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Study of genetic diversity of chilli germplasm conserved in bangladesh through multivariate analysis

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Abstract

Objective: This research work has been aimed to reveal genetic diversity in morphological traits. It is also important for genotype selection for breeding purpose, even in evaluation and conservation of their diverse gene pool.

Method: Genetic variation was evaluated in Eighty-two chilli (*Capsicum* spp.) genotypes for fifteen morphological traits through descriptive statistics, PCA, and cluster analysis. 3.1. Mapping of locations and germplasm distribution using DIVA-GIS computer program.

Result: DIVA-GIS computer program has localized accessions into 10 districts (administrative units). The descriptive statistics revealed substantial variations in the traits yield plant^{-1}, number of fruit plant^{-1}, individual fruit weight, days to 50% flowering, days to 50% fruiting, fruit width, and 1000 seed weight. Multivariate analysis grouped the collection into 5 clusters. Inter-cluster distance between clusters III and IV was found to be maximum followed by between clusters I- III and III -V. Cluster III possessed genotypes having maximum yield, earliness, and dwarf plant stature.

Conclusion: Considering group distance, mean performance, and variability the inter-genotypic crosses between Cluster III and Cluster IV, Cluster I and Cluster III, and Cluster III cluster V may be suggested to use for future hybridization programs.

Keywords: Capsicum; Genebank; Chilli; Germplasm; PGRC; BARI; Bangladesh

1. Introduction

Chilli peppers are varieties of the berry fruit of plants from the *genus Capsicum* which are members of the nightshade family *Solanaceae* cultivated for their pungency [1]. Chilli is one of the principal ingredients of Bangladesh kitchen. It is used both in green and red-dried states; dried chilli is used as curry powder and paste. Harvesting of chilli is done at the mature green stage for fresh consumption or as a spice in culinary preparation while matured-red chilli is dried and used for making powder and paste. It adds a well-relished spicy taste to the food with a pungency flavor. The presence of alkaloid compounds *Capsaicinoids* is responsible for the hot taste [2, 3]. Chilli belongs to the family *Solanaceae* and includes 27-30 species. Only five species are domesticated which are *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens* [4, 5].

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Chilli is grown across the country, throughout the year and many landraces with sufficient genetic diversity have been developed in Bangladesh. Landraces and local cultivars represent a tremendous genetic reservoir for breeding varieties with desired attributes. However, modern agriculture has accelerated the replacement of traditional varieties. The government of Bangladesh has adopted specific plans and policies for maintaining the diversities of plant genetic resources, their conservation, and their sustainable utilization to secure national food security. Research institutes especially Bangladesh Agricultural Research Institute (BARI) play an important role in the implementation of government-adopted plans and policies for germplasm conservation and utilization. The Plant Genetic Resources Centre (PGRC), BARI has collected and conserved 11081 germplasm of 83 crops since 1983 of which chilli germplasm consists of 335 accessions and breeding lines [6]. This genetic diversity is untapped, underutilized, and represents the entire chilli diversity of Bangladesh and has not been systematically incorporated into chilli breeding programs. Characterization of germplasm based on morphological and agronomic traits are the way forward to effective utilization of germplasm for crop improvement [7]. By considering the above aspects, the study was undertaken to investigate the variability of germplasm accessions using descriptive statistics, PCA, and cluster analysis in selecting genotypes that meet the objectives of the breeding program [8].

2. Materials and Methods

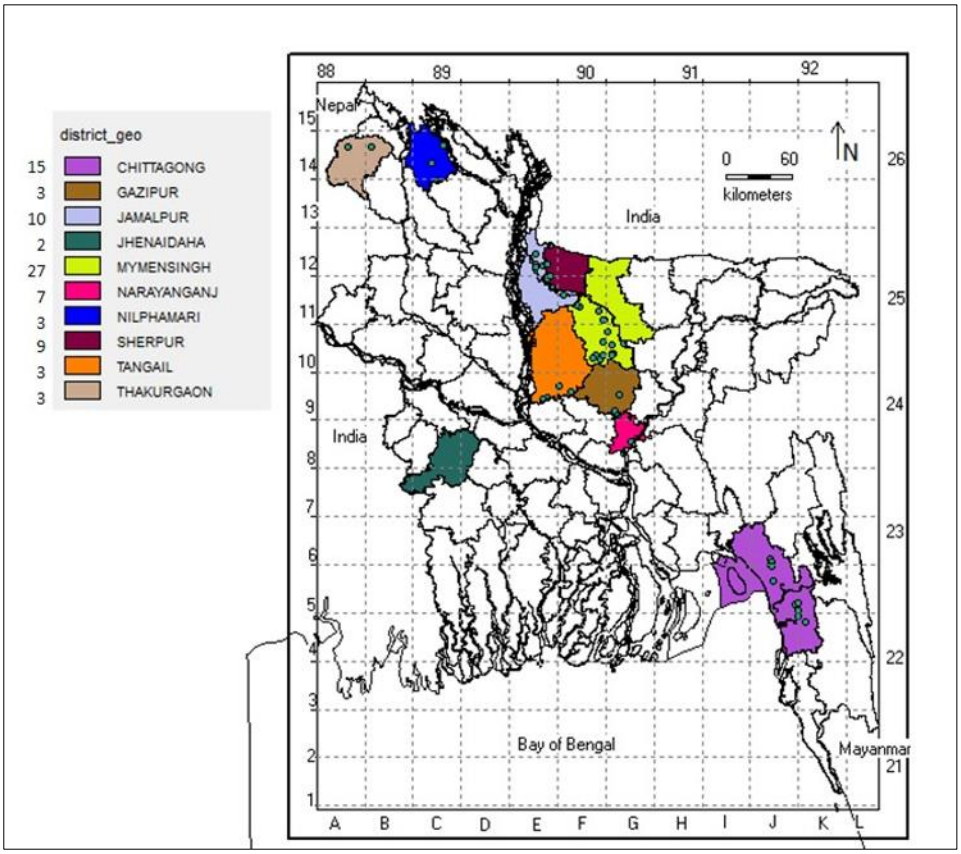

Figure 1 Spatial distribution map showing locations where germplasm was localized after superimposing of Bangladesh Map

The candidate germplasm for the study was selected based on monitoring data of day-to-day management of the Genebank. Germplasm of viability below 80% and seed stocks less than the adequate quantity/number or both were selected for the study. Considering available resources, we included 82 germplasm in the study (Table 1). The germplasm was grown at the central research farm of the Plant Genetic Resources Centre (PGRC), Bangladesh Agricultural Research Institute (BARI) in RCBD design with three replications, during the Rabi season of 2016-17 (October to May). Each replication included 5 seedlings per accession. Thirty-five-day-old seedlings were transplanted in the well-prepared seedbeds on 23rd October 2016. Each germplasm was planted in a plot of three rows having five pits in each plot. Row-to-row and plant-to-plant distances were maintained at 70×60 cm. Recommended doses of manures and fertilizer were applied following standard application methods. Weeding and mulching were done four times at 20-day intervals starting from mid-November. Plant protection measures were taken as and when necessary.

Qualitative and quantitative data were recorded as per the IPGRI descriptor [9].. Data on seed germination, stem morphology, plant height (cm), leaf morphology, number of secondary branches/plant, flower morphology days to first flowering, days to 50% flowering, fruits/plant, 5 fruits weight (g), fruit length (cm), fruit diameter (cm), seeds/fruit, 1000 seed weight (g) and yield/plant (g) were recorded on individual plant basis from the 5 plants selected at random per plot. Principal component analysis (PCA) was carried out by the software IBM SPSS Statistics 25. A dendrogram was generated through PCA and the genotypes were grouped into clustering at1-5 scale of the dendrogram (Figure 3). The quantitative data for vegetative, inflorescence, and fruit characters were subjected to descriptive analysis including maximum, minimum, mean values, and coefficient of variation (CV). Analytical functions of the DIVA-GIS (Geographic Information System) computer program were used to localize the accessions to administrative boundaries (Districts). Grids of approximately 20 x 20 km were marked on a DIVA-GIS-generated map along the longitude and latitude division/degrees.

Table 1 List of chilli genotypes along with sources of collection

Sl No.	Collector's number	Latitude	Longitude	District	Sl. No.	Collector's number	Latitude	Longitude	Collection area/source
1	AMA-187	24.37	90.33	Mymensingh	42	MAH-29	23.90	90.49	Gazipur
2	AMA-191	24.34	90.30	Mymensingh	43	MAH-36	23.87	90.51	Narayanganj
3	AMA-225	24.47	90.56	Mymensingh	44	RAI-100	22.65	91.79	Chattogram
4	AMA-226	24.47	90.56	Mymensingh	45	RAI-156	22.65	91.79	Chattogram
5	AMA-240	24.47	90.56	Mymensingh	46	RAI-160	22.30	91.99	Chattogram
6	AMA-246	24.04	90.52	Mymensingh	47	RAI-190	22.26	92.01	Chattogram
7	AMA-254	24.78	90.20	Mymensingh	48	RAI-232	22.26	92.01	Chattogram
8	AMA-285	24.87	90.07	Jamalpur	49	RAI-259	25.82	89.02	Rangpur
9	AMA-297	25.03	89.96	Sherpur	50	RAI-260	26.11	88.27	Thakurgaon
10	AMA-320	25.98	90.01	Jamalpur	51	RAI-263	26.11	88.46	Thakurgaon
11	AMA-325	25.98	90.01	Jamalpur	52	RAI-83	24.75	91.75	Chattogram
12	AMA-335	25.00	89.93	Sherpur	53	AMA-139	24.34	90.37	Mymensingh
13	AMA-343	25.21	89.82	Jamalpur	54	AHI-1	23.44	88.96	Jhenaidaha
14	AMA-349	25.21	89.82	Jamalpur	55	AHI-6	23.44	88.96	Jhenaidaha
15	AMA-358	25.12	89.89	Jamalpur	56	AMA-23	24.07	90.12	Tangail
16	AMA-361	25.13	89.93	Jamalpur	57	AMA-163	24.39	90.46	Mymensingh
17	AMA-362	25.13	89.93	Jamalpur	58	AMA-164	24.39	90.46	Mymensingh
18	AMA-415	25.28	89.95	Sherpur	59	AMA-175	24.37	90.47	Mymensingh
19	MAH-18	23.56	90.56	Narayanganj	60	AMA-199	24.37	90.38	Mymensingh
20	RAI-115	22.63	91.78	Chattogram	61	AMA-248	24.78	90.20	Mymensingh
21	RAI-261	26.11	88.27	Thakurgoan	62	AMA-283	24.78	90.17	Mymensingh
22	RAI-292	26.12	89.27	Rangpur	63	AMA-307	25.02	89.93	Sherpur
23	RAI-80	22.69	91.78	Chattogram	64	AMA-333	25.02	89.93	Sherpur
24	RAI-95	22.65	91.79	Chattogram	65	AMA-361	25.01	89.89	Jamalpur
25	AMA-112	24.57	90.42	Mymensingh	66	AMA-391	25.27	89.95	Sherpur
26	AMA-118	24.57	90.42	Mymensingh	67	AMA-408	25.28	89.95	Sherpur
27	AMA-128	24.57	90.42	Mymensingh	68	AMA-416	25.28	89.95	Sherpur
28	AMA-146	24.39	90.46	Mymensingh	69	MAH-11	23.66	90.62	Narayanganj

29	AMA-153	24.39	90.46	Mymensingh	70	MAH-38	23.87	90.51	Narayanganj
30	AMA-174	24.38	90.47	Mymensingh	71	MAH-39	23.87	90.51	Narayanganj
31	AMA-203	24.45	90.46	Mymensingh	72	MAH-40	23.87	90.51	Gazipur
32	AMA-296	24.93	89.97	Sherpur	73	MAH-41	23.87	90.51	Gazipur
33	AMA-344	25.21	89.82	Jamalpur	74	MAH-42	23.87	90.51	Narayanganj
34	AMA-37	24.07	90.12	Tangail	75	MAH-43	23.87	90.51	Narayanganj
35	AMA-51	24.12	90.02	Tangail	76	RAI-67	23.62	91.79	Chattogram
36	AMA-56	24.49	90.39	Mymensingh	77	RAI-145	22.32	92.00	Chattogram
37	AMA-73	24.66	90.40	Mymensingh	78	RAI-188	22.21	92.01	Chattogram
38	AMA-74	24.66	90.40	Mymensingh	79	RAI-205	22.16	92.07	Chattogram
39	AMA-75	24.66	90.40	Mymensingh	80	RAI-228	22.50	91.80	Chattogram
40	AMA-90	24.66	90.39	Mymensingh	81	RAI-231	22.50	91.80	Chattogram
41	AMA-95	24.57	90.42	Mymensingh	82	RAI-258	25.82	89.01	Rangpur

3. Results

3.1. Mapping of locations and germplasm distribution

DIVA-GIS computer program has localized accessions into 10 administrative units (Districts). Jamalpur, Mymensingh, and Sherpur are adjacent districts and the geographic distance between them is not much more while other districts are in distance proximities (Figure 1).

3.2. Distribution of germplasm

The data in Table 1 reveals that the abundance of germplasm was maximum Jamalpur (46) followed by, Mymensingh (27), Chattogram (15), and Sherpur (9). The remaining 22 germplasms were distributed in the remaining 6 districts. The germplasm was selected for the trial based on seed viability and/or the amount of seed stock in the active collection of Genebank. The result directly indicates that adequate collection might have not been done so far from many districts. However, the selection strategy of germplasm for the trial might also be the cause of this disparity. For example, a sufficient number of germplasm of an area exists in the Genebank but was not selected or included for the trial due to a satisfactory germination rate or desired seed quantity. However, the generated information can benefit future germplasm collection strategies [10].

3.3. Morphological diversity through univariate analysis

Fruit length, fruit width, fruit weight, plant height, number of fruits/plant, 1000 seed weight and yield, etc. are the most important yield-attributing characteristics that contribute production and marketing value of chilli. Range, mean, and standard deviation including the percentage of coefficient of variation (CV) were analyzed to compare the diversity existing in each trait (Table 2). The CV measures the relative dispersion of the data sets with different units around the means based on variability. The estimated CV% of the studied traits revealed that the trait yield plant^{-1} (CV=72.87%) exhibited maximum variation followed by the number of fruits plant^{-1} (CV=67.64%) individual fruit weight (CV=39.95%), days to 50% flowering (CV= 37.33%), days to 50% fruiting (CV= 31.53%), fruit length (CV= 28.36%), fruit width (CV=24.16%) and 1000 seed weight (CV=21.56%). Minimum variation was observed in plant height (CV= 20.90%). The CV of vegetative traits ranged from 11.55 (leaf length/wide ratio) to 32.45% (leaf length). The results indicate that the seed weight of chilli is relatively stable while the fruit yield is unstable and may be influenced by different climatic factors particularly annual climate variation and cultural management practices [11].

The frequency distribution graphs of fruit traits are positively skewed with some outliers (Figure 2 a-c). The frequency graph of 1000 seed weight on the other hand showed a symmetrical distribution. The results correlate with the estimated CV% as stated in Table 2. Thus, our results have exhibited that all these traits have higher amounts of exploitable genetic variabilities.

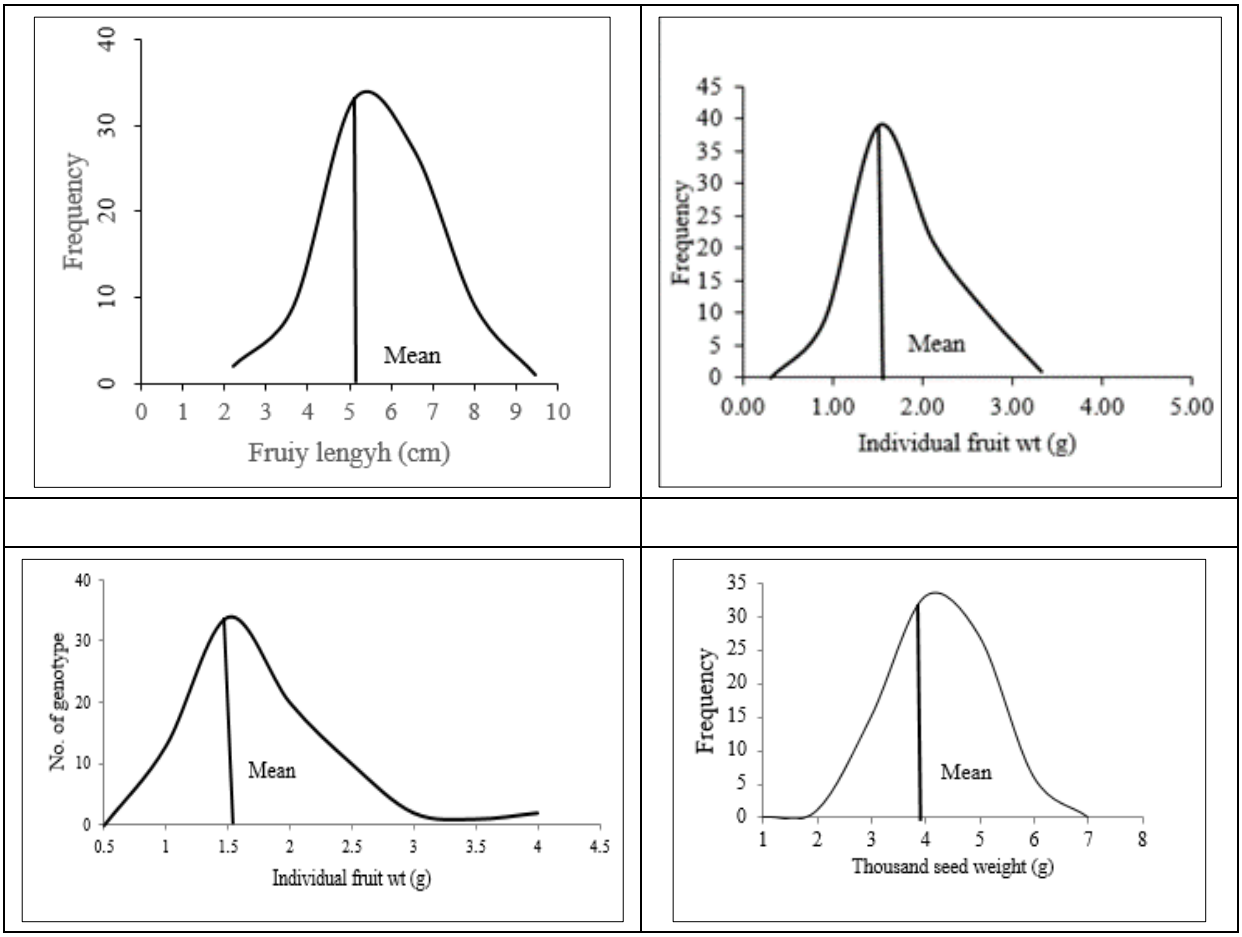

Figure 2 Distribution of fruit traits among the germplasm (a) fruit length (b) fruit width (c) individual fruit weight and (d) thousand seed weight

Table 2 Descriptive statistics of different characters in chilli

Character(s)	Range		Mean	SD	CV%
	Min	Max.			
Stem length (cm)	1.40	7.54	3.48	1.04	29.87
Mature leaf length (cm)	3.48	8.66	5.51	1.26	22.84
Mature leaf width (cm)	1.56	4.76	2.54	0.82	32.45
Leaf length / wide ratio	1.47	2.90	2.23	0.26	11.55
Days to 50% flowering	35.00	128.00	68.63	25.76	37.53
Days to 50% fruiting	58.00	140.00	84.16	26.26	31.21
Fruit length (cm)	1.90	10.44	5.11	1.45	28.36
Fruit width (cm)	0.60	1.92	0.94	0.23	24.16
Fruit weight (g)	0.69	3.84	1.51	0.61	39.95
Plant height (cm)	21.72	108.90	52.02	22.76	20.90
No. of fruits/plant	15.83	182.93	32.69	22.07	67.64
1000 seed wt. (gm)	1.80	5.70	3.87	0.83	21.56
Yield/plant (g)	19.21	202.50	40.03	29.17	72.87

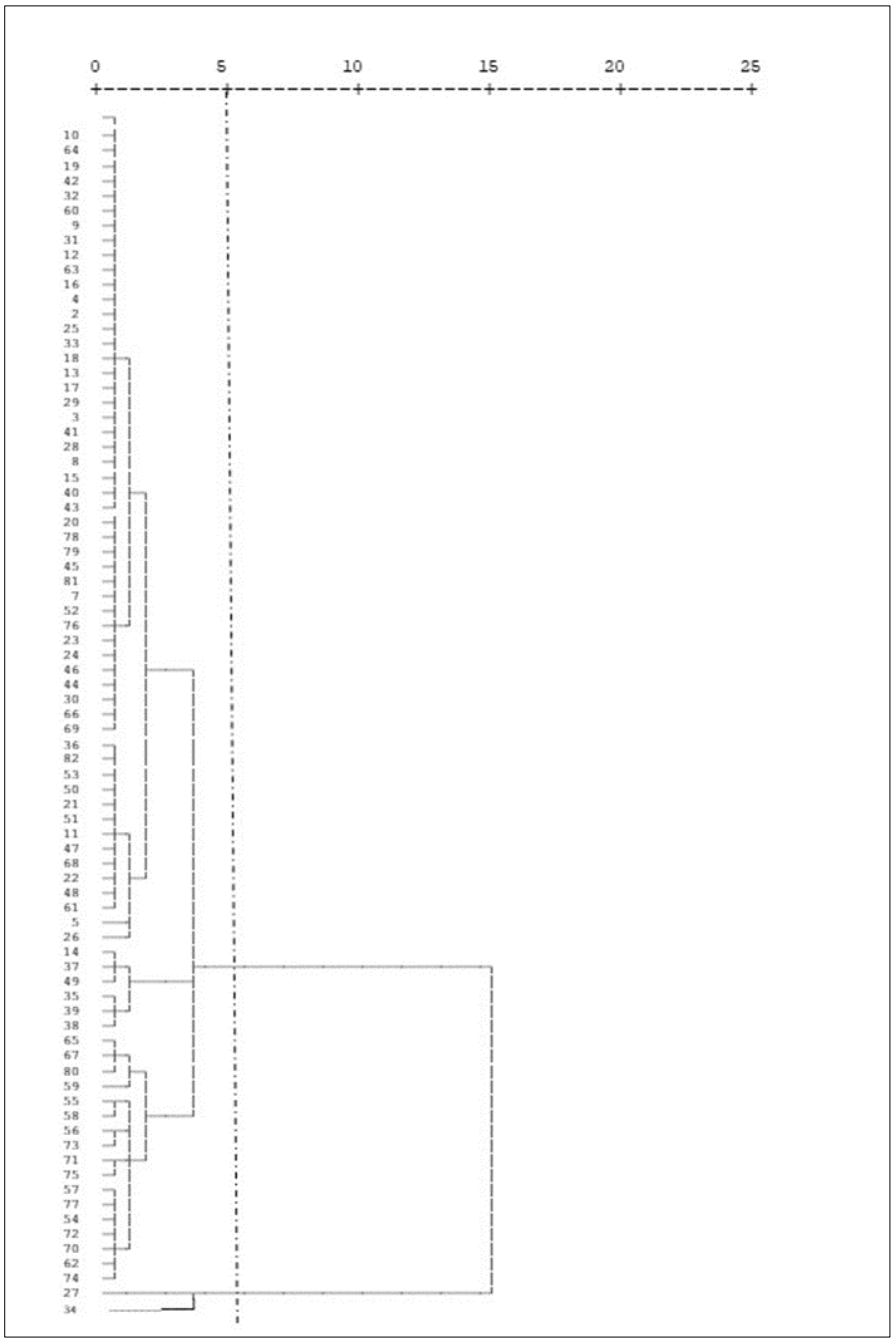

Figure 3 Dendrogram constructed by hierarchical analysis showing the relationship among the evaluated chilli genotypes using 21 qualitative traits

3.4. Multivariate analysis

To examine the relationship among quantitative variables, principal component analysis (PCA) was carried out. PCA explains the variability of a set of random variables in terms of new set of variables with reduced dimensionality and with as little loss of information as possible. The first five linear combinations were identified accounting for a maximum 83.21% variation of total diversity (Table 3). Factor loading analysis showed that Prin1 comprised five characters namely, leaf width, leave length, days to first flowering, days to first fruit set, and plant height; Prin2, factor loading included fruit weight, fruit width, fruit length, and stem length, Prin3 included plant height, stem length, and Prin4 included fruit length, days to flowering and fruit set (Table 4).

Table 3 Eigenvalues of the Covariance Matrix of 13 principal components for quantitative characters

Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	4.712	36.249	36.249
2	2.231	17.164	53.413
3	1.902	14.632	68.045
4	1.155	8.882	76.927
5	0.817	6.286	83.212
6	0.789	6.067	89.280
7	0.586	4.506	93.786
8	0.324	2.494	96.280
9	0.263	2.019	98.299
10	0.152	1.170	99.469
11	0.037	0.285	99.754
12	0.025	0.195	99.948
13	0.007	0.052	100.000

Extraction Method: Principal Component Analysis.

Table 4 Component Matrix (Rescaled)

Character(s)	Component			
	1	2	3	4
Leaf width	0.946	0.073	0.011	-0.158
Leaf length	0.903	0.220	0.210	-0.021
Days to 1st fruit set	0.889	-0.032	-0.036	0.320
Days to 1st flowering	0.865	-0.057	0.015	0.370
Plant height (cm)	0.839	0.270	0.299	0.006
Leaf weight	-0.670	0.224	0.284	0.378
Individual fruit weight	0.138	0.834	-0.342	0.023
Fruit width	-0.068	0.732	-0.274	-0.446
Fruit per plant	0.032	0.011	0.806	-0.415
Stem length	-0.368	0.421	0.424	-0.014
Fruit length	-0.369	0.480	0.223	0.579

Extraction Method: Principal Component Analysis.

3.5. Cluster analysis

From cluster analysis, no relationship was found between the origin of the cultivars and cluster pattern (Table 5). Cluster I contained the highest 42 genotypes, followed by 19, 12 and 7 in Clusters V, IV and II respectively. Cluster III comprises only 2 genotypes of chilli (Table 6). The cluster pattern suggests that the chilli accessions under study could easily adapt to different cultivation areas of Bangladesh as the climatic conditions of the experimental field were different from the original site.

Table 5 Intra (bold) and inter-cluster distances (D2) in 82 genotypes of chilli

Cluster	**I**	**II**	**III**	**IV**	**V**
I	23.00	78.023	166.69	97.325	50.438
II		29.65	136.95	48.071	63.319
III			31.89	177.44	116.425
IV				27.21	101.690
V					24.34

Table 6 No. of germplasm in each of 5 clusters

Cluster	**No. of genotype**	**Genotype**	**No. of Germplasm from each source**	**Important characters**
I	42 (23.00)	AMA-187, AMA-191, AMA-225, AMA-226, AMA-246, AMA-254, AMA-285, AMA-297, AMA-320, AMA-335, AMA-358, AMA-361, AMA-415, MAH-18, RAI-115, RAI-80, RAI-95, AMA-112, AMA-118, AMA-146, AMA-174, AMA-203, AMA-296, AMA-344, AMA-90, AMA-95, MAH-29, MAH-36, RAI-100, RAI-156, RAI-160, RAI-83, AMA-175, AMA-199, AMA-307, AMA-333, AMA-391, MAH-11, RAI-67, RAI-188, RAI-205, RAI-231	Chattogram-10 Gazipur-1 Jamalpur-5 Mymensing-15 Sherpur-7 Narayanganj-3 Rangpur-1	Medium leaf size, low yielder, medium fruit weight
II	7 (29.65)	AMA-51, AMA-74, AMA-75, AHI-6, AMA-164, MAH-39, MAH-43, MAH-39, MAH-43	Jhenaidah-1 Mymensingh-3 Narayanganj-2 Tangail-1	Taller plant, bigger leaf size, early to medium flowering, flower took longer time to fruit set, bigger size fruit
III	2 (31.89)	AMA-37, AMA-128	Mymensingh-1 Tangail-1	High yielder, medium to tall plant
IV	12 (27.21)	AHI-6, AMA-23, AMA-163, AMA-283, AMA-361, AMA-408, MAH-38, MAH-40, MAH-41, MAH-42, RAI-145, RAI-228	Jhenaidah-1, Tangail-1 Mymensingh-2 Jamalpur-1 Sherpur-1 Narayanganj-2 Gazipur-2 Chattogra-2	Delay in flowering and flower took fewer days to fruit set, low yielding
V	19 (24.34)		AMA-240, AMA-325, AMA-343, AMA-349, AMA-362, RAI-261, RAI-292, AMA-153, AMA-56, AMA-73, RAI-190, RAI-232, RAI-259, RAI-260, RAI-263, AMA-139, AMA-248, AMA-416, RAI-258	Smaller leaf, early flowering and fruiting

(Figures within the parenthesis are intra-cluster distances)

Intra- and inter-cluster distances also influence the shapes of clusters. A maximum intra-cluster distance of 29.65 was measured in cluster II indicating heterogeneity of cluster members. The minimum intra-cluster distance is 23.00 in Cluster I indicating homogeneity of cluster members. Differences in cluster means existed in almost all the characters studied (Table 5). Cluster distance between IV and III was the highest followed by between clusters distance I and III and, between cluster II and III, between cluster III and V, and between cluster IV and V. Desirable parents might be chosen from the above clusters for effective crossing programs. The minimum distance was recorded between Clusters II and IV indicating that the genotypes of these clusters are genetically close and selection of genotypes from these clusters for future chilli breeding programs has no genetic gain in the offspring. Previous reports on chilli improvement programs also resulted in high heterotic hybrids and broad spectrum variability in segregating generations using genetically diverse parents in hybridization programs [12-15].

The highest mean values for individual fruit weight and plant height were observed in Cluster II which means the genotypes fallen in Cluster II have the genetic potential to contribute to yield maximization and individual fruit weight. Cluster III possessed genotypes with maximum yield coupled with earliness and dwarf plant stature indicating selection of genotypes from these clusters for future chilli breeding programs has a positive impact on short plant type, earliness, and higher yield. Clusters I and V had the genotypes that showed the lowest mean value for almost all the characters under study and indicating the selection of genotypes from these clusters for future chilli breeding programs has no chance of positive impact except for short plant type, earliness, and yield (Table 7). The results also correlate with the estimated CV% presented in Table 5.

Table 7 Initial Cluster Centers of diversity contributing characters

Traits	**Cluster**				
	I	**II**	**III**	**IV**	**V**
Leaf length	4.78	7.8	7.6	6.3	4.45
Leaf width	1.8	4.04	3.18	3.68	2.15
Days to flowering	64	85	74	128	46
Days to fruit set	76	111	88	140	59
Individual fruit weight	1.37	2.41	1.11	0.77	1.8
Plant height	38.72	108.9	89.98	58.47	30.47
Number of fruit/plant (Yield)	3.97	11.59	202.5	4.26	87.99

4. Discussion

Smallholders of Bangladesh have adopted cultivation practices that allow unintentional racial crossing as they grow different chilli cultivars and landraces in small areas close to each other and facilitate pollen exchange. At the same time, smallholder farmers usually exchange seeds that promote gene flow through the dispersal of different cultivars among geographically distant localities. Moreover, the introduction of commercial or improved germplasm has also contributed to blurring racial boundaries [16,17]. The mixing of such germplasm with native landraces produces creolized varieties that farmers would later identify as “local” [18].. In our study, the clustering of accession irrespective of collection sources might be the result of the gene flow of seed exchange among the farmers and the unintentional crossing of local cultivars of chilli in the community farming systems. Genetic diversity and cluster relationships of germplasm suggest chilli accessions of Bangladesh could easily adapt to different cultivation areas as the climatic conditions of the experimental field were different from the original sites. Climates are the product of many factors, including latitude, elevation, topography, distance from the ocean, and location on a continent. The closer proximity reduces environmental variability, and minimizes ecosystem differences, while a larger spatial scale increases heterogeneities of the environment and soil properties [19]. The environmental factors change the functional traits and adaptive processes of plants. Trait variations of genotypes along climatic gradients are indicative of geographic diversity and genetic adaptation [20]. The morphological characteristics and agronomic performance of 82 chilli germplasms showed a significant genotypic difference from the seedling stage. The presence of anthocyanin or any pigmentation at the stem or anthocyanin coloration of hypocotyl was reported before as the response of plants to abiotic stresses such as drought, low temperature, and ultraviolet radiation [21]. Thus our results have exhibited that all these traits have higher amounts of exploitable genetic variabilities.

5. Conclusion

GIS map provides an accurate description of the abundance of germplasm in each district and the generated information can benefit future collection strategies of the Genebank. Considering group distance, mean performance, and variability the inter-genotypic crosses between Cluster III and Cluster IV, Cluster I and Cluster III, and Cluster III cluster V may be suggested to use for future hybridization programs.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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