. annum*, *C. frutescence* and *C. chinense* Jacq. belongs to the *annuum* complex.

Green fruits of chilli are used as vegetable whereas ripe dried fruits as a spice because of its pungency and pleasant flavour [3]. Mature fruits of chilli contain carotenoid pigments, flavonoids and significant amounts of vitamin C, B1 and B2. The capsaicinoid compounds, which are responsible for the pungent flavour, are widely used for medicinal applications [4] such as cure of pharyngitis, lymph node swellings or boils and pain relief. In addition to the various medicinal uses, various forms of chilli with a whole host of colours also make it an important ornamental plant.

Plant biodiversity or the plant genetic wealth of an area is the variability between the plant kingdom and the ecosystem complex in which they occur. *Capsicum* is a highly polymorphic genus with much morphological and genetic diversity at both intra- and inter-specific level. The existence of extensive variability has been reported in local populations but with no such systematic report. In India, a rich diversity in chilli exists due to its diverse geo-climatic regions and the reproductive behaviour of the crop. Moreover, genetic diversity may arise due to the environmental influence and may be determined by the magnitude and nature of their genetic variability in which they are grown [5].

Maximum diversity arises within cultivars grown in India with respect to shape, size, yield and other quality traits. This may be due to 2-40 % outcrossing which occurs due to entomophily. In order to access the source of genetic diversity adequate knowledge on the population structure of the particular species is very much essential. Diversity analysis among the working collections of germplasm enhances the efficacy of breeding and germplasm management. The knowledge on diversity and genetic distances of variable forms of the germplasm available at a particular region is a prerequisite for improving cultivars and developing cultivars suited for various needs. Development of improved high yielding varieties is based on only few parents and most of the useful genes present in local landraces and wild relatives will be lost. This will have undesirable consequences like genetic erosion and increased vulnerability to biotic and abiotic stresses. Conservation and sustainable use of the variable forms of chilli is the need of the hour for persistent improvement of this crop. Keeping in view the importance of diversity analysis, the present study was done to characterize the local chilli genotypes collected, selected and maintained at Assam Agricultural University, Jorhat.

## Materials and Methods

### Plant Materials

A set of thirty-seven Assam chilli genotypes was considered for morphological characterization which included 30 genotypes of *Capsicum annum*, 3 belonging to *Capsicum chinense* viz., Manipuri Bhut, Bor Bhut and Lota Bhut and 4 were unknown species. Among the thirty *Capsicum annum* types K-L-1, K-L-2, K-L-3, K-L-4, K-L-13, K-L-14, K-L-31, K-L-35, K-S-4, K-S-9, K-S-11, K-S-32, K-S-37, K-S-38, K-S-41, K-S-47, Krishna L (different variable forms of black chilli), Khorika, KA-2, 2016/CHIVAR-2, CHIV AR-3, CHIVAR-4, CHIVAR-5, CHIVAR-6, CHIVAR-7 and CHIVAR-8 belonged to common chilli botanical variety *acuminatum*; Bireek-1, Bireek-2, B-1-P Sel, B1 x B2 belonged to cone chilli types *Capsicum annum* var. *conooides*; and Bhekuri Jolokia to type cherry pepper *Capsicum annum* var. *cerasiforme*. Two local chilli genotypes Mem Jolokia and Haitha Jolokia belonged to *Capsicum annum* but with perennial growth habits.

Except for 2016/CHIVAR-2, CHIVAR-3, CHIVAR-4, CHIVAR-5, CHIVAR-6, CHIVAR-7 and CHIVAR-8 which were provided by IIVR, Varanasi under AICRP (Vegetable Crops) varietal trial, and the other germplasm were locally collected from different places of Assam (Table 1).

#### Qualitative and Quantitative Observations

The experimental area is situated between 26°46' N latitude and 94°13' E longitude at an elevation of 86.56 m from the mean sea level. The soil of the experimental area belongs to Inceptisols derived from the alluvial deposits of the river Brahmaputra and its tributary Bhogdoi. The soil is light textured, acidic (pH: 4.8) and medium in organic matter content. Agricultural meteorological data during the experimentation period recorded an average maximum temperature of 27.22°C and a minimum temperature of 16.38°C, an average relative humidity of 94.6 percent and 20.98 mm rainfall.

The fertilizers were applied at 120:60:60 kg N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O per ha in the form of Urea, Single Super Phosphate and Muriate of Potash at final land preparation. Irrigation was done as and when necessary. Prior to the experimentation, seeds of the genotypes were obtained from previous crops maintained through temporally and spatially isolated plots in poly houses.

Morphological data were obtained from the plants on the all thirty accessions assembled from different places of India. Twenty-seven different traits related to plant habit (5), leaf (6), flower (2), fruit (13) and biotic stress (1) were recorded as per NBPGR Minimal Descriptors for Agri-Horticultural Crops, Part-II: Vegetable Crops [6]. Observations were taken on five randomly selected plants from the genotypes for the quantitative traits.

#### Statistical Analysis

The statistical calculations for mean, range, the coefficient of variation were performed in Microsoft Office Excel 2007. The qualitative and quantitative data were standardized using the mean and standard deviation of the samples and used to work out the Euclidian distances based dissimilarity matrix. An unrooted phylogenetic tree was then produced using Unweighted Paired Group Method using Arithmetic Average (UPGMA) of hierarchical clustering based on dissimilarity matrix as implemented in the DARwin

software version 6.0. Principal component analysis (PCA) was performed following the method of [7] using the Analyse-it software Microsoft Excel 32 bit and the biplot diagram was generated using PC1 and PC2.

## Results and Discussion

#### Mean Performance, Variability and Correlation

Abundant diversity was observed for all the quantitative traits considered in the study. Plant architecture was studied using plant height, number of branches per plant, leaf length and leaf breadth, plant growth habits, stem colour, leaf size, shape, margin and colour. Plant height ranged from 33 to 315 cm with a maximum height recorded in Manipuri Bhut (*C. chinense*) and a minimum plant height recorded in KA-2 (*Capsicum annuum* var. *acuminatum*). The number of primary branches varied from 3.6 to 11, with a maximum value being shown by Khorika (*Capsicum annuum* var. *acuminatum*) and a minimum by Lota bhut (*C. chinense*). Leaf length also showed a wide range from 3.6 to 17.5 cm. Maximum leaf length was observed in Manipuri Bhut (*C. chinense*) and the minimum leaf length was recorded in KS-38 (*Capsicum annuum* var. *acuminatum*). Similarly, leaf breadth also showed variation with values ranging from 1.4 to 7.6 cm. The broadest leaf was observed in Manipuri Bhut (*C. chinense*) while narrowest leaves were observed in Krishna L (*Capsicum annuum* var. *acuminatum*). Great diversity in fruit characters like fruit length, breadth, weight and number of fruits per plant was also observed. Fruit length, fruit breadth and fruit weight ranged from 1.32 to 10.2 cm, 0.37 to 2.23 cm and 1.5 to 10.2 g respectively. A wide diversity in the number of fruits per plant was observed ranging from 28 fruits in KL-2 to 846 fruits in Manipuri Bhut (*C. chinense*). Maximum yield per plant was obtained in Manipuri Bhut (*C. chinense*) 5761.26 g while the minimum yield of 146.4 g was observed in KS-11 (*Capsicum annuum* var. *acuminatum*). The maximum mean value was observed in yield per plant, followed by the number of fruits per plant and followed by plant height (Table 3). Based on the range and CV, the most variable character was fruit yield per plant (138.74%) followed by the number of fruits per plant (91.7%) and plant height (80.8%). The genotypes of *C. chinense* yielded higher than the *C. annuum* genotypes. The presence of wide range of variation in the component traits such as plant height

Table 1. Details of the chilli genotypes with geographical coordinates

S No.	Genotype name	Botanical name	Pedigree	Origin	Geographical coordinates
1-16	K-L-1, K-L-2, K-L-3, K-L-4, K-L-13, K-L-14, K-L-31, K-L-35, K-S-4, K-S-9, K-S-11, K-S-11, K-S-32, K-S-37, K-S-38, K-S-41, K-S-47	<i>Capsicum annuum</i> var <i>acuminatum</i>	Pureline selection from Krishna	Assam, India	26° 47' N latitude 94° 12' E longitude
17	Bireek-1	<i>Capsicum annuum</i> var <i>conoides</i>	Landrace	Assam, India	25° 82' N latitude 93° 72' E longitude
18	Bireek-2	<i>Capsicum annuum</i> var <i>conoides</i>	Landrace	Assam, India	Same as above
19	B-1-P Sel	<i>Capsicum annuum</i> var <i>conoides</i>	Pureline selection from Bireek 1	-	26° 47' N latitude 94° 12' E longitude
20	B1 x B2	<i>Capsicum annuum</i> var <i>conoides</i>	F <sub>1</sub> of Bireek 1/ Bireek 2	-	26° 47' N latitude 94° 12' E longitude
21	Krishna L	<i>Capsicum annuum</i> var <i>acuminatum</i>	Landrace	Assam, India	26° 47' N latitude 94° 10' E longitude
22	Khorika	<i>Capsicum annuum</i> var <i>acuminatum</i>	Landrace	Assam, India	26° 47' N latitude 94° 10' E longitude
23	KA-2	<i>Capsicum annuum</i> var <i>acuminatum</i>	Unknown	-	-
24	Moni Jolokia	<i>Capsicum sp.</i>	Landrace	Assam, India	-
25	Manipuri Bhut	<i>Capsicum chinense</i>	Landrace	Manipur, India	25° 12' N latitude 93° 80' E longitude
26	Bor Bhut	<i>Capsicum chinense</i>	Landrace	Assam, India	-
27	Lota bhut	<i>Capsicum chinense</i>	Land race	Assam, India	-
28	Haitha Jolokia	<i>Capsicum sp.</i>	Landrace	Assam, India	26° 47' N latitude 94° 13' E longitude
29	Bhekuri Jolokia	<i>Capsicum sp.</i>	Landrace	Assam, India	26° 47' N latitude 94° 13' E longitude
30	Mem Jolokia	<i>Capsicum sp.</i>	Land race	Assam, India	26° 47' N latitude 94° 12' E longitude
31-37	2016/CHIVAR-2, 2016/CHIVAR-3, 2016/CHIVAR-4, 2016/CHIVAR-5, 2016/CHIVAR-6, 2016/CHIVAR-7, 2016/CHIVAR-8	<i>Capsicum annuum</i> var <i>acuminatum</i>	Unknown	-	-

Table 3. Range, mean and variation coefficient for the nine quantitative characters

Character	Range	Mean $\pm$ SEM	CV (%)
Plant height (cm)	33.00 - 315.40	80.71 $\pm$ 10.87	80.77
Number of branches/plant	3.60 - 11.00	6.63 $\pm$ 0.25	22.22
Leaf length (cm)	3.60 - 17.50	7.16 $\pm$ 0.54	45.38
Leaf breadth (cm)	1.42 - 7.60	2.97 $\pm$ 0.28	55.9
Fruit length (cm)	1.32 - 10.20	6.14 $\pm$ 0.37	35.84
Fruit breadth (cm)	0.37 - 2.23	1.27 $\pm$ 0.07	35.48
Fruit weight (g)	1.50 - 10.20	4.38 $\pm$ 0.36	49.74
Number of fruits/plant	28.00 - 846.00	184.94 $\pm$ 31.59	91.66
Fruit yield/plant (g)	146.40 - 5761.26	762.77 $\pm$ 172.28	138.74

(80.8%), number of branches per plant (22.2%), fruit length (35.8%), fruit breadth (35.5%), fruit weight (49.7%), number of fruits per plant (91.7%), leaf length (45.4 %) and leaf breadth (55.9 %), corroborated the greater yield differences among the genotypes.

Pearson's correlation coefficient among the quantitative traits revealed a significant positive correlation of plant height (PH), leaf length (LL), leaf breadth (LB) and number of fruits per plant (FN) with fruit yield in the chilli genotypes (Table 4). This suggested that the genotypes which were tall, with longer and broader leaves bore more number of fruits giving higher yield. These traits were also important for distinguishing the genotypes in the species and sub-species level. Significant negative correlation between fruit breadth and number of branches per plant indicated that the genotypes with more number of branches bore fruits with narrower fruit breadth. These traits could be important criteria for improvement of yield in chilli.

The number of phenotypic variants for the 37 genotypes as per NBPGR Minimal Descriptors for Horticultural Crops revealed a lot of variation for the studied traits (Table 2). Among the eighteen qualitative characters, an annual life cycle was exhibited by all the genotypes except the *C. chinense* genotypes, Moni Jolokia, Bhekuri Jolokia, Mem Jolokia and Haitha Jolokia

which were semi-perennial to perennial. Stem colour showed 17 genotypes with green phenotype, 11 with purple and 9 with green with purple stripes. Plant growth habit among the 37 genotypes exhibited 17 with prostrate, 13 with erect and 7 with intermediate growth.

Among the leaf characteristics, the maximum number of phenotypic variants were found for leaf size (16 small, 15 medium and 6 large) and colour (21 green, 10 dark green and 6 purple). Phenotypic variation for leaf shape gave 24 variants with lanceolate leaves and 13 with ovate leaves and 35 variants had entire leaf margin and 2 had undulating leaf margin. Similarly, for flower characteristics, 25 phenotypic variants were observed with white corolla and 12 variants were with different shades of a purple corolla. A wide variation was observed for anther colour showing phenotypic variants with 1 white, 1 yellow, 14 pale blue, 4 blue, 1 bluish yellow and 16 purple anther.

Among fruit characteristics, fruit colour exhibited enormous variation among genotypes. Phenotypic variants for fruit colour were 1 white, 15 green, 8 purple, 1 deep purple, 12 black matured, 1 lemon yellow, 1 orange-yellow, 2 pale orange, 4 light red, 8 red, 19 dark red and 2 brown ripe. Fruit shape variants among genotypes included 27 long, 2 very short, 1 tapering, 3 conical and 4 oval. The number of genotypes with variation in fruit shape at pedicel attachment were

Table 2. Phenotypic variants observed for the various qualitative traits across the 37 genotypes

S No.	Trait	Score	Phenotype	No. of genotypes	S No.	Trait	Score	Phenotype	No. of genotypes		
1	Life cycle	1	Annual	32	11	Ripe fruit colour	3	Pale Orange Yellow	-		
		2	Biennial	-			4	Orange Yellow	1		
		3	Perennial	5			5	Pale Orange	2		
2	Stem colour	1	Green	17			6	Orange	-		
		2	Green with purple stripes	9			7	Light Red	4		
		3	Purple	11			8	Red	8		
3	Plant growth habit	3	Prostrate	17			9	Dark Red	19		
		5	Intermediate	7			10	Brown	2		
		7	Erect	13			11	Purple	-		
4	Leaf size	3	Small	16			12	Fruit shape	12	Black	-
		5	Medium	15					1	Long	27
		7	Large	6	2	Very short			2		
5	Leaf shape	1	Deltoid	-	3	Tapering			1		
		2	Ovate	13	4	Conical			3		
		3	Lanceolate	24	5	Oval			4		
6	Leaf margin	1	Entire	35	13	Fruit shape at pedicel attachment			1	Acute	10
		2	Undulate	2					2	Obtuse	18
		3	Ciliate	-					3	Truncate	8
7	Leaf colour	1	Green	21					4	Cordate	1
		2	Dark Green	10					5	Lobate	-
		3	Purple	6	14	Fruit shape at the blossom end	1	Pointed	24		
8	Corolla colour	1	White	25			2	Blunt	9		
		2	Yellow	-			3	Shrunken	-		
		3	Purple	12			4	Shrunken and pointed	4		
9	Anther colour	1	White	1	15	Fruit position	3	Pendant	23		
		2	Yellow	1			5	Semi Pendent	3		
		3	Pale Blue	14			7	Erect	11		
		4	Blue	4	16	Adherence of calyx to fruit	3	Loose	11		
		5	Bluish Yellow	1			5	Semi-hard	24		
		6	Purple	16			7	Hard	2		
10	Mature fruit colour	1	White	1	17	Fruit surface	1	Smooth	19		
		2	Yellow	-			2	Semi wrinkled	15		
		3	Green	15			3	Wrinkled	3		
		4	Orange	-	18	Biotic stress	1	Very low	26		
		5	Purple	8			3	Low	10		
		6	Deep Purple	1			5	Intermediate	1		
		7	Black	12			7	High	-		
11	Ripe fruit colour	1	White	-	9	Very high	-				
		2	Lemon Yellow	1							

Table 4. Pearson correlation coefficients among the quantitative traits in chilli

Character	PH	BR	LL	LB	FL	FB	FW	FN
BR	-0.47							
LL	<b>0.87</b>	-0.49						
LB	<b>0.73</b>	-0.43	<b>0.91</b>					
FL	-0.47	<b>0.52</b>	-0.42	-0.49				
FB	<b>0.52</b>	<b>-0.56</b>	0.47	0.38	-0.14			
FW	-0.01	-0.02	0.12	-0.01	0.46	0.36		
FN	<b>0.71</b>	-0.20	<b>0.73</b>	<b>0.74</b>	-0.48	0.20	-0.11	
FY	<b>0.79</b>	-0.24	<b>0.77</b>	<b>0.62</b>	-0.20	0.41	0.29	<b>0.81</b>

PH: Plant height (cm); BR: Branches per plant; LL: Leaf length (cm); LB: Leaf breadth (cm); FL: Fruit length (cm); FB: Fruit breadth (cm); FW: Average fruit weight (g); FN: Fruit number per plant; FY: Fruit yield per plant (g)

Bold figures indicate significant correlation

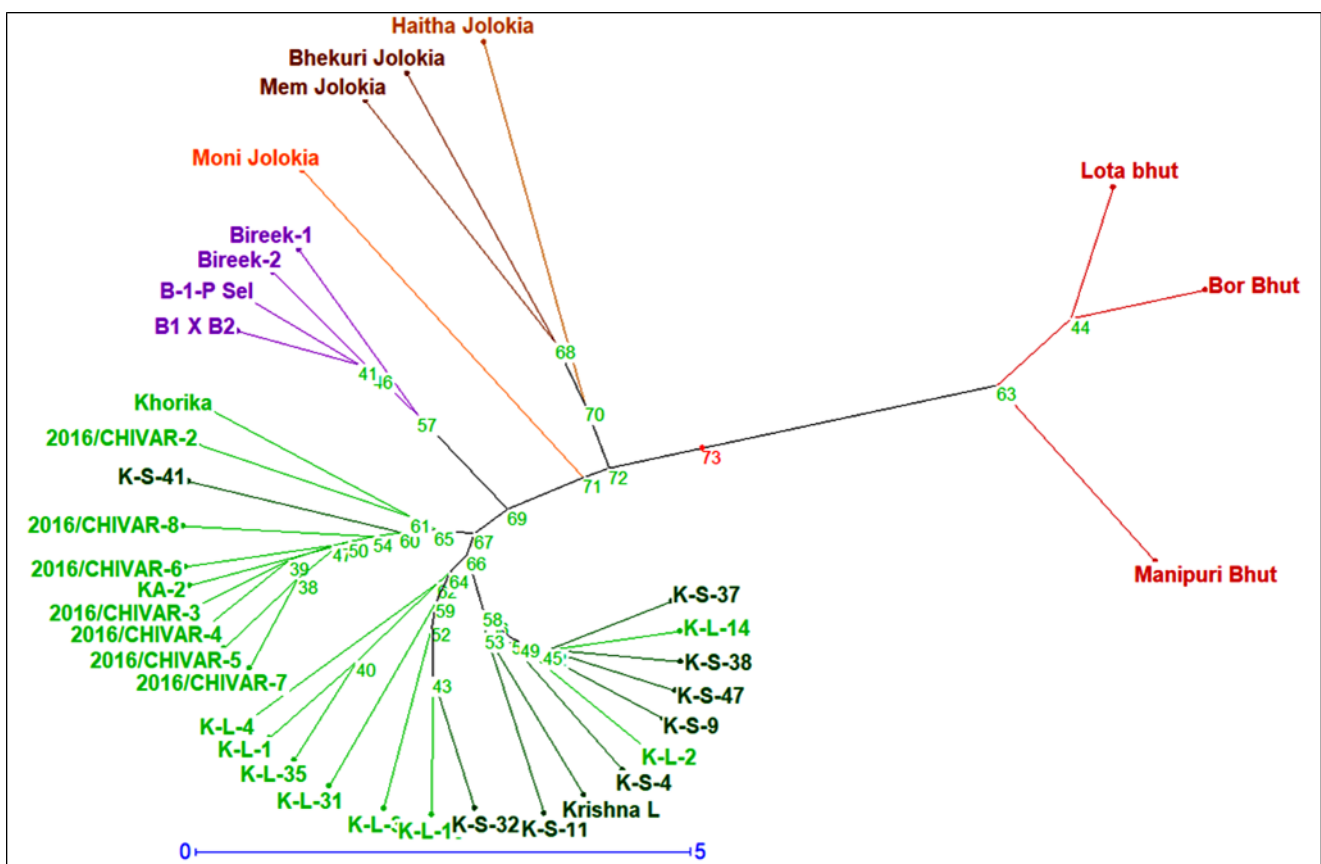


Figure 1. Unrooted phylogenetic tree of chilli genotypes based on qualitative and quantitative traits (The tree was generated using UPGMA based on the dissimilarity among the 37 chilli genotypes)

10 acute, 18 obtuse, 8 truncate and 1 cordate. The blossom end fruit shape was observed to be pointed in 24, blunt in 9 and shrunken-pointed in 4 genotypes. Twenty-three of the phenotypic variants held a pendant position in the stem followed by erect and semi-pendent in 11 and 3 genotypes, respectively. The adherence of calyx to fruits was loose for 11, semi-hard for 24 and hard for 2 genotypes. The fruit surface was found to be smooth, semi-wrinkled and wrinkled for 19, 15 and 3 genotypes, respectively. A very low to intermediate biotic stress was observed in the genotypes with very low to no incidence in 26 variants, low incidence in 10 and intermediate incidence in 1 variant.

### Phylogenetic Analysis

Phylogenetic analysis was performed for the 37 genotypes using standardized values for the qualitative and quantitative traits. Dissimilarity matrix of usual Euclidean distances gave a minimum dissimilarity value of 2.065 and a maximum of 13.311 indicating the diverse nature of genotypes under study. The unrooted phylogenetic tree constructed with dissimilarity values using UPGMA method grouped the genotypes into five major clusters (I, II, III, IV and V) clockwise from right to left (Figure 1). A big clustering (cluster I) was formed at node no 67 with the genotypes belonging to *Capsicum annuum* var. *acuminatum*. Based on the nodal separation of the branches of this cluster (I) at nodes 64, 65 and 66, it was further separated into three sub-clusters. The sub-cluster formed at node 66 comprised of Krishna L and eight other genotypes all having mature fruit colour purple to black and other similar fruit characteristics, similar plant architecture and leaf characteristics. It is remarkable to note that this cluster comprised of the genotypes with anthocyanin pigmentation in their stems and leaves to varying degree (Plate1). Flowers showed different shades of purple. Fruits borne were pendent and long with pointed tips (Plate 2). These variant forms might have arisen due to natural outcrossing with Krishna L which is a landrace of Assam, India. This landrace has been under cultivation from ages and is a heavy bearer of pungent dark purple to black coloured matured fruits. The sub-cluster at node 64 comprised of the variant types of purple to black chilli genotypes, with long, semi-wrinkled pointed tip fruits, borne erect to semi-pendent on the stems. The flowers were with white corolla with a purple

tinge and purple anthers. Green chilli genotypes formed sub-cluster at node 65. The genotypes bore fruits which were long, tapering with pointed ends and acute shape at pedicel attachment (Plate 2). These genotypes were green with very less purple pigmentation. Leaves were mostly lanceolate and smaller in size. Flowers were with white corolla and pale blue to purple anthers.

Cluster II at node no. 69 in the unrooted phylogenetic tree (Figure 1) consisted of the genotypes of *Capsicum annuum* var. *conoides* collected from Karbi Anglong district of Assam, India and two genotypes developed at AAU, Jorhat, Assam, India. Fruits were cone-shaped, borne erect on the pedicel, both green and purple in colour (Plates 1 and 2). These genotypes were tall with a lesser number of branches per plant; green stems and leaves. Cluster III (node no. 71) included one genotype, Moni Jolokia which was a perennial shrub, bearing cone-shaped globose erect fruits (Plates 1 and 2). The genotype bore big ovate leaves which were green in colour. The corollas were white with greyish blue anthers. This genotype was found to be clustering in between the other local *C. annuum* sp. Bireek and Mem Jolokia, but was not seen to form groups with these genotypes. The fourth cluster (IV) at node no. 72 included the local chilli genotypes Mem Jolokia, Bhekuri Jolokia and Haitha Jolokia. These three genotypes were perennial, with green stem and leaves. The two genotypes Mem Jolokia and Bhekuri Jolokia bore erect fruits, while Haitha Jolokia bore semi-pendent fruits (Plate 1). Cluster V included the *C. chinense* genotypes consisting of Manipuri Bhut, Bor Bhut and Lota Bhut (Plates 1 and 2). The presence of maximum diversity in the genotypes belonging to *C. annuum* was in accordance with the findings of [8].

The plant architecture, leaf, flowers and fruit characteristic distinctly separated the genotypes belonging to the species *Capsicum chinense* from the other capsicum genotypes. The observed quantitative traits were also variable from the other capsicum genotypes. These genotypes showed a perennial growth habit and were high yielder bearing distinct, tapering, wrinkled surface, orange coloured ripe, highly pungent fruits (Plate 3). The UPGMA cluster analysis assumes a constant evolutionary rate among accessions and is typically most appropriate for diversity study within species [9]. The clustering of the genotypes showed that





Plate 1. Plant types of genotypes in clusters I(1-14), II(15), III(17), IV(16,20) AND V(18,19)

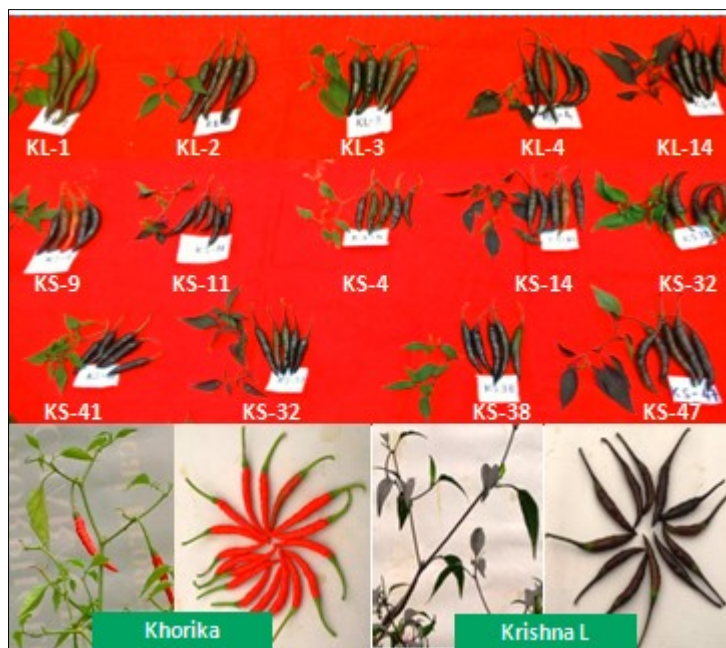


Plate 2. Fruit and leaf type of some genotypes in Cluster I



Plate 3. Flower, fruit and leaf types of some genotypes in different clusters

within *C. annuum* genotypes, three sub-clusters originated based on the minor morphological variations. It was remarkable to note that *Capsicum annuum* var. *acuminatum* and *Capsicum annuum* var. *conoides* were found to be closely related to each other based on stem, leaf, flower and fruit characteristic (Plate 2) except that the genotypes under var. *conoides* which were late maturing as compared to the genotypes of var. *acuminatum*. Moreover, fruit surface, position and shape of the fruits of the var. *conoides* were quite distinct from those of the var. *acuminatum* (Plates 1 and 2). The local chilli genotypes Moni, Mem, Bhekuri and Haitha appeared to be close to the *C. annuum* genotypes and might be used as a source for gene introgression into the cultivated types as these local genotypes are rich sources of many useful genes including resistance to biotic and abiotic stresses as revealed by resistant performances during stresses both *ex situ* and *in situ*. These species also appeared to be in between the *C. annuum* and *C. chinense* genotypes which could be a potential source for chilli improvement as a whole. Among the domesticated species of *Capsicum*, *C. chinense* has been reported to have a better crossability with *C. annuum* [10, 11], providing scope for chilli improvement. The genotypes of these two species could be used as diverse parents for the transfer of many desirable traits. Similarly, the two landraces Khorika and Krishna L also appeared to be dissimilar for many morphological traits. So even when placed in one big cluster I (at node 67), they were grouped in two separate sub-clusters. These genotypes exhibited good adaptability with respect to biotic and abiotic stresses

and were high yielders. Thus, the cluster analysis resulted in a vivid picture implicating the usefulness of the genotypes for crop improvement programme. In an analogous study on the phenotypic divergence among 24 accessions belonging to six species of *Capsicum*, using 12 quantitative and qualitative traits, resulting data grouped the species into six clusters. Three traits among these were the most discriminative of these species, *i.e.*, fruit diameter, number of fruits per plant and leaf diameter. They were reported useful as morphological markers for yield improvement and for obtaining good segregants in chilli breeding programs [12].

#### Principal Component Analysis (PCA)

PCA explained the proportion of relative contribution of the various characters to the total variance of chilli genotypes under study. The study of many qualitative and quantitative traits is important for the assessment of differences between genotypes and their breeding potential. PCA combines the capacity to provide a synthetic summary of the most relevant traits and assessment of the relative contribution of different characters to the total variability of the population [13]. The PCA contributed 99.7 % to the total variability of chilli genotypes up to twenty-two principal components. The largest contribution to variability (34.93%) was made by the first component and smallest contribution (1%) by the twenty-second component. The first six components accounted for 81.44% of the total variability and these six components were considered for discussion.

The highest proportion of total variability exposed by the first component (34.93%) was contributed by a large number of traits with high positive values - plant height, leaf length, leaf breadth, fruit number per plant, fruit yield, fruit shape at blossom end, leaf size, life cycle and traits with low positive values - leaf margin, fruit shape, fruit shape at pedicel attachment and fruit surface (Table 5). The variability in this component was mostly linked to leaf and fruit characteristics which were mostly quantitative in nature. Correlation matrix of these traits revealed that fruit characters in this component showed significant positive correlation with leaf length, breadth and plant height suggesting that these characteristics could complement each other, and also appeared to be important for morphological characterization of the chilli genotypes. The high negative values in this component were observed for mature fruit colour, fruit length and branches per plant.

About 15% of the variability was contributed by the second principal component with high positive values for fruit breadth and fruit weight, and low positive values for plant height, leaf length, fruit length, fruit yield, stem colour, leaf shape, leaf colour, corolla colour, colour of matured fruit/ripe fruits, fruit surface, fruit shape at pedicel attachment and at blossom end, and fruit shape in general (Table 5). High negative values were obtained for leaf margin and fruit position. The correlation matrix (Table 4) also showed a significant positive correlation of plant height with leaf length, leaf breadth, fruit breadth, fruit yield per plant, fruit shape at the blossom end and fruit surface, suggesting effective complementation among the traits useful for crop improvement. The third PC contributing 12.76 % to the total variability showed a greater influence of the plant traits *viz.*, number of branches per plant, leaf shape and fruit surface. The fourth and the fifth PC contributed 8.04% (leaf shape, leaf colour, corolla colour, number of fruits per plant, plant growth habit) and 6.08 % (leaf breadth, leaf margin, adherence of calyx to fruits, anther colour, stem colour), respectively to the variability (Table 5). The variability of these components was mostly linked to the qualitative traits and a few quantitative traits. Morphological characterization of 18 *C. annuum* L. accessions from southern Mexico using 47 morphological descriptors and

response to *Bemisia tabaci* - Begomovirus complex resulted in 12 principal components accounting for 94% of the variation with 3 major clusters and 7 sub-clusters [14].

The PCA biplot diagram of the species drawn on the basis of PC1 and PC2, accounting for a total of 49.9% total variability (Figure 2) identified the traits that contributed profoundly to PC1 (plant height 83%, fruit shape at blossom end 81%, leaf length and life cycle 80%, leaf breadth 79%, colour of matured fruits 69% and fruit number 67%) in characterizing the different genotypes. These traits contributing to the PC1 (plotted on X-axis) distinctly grouped all the genotypes belonging to *Capsicum annuum* in a wide grouping. The genotypes of *Capsicum annuum* var. *acuminatum* and *Capsicum annuum* var. *conoides* did not show separate grouping. The local chilli genotypes Bhekuri, Haitha and Mem Jolokia were plotted near the *Capsicum annuum* group but at a distance. PCA biplot diagram plotted Mem Jolokia near the *Capsicum annuum* var. *acuminatum* genotypes as it might be an ecotype-specific genotype which was tall and perennial bearing innumerable, small and erect fruits. Haitha and Bhekuri were plotted at a distance which appeared to be the ecological variants with similar leaf patterns, but with vast differences in the stem, flower and fruit characteristics than *Capsicum annuum*. Haitha bore characteristic of semi-pendent flowers with white corolla, light green matured fruits and lemon yellow-ripe fruits whereas, Bhekuri was characterised by purple pigmentation in the stem, small white erect flowers and unique small, erect, globose fruits. The colour transformation in the fruits of this type was remarkable as immature fruits bore purplish tinge which gradually turned to white, matured fruits turned to yellow and finally turned red on ripening. The PCA biplot isolated the two *C. chinense* genotypes, Manipuri bhut and Bor bhut into a separate group perhaps because of the similarities in most of the morphological traits of these two genotypes except for some minor variations. Moni Jolokia was unique from the other groups but was close to the *C. chinense* genotypes most probably because of the similarities in fruit characteristics like fruits per plant, leaf characteristics and life cycle. This grouping was in accordance with cluster analysis with one exception *C. chinense* genotype Lota bhut which was

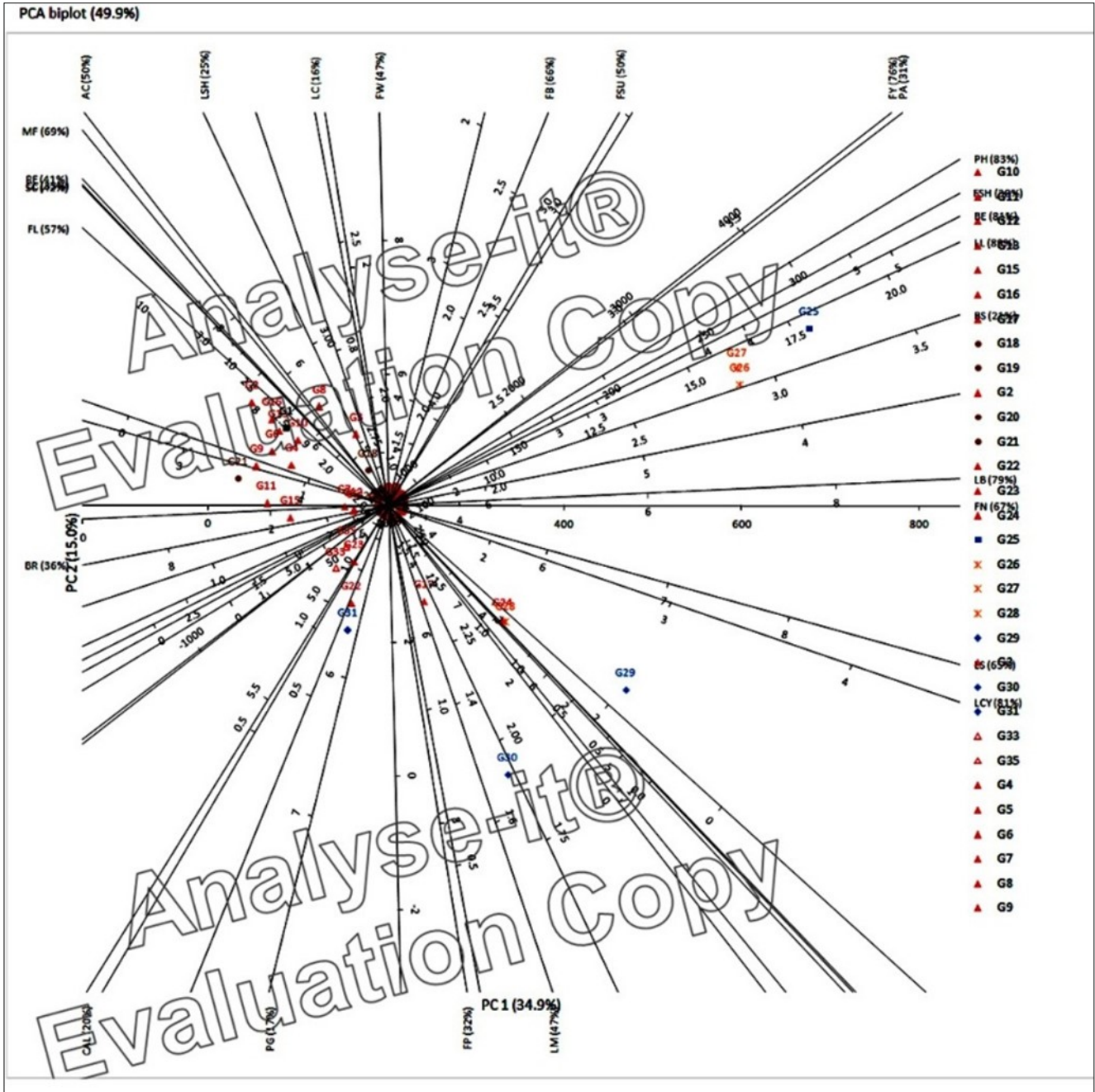


Figure 2. PCA Biplot diagram of chilli genotypes based on PC 1 and PC 2

Table 5. Matrix of eigenvectors and values of principal components of the 27 chilli traits

Trait	Principal component					
	C1	C2	C3	C4	C5	C6
Plant height (cm)	0.275	0.166	-0.002	0.153	-0.024	0.17
No of branches per plant	-0.193	-0.038	0.33	0.149	-0.002	-0.004
Leaf length (cm)	0.294	0.135	0.01	0.103	0.11	-0.113
Leaf breadth (cm)	0.289	0.013	0.02	0.045	0.28	-0.136
Fruit length (cm)	-0.211	0.19	0.238	-0.032	0.122	-0.182
Fruit breadth (cm)	0.14	0.343	-0.138	-0.12	0.048	-0.01
Fruit weight (g)	-0.008	0.342	0.119	-0.257	0.153	-0.235
No of fruits per plant	0.266	0	0.108	0.205	0.035	0.125
Fruit yield per plant (g)	0.252	0.196	0.138	0.11	-0.041	0.082
Lifecycle	0.286	-0.098	-0.001	0.059	0.205	0.096
Stem colour	-0.174	0.18	-0.261	0.079	0.209	0.04
Plant growth habit	-0.047	-0.191	-0.348	0.223	-0.087	0.246
Leaf size	0.258	-0.072	-0.103	0.053	0.13	-0.257
Leaf shape	-0.095	0.202	0.252	0.252	-0.112	0.294
Leaf margin	0.102	-0.302	-0.057	0.126	0.427	-0.038
Leaf colour	-0.036	0.192	-0.209	0.368	0.181	0.091
Corolla colour	-0.154	0.161	-0.247	0.28	0.138	0.111
Anther colour	-0.176	0.227	-0.266	0.006	0.24	-0.06
Mature fruit colour	-0.212	0.259	-0.085	-0.073	0.14	-0.085
Ripe fruit colour	-0.17	0.181	-0.21	-0.182	-0.067	0.123
Fruit shape	0.167	0.091	-0.332	-0.071	-0.357	-0.075
Fruit shape at pedicel attachment	0.161	0.123	-0.092	-0.419	0.114	0.292
Fruit shape at blossom end	0.278	0.14	-0.149	-0.014	-0.057	0.053
Fruit position	0.044	-0.272	-0.267	-0.246	-0.006	-0.314
Adherence of calyx to fruit	-0.099	-0.159	0.113	-0.211	0.532	0.274
Fruit surface	0.155	0.262	0.206	0.059	0.013	-0.274
Biotic stress	0.146	0.048	0.096	-0.35	-0.022	0.463
Total variance	9.43	4.052	3.446	2.17	1.643	1.247
Variance explained (%)	34.926	15.008	12.763	8.039	6.085	4.619
Cumulative (%)	34.926	49.934	62.697	70.736	76.821	81.44

separated from its group and was placed quite near a *C. annuum* genotype KA-2. These traits appeared to be important in characterizing the genotypes at the species level. The maximum contribution to PC2 was made by fruit yield (76%) followed by fruit breadth (66%) (Figure 2). The various traits explained by PC1 and PC2 was capable of resolving the genotypes to their respective species level. Cluster analysis and principal component analysis were used to group 54 chilli genotypes (*Capsicum annuum* L.) into seven clusters [3]. Based on mean, variance and group distance, in his study, clusters I and III along with other clusters were suggested for hybridization programme. In the present study, the grouping of the chilli genotypes based on morphological characteristics to their respective species level through PCA analysis and cluster analysis was in general agreement.

### Conclusion

The results presented here demonstrate the utility of cluster analysis and PCA in partitioning the genetic variation among chilli genotypes and in identifying different genotypes of chilli which would serve as potential sources of unique breeding material for future crop improvement. The study also provides guidance for future analysis of genetic diversity using the more reliable molecular markers to facilitate efficient management and utilization of the available germplasm. Genetic fingerprinting of local germplasm would protect the plant genetic resources of the nation in light of the PPVFRA and Convention on Biological Diversity. A core collection could also be created with the morphological diversity for utilization in the future breeding programme.

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### Conflict of Interest

The authors declare no conflict of interest.

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