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GENETIC DIVERSITY OF CHILI PEPPER (*Capsicum* spp.) GENOTYPES GROWN IN BHUTAN BASED ON MORPHOLOGICAL CHARACTERS

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SUMMARY

Chili (*Capsicum* spp.) is an important spice and vegetable crop grown across the world. It is an integral part of Bhutanese cuisine and culture and is one of the most important vegetables grown in Bhutan. Although many chili varieties are grown across the country, information on their genetic diversity is limited. Therefore, the objective of this study was to determine the genetic variation of chili accessions grown in Bhutan by using morphological characterization. Twenty-seven accessions from different locations were evaluated on the basis of 50 morphological descriptors. Results showed that the all the accessions, except for the out-group based on *Capsicum frutescens* L., evaluated in this study were *Capsicum annuum* L. accessions. High variations in qualitative and quantitative characters were observed among the *C. annuum* accessions. Fruit components, specifically, fruit width (54.99%), fruit length (50.87%), and fruit wall thickness (148%), showed high coefficients of variation. Hierarchical cluster analysis allocated the accessions into 10 clusters at a Euclidian distance of 5. Eleven accession lines that were characterized by slender elongated fruits with pointed blossom ends and semiwrinkled fruits were located in one cluster. Fourteen components were selected as meaningful attributes with eigenvalues greater than 1 in principal component analysis. These components accounted for 83% of the variation. Therefore, the *C. annuum* accessions grown in Bhutan exhibited wide variation. This variation could be attributed to diverse ecological zones and microclimates. However, studies at the molecular level are recommended to confirm the diversity of the accessions.

Keywords: Chili, germplasm, quantitative and qualitative traits

Key findings: The main chili varieties grown in Bhutan were *C. annuum* accessions, and high variation within species was observed.

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INTRODUCTION

The chili pepper (*Capsicum* spp.) is one of the most popular spice and vegetable crops worldwide. It originated in the Central and South Americas. It has spread across continents and now occupies an important position in the food and culture of many countries. The genus *Capsicum* belongs to the Solanaceae family and consists of approximately 30 known species, among which only five are domesticated (Bosland and Votava, 2000). These five species include *Capsicum annuum* L., *Capsicum frutescens* L., *Capsicum chinense* Jacq., *Capsicum baccatum* L., and *Capsicum pubescens* Ruiz and Pav. Approximately 150 different types of chili exist and are characterized on the basis of color, shape, and pungency (Berke, 2002).

Chili is an important cash crop for smallholder farmers in developing countries, such as Ethiopia, Nigeria, Ghana, China, India, Pakistan, Indonesia, Cambodia, and Thailand (Ebert *et al*., 2013). In Bhutan, chili is grown as a vegetable, and chili prepared with cheese is a popular local dish known as *ema datshi.* Given the increasing commercial importance of chili as a source of cash income, growers in Bhutan are turning toward growing only varieties with high market value. This trend, coupled with the rapid degradation of habitats and the introduction of high-yielding varieties, could eventually lead to the extinction of traditional cultivars. Pradhan (1996) reported 18 landraces of *Capsicum* species in Bhutan. The most popular varieties are Sha ema, Baegup ema, Parop ema, Dallay ema, and Urkabangala (Dem *et al.,* 2008). Thapa (2012) also reported 40 different location-specific varieties. Several location-specific varieties are cultivated in Bhutan. However, information reported on the phenotypic descriptions of the few varieties is limited and contradictory. Moreover, although chili peppers are considered autogamous, several studies have reported high cross-pollination rates of 7%–90%, which could lead to changes in the genetic identity of chili landraces (Bozokalfa *et al*., 2009), thus increasing heterogeneity and resulting in the loss of original attributes. Given that the chili is an integral part of Bhutanese cuisine and culture, the assessment of genetic variability among chili genotypes is important. It is useful for the conservation of genetic resources and for broadening genetic bases for breeding and is crucial for cultivar protection (Yuzbasioglu *et al.*, 2006). In addition, traditional varieties represent an important source of germplasm that is needed to develop adaptation strategies to climate changes (NBC, 2008). The traditional Bhutanese chili varieties are conserved in the Asian Vegetable Research and Development Center, Taiwan, and in the National Biodiversity Center in Bhutan. Limited studies have been done on chilies. Genetic diversity is required for populations to evolve in response to environmental changes, such as climate change and new or altered diseases (Galetti, 2018). Geleta *et al*. (2004) reported that although very closely or distantly related parents show low heterosis, crosses between parents of intermediate divergence classes exhibit increased heterosis effects for fruit yield, fruit length, and fruit weight. Therefore, in any crop breeding program, information on genetic divergence in a population helps in the selection of suitable parents for utilization and reduces the number of needed crosses (Guerra *et al.,* 1999).

Bertan and Oliveira (2007) stated that despite the many advances in biotechnology and bioinformatic tools, breeders still commonly select parents on the basis of their phenotypic performance in terms of yield components, vegetative and reproductive cycles, and pest and disease resistance. Hence, germplasm characterization is important for the conservation and utilization of plant genetic resources (Thul *et al.,* 2012). Accessions for conservation in gene banks must be properly evaluated and made available to breeding programs (Sudre´ *et al.,* 2010). Germplasm conservation provides the raw material that can be utilized by scientists to improve crop productivity and diversify production systems (Schreinemachers *et al*., 2014).

Chili has been characterized by using morphological; cytogenetic; and molecular markers, such as restriction fragment length polymorphism, random amplified polymorphic DNA and amplified fragment length polymorphism markers (Bozokalfa *et al.,* 2009). Despite the accuracy in estimating genetic divergence among accessions by using molecular markers, knowledge on the phenotype given by morphological and agronomical descriptors remains important. Therefore, the objective of this study was to determine the extent of the genetic variation of chili accessions grown in Bhutan by using morphological characteristics.

MATERIALS AND METHODS

Plant material

Twenty-seven accessions were collected from districts across the country (Figure 1). Chili pods were collected mainly from farmer's fields, and some were collected from local markets. The chili accessions were collected on the basis of their shape, size, relative pungency, and popularity in local areas. In provinces where more than one popular variety is grown, seeds were collected by maintaining a minimum distance of 5 km (Yunam *et al.,* 2012). According to Baral *et al.* (2002), out-group accessions are necessary to confirm monophyletic relationships among *C. annuum* var *annuum* accessions. Therefore, one accession of *Capsicum frutescens* was used. As shown in Table 1 and Figure 2, local names were given for the collected accessions, and numbers were given for those from the same locality.

Field experiment

The field experiment was carried out at the agricultural farm of the College of Natural Resources (CNR), Royal University of Bhutan, Lobesa Punakha, from April 2016 to September 2016. The farm lies at an altitude of 1440 masl. The study region is located in a

Table 1. Accessions of chili peppers (*Capsicum annuum* L.) and their collection sites.

Entry No.	Local name	Seed collection/data collection	Coordinates	Elevation (m)
1	Urka bangala	Bumdeling, Trashiyangtse	27°39''31.1''N 90°27'20.59''E	1915.9
2	Khasadrapchu ema	Khasadrapchu, Thimphu	27°23`'07.75''N 89°34'54.08''E	2215.5
3	Thinleygang II	Punakha	27°31'39.24''N 89°48'08.24''E	1796.4
4	Upper Nubi	Nubi, Trongsa	27°29' 42.59''N 90°51'29.43''E	3293.3
5	Thinleygang I	Punakha	27°31'30.15''N 89°40'09.75''E	1845.5
6	Lower Nubi	Nubi, Trongsa	27°31'17.00''N 90°27'26.26''E	1916.5
7	Paro IV	Lamgong, Paro	27°26'56.02''N 89°22' 15.83''E	2366.1
8	Sarpang local	Dekiling, Sarpang	26°52'57.95''N 90°20'14.03''E	350.82
9	Punakha ema	Kabesa Punakha	27°32'34.87''N 89°59'54.25''E	1983.9
10	Lhuentse semi oro	Khoma, Lheuntse	27°34'04.20''N 92°55'35.78''E	1224.9
11	Khoma ema	CNR, Lobesa, Punakha	27°30'55.43''N 89°51'59.72''E	1387.1
12	Emaotosum_Paga	Chapcha, Chukha	27°11'36.09''N 89°32'16.69''E	2409.0
13	Namseling	Mewang, Thimphu	27°24' 20.71''N 89°36' 47.51''E	2234.4
14	Sha ema	Kazhi, Wangduephodrang	27°32'34.87''N 89°59'54.25''E	1983.0
15	Boegaap ema	Kazhi, Wangduephodrang	27°32'34.87''N 89°59'54.25E	1983.0
16	Khoma solo	Khoma, Lhuentse	27°34'04.20''N 92°55'35.78''E	1224.0
17	Nubi_Nubi	Nubi, Trongsa	27°31'04.69''N 90°27'43.74''E	1866.9
18	Tharay Khursani	Barshong, Tsirang	27°18'52.69''N 89°51'22.37''E	2624.0
19	Lobesa ema	Lobesa, Punakha	27°30'55.43''N 89°51'59.72''E	1387.0
20	Sailey Khursani	Barshong, Tsirang	27°18'52.69''N 89°51'22.37''E	1866.9
21	Jerey Khursani	Barshong, Tsirang	27°18'52.69''N 89°51'22.37''E	1866.9
22	Khasadrapchu I	Mewang, Thimphu	27°23`'07.75''N 89°34'54.08''E	2215.5
23	Paro I	Lamgong, Paro	27°26'56.02''N 89°22'15.83''E	2366.0
24	Paro II	Lamgong, Paro	27°27'26.03''N 89°21'49.76''E	2492.3
25	Paro III	Lamgong, Paro	27°27'26.03''N 89°21'05.74''E	2342.0
26	Dalle Khursani	Dekiling, Sarpang	26°39'33.77''N 93°50'46.29''E	350.82
27	Pakshikha ema	Pakshikha, Chukha	26°55'09.22''N 89°31' 24.55''E	2022.6

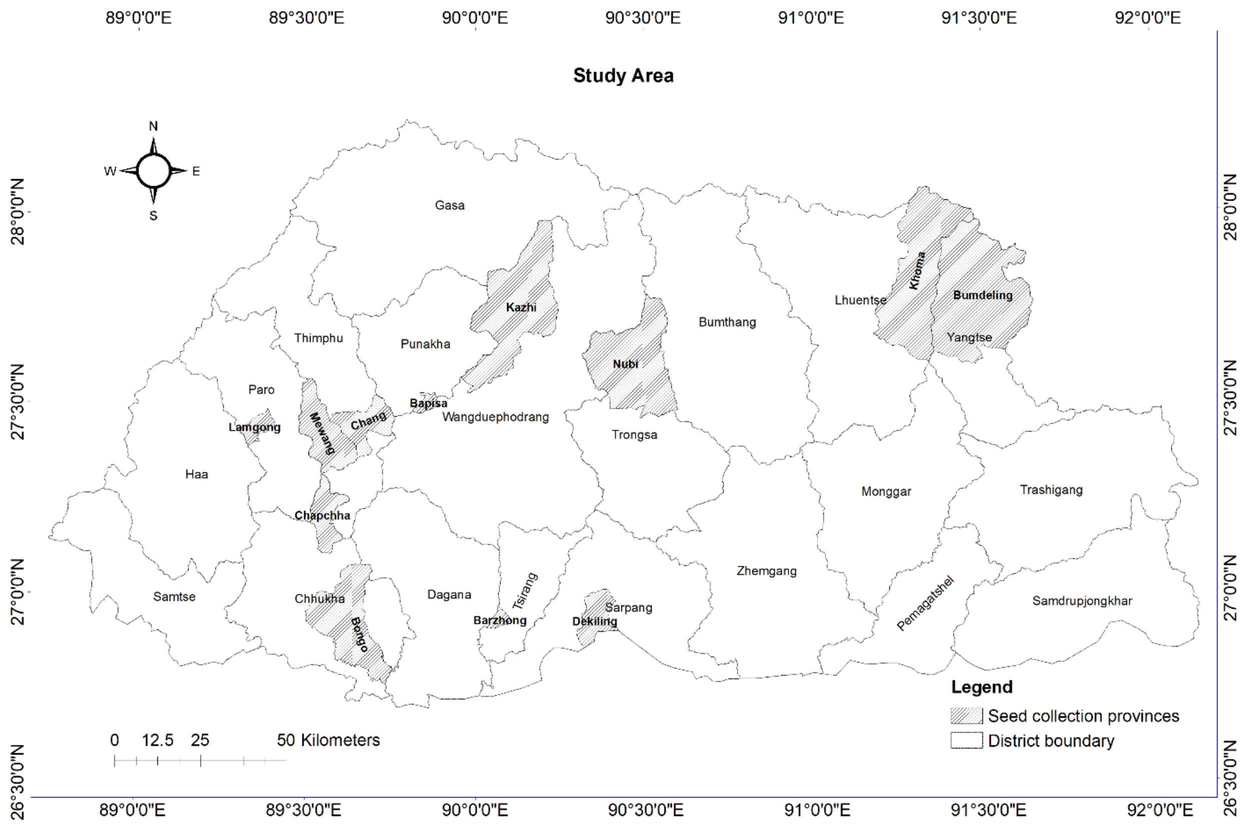

Figure 1. Geographical regions of seed and data collection.

dry subtropical belt with hot and dry summers and moderate winters. All cultural practices were performed in accordance with the recommended practices for chili. Among the 27 accessions, 10 showed poor growth in the CNR farm environment. Therefore, data for these accessions were collected from farmers' fields during the same season.

Data collection and analysis

Five plants were randomly selected for data collection. Data were collected in accordance with *Capsicum* descriptors (IPGRI 1995). Each entry was characterized at the vegetative stage, reproductive stage, and fruiting stage (Table 2). Scores were assigned on the basis of *Capsicum* descriptors. Data for quantitative characters were analyzed by calculating means, minima, maxima, standard deviations, and coefficients of variation (CV), and qualitative data were characterized on the basis of frequency and percentage. Cluster analysis based on the nearest-neighbor method with a squared Euclidian distance of 5 was performed, and a dendrogram was constructed. All analyses were conducted by using IBM SPSS Statistics 21. Principal component analysis (PCA) was run in R software version 3.5.2.

Figure 2. Accessions of *Capcisum* spp. evaluated in the study. A. Dallay Khorsaney (fruit scale = 1 cm); B. Jerey Khorsaney (scale = 0.5 cm); C. Sailey Khorsaney (scale = 1 cm); D. Sarpang local (scale = 2 cm); E. Pakshika ema (scale = 5 cm); F. Lobesa ema (scale = 1.5 cm); G. Khasadrapchu ema (scale = 2.5 cm); H. Tharay (scale = 1 cm); I. Paro I (scale = 5 cm); J. Paro II (scale = 5 cm); K. Paro III (scale = 5 cm); L. Khoma (scale = 4 cm); M. Lower Nubi (scale = 5 cm); N. Khasadrapchu ema (scale = 5 cm); O. Urka Bangala (scale = 3 cm); P. Sha ema (scale = 5 cm); Q. Baegap ema (scale = 4 cm).

Table 2. Morphological characters studied.

Plant Characters
Life cycle: Annual, biennial, perennial
Stem color: Green, green with purple stripes
Nodal anthocyanin (whole plant): green, light purple, purple, dark purple
Stem shape: Cylindrical, angled, flattened
Stem pubescence: Sparse, intermediate, dense
Plant height (cm): <25, 25–45, 46–65, 66–85, >85
Plant growth habit: Prostrate, intermediate, compact, erect
Plant canopy width (cm): At the widest point (immediately after first harvest)
Stem length: Height to first bifurcation (immediately after first harvest)
Stem diameter: Measured in the middle part to the first bifurcation (immediately after first harvest)
Leaf characters
Leaf density: Sparse, intermediate, dense
Leaf color: Yellow, light green, green, dark green, light purple, purple, variegated
Leaf shape: Deltoid, ovate, lanceolate
Lamina margin: Entire, undulate, ciliate
Leaf pubescence: Sparse, intermediate, dense
Matured leaf length (cm)
Matured leaf width (cm)
Inflorescence characters
Number of flowers per axil: One, two, three or more
Flower position: Pendent, intermediate, erect
Corolla color: White, light yellow, yellow, yellow-green, purple with white base, white with purple base, white with purple margin, purple
Corolla spot color: White, yellow, green-yellow, green, purple
Corolla shape: Rotate, campanulate
Corolla length (cm): <1.5, 1.5–2.5, >25
Anther color: White, yellow, pale blue, blue, purple
Anther length (mm)
Filament color: White, yellow, green, blue, light purple, purple
Filament length (mm)
Fruit characters
Calyx pigmentation: Absent, present
Calyx margin: Enveloping, non-enveloping
Calyx annular constriction: Absent, present
Days to fruiting
Anthocyanin spots or stripes: Absent, present
Fruit color at intermediate stage: White, yellow, green, orange, purple, deep purple
Fruit set: Low, intermediate, high
Fruit color at mature stage: White, lemon-yellow, pale orange yellow, orange-yellow, pale orange, orange, light red, red, dark red, purple, brown, black
Fruit shape: Elongate, almost round, triangular, campanulate, blocky
Fruit length (cm)
Fruit width (cm)
Fruit weight (g)
Fruit pedicel length (cm)
Fruit wall thickness (mm)
Fruit shape at pedicel attachment: Acute, obtuse, truncate, cordate, lobate
Neck at base of fruit: Absent, present
Fruit shape at blossom end: Pointed, blunt, sunken, sunken and pointed
Fruit blossom end appendage: Absent, present
Fruit cross- sectional corrugation: Slightly corrugated, intermediate, corrugated
Number of locules
Fruit surface: Smooth, semiwrinkled, wrinkled
Pedicel with fruit: Slight, intermediate, persistent
Placenta length: <1/4 fruit length, 1/4–1/2 fruit length, >1/2 fruit length

RESULTS

Qualitative characteristics

Plant characters (6), leaf characters (5), inflorescence characters (6), and fruit characters (16) were used to evaluate the variation in the qualitative characters of the chili accessions grown in Bhutan. The results are shown in Table 3. A total of 23 accessions had an annual life cycle, and four were perennial. The landraces were classified into two types on the basis of stem color: 15 accessions had green stems, and 12 accessions had green stems with purple stripes. Similarly, the accessions were classified into two groups on the basis of stem shape: 20 accessions had cylindrical stems, and seven had angular stems. Four types of nodal anthocyanins were observed: 12, eight, four, and three accessions had light purple, purple, dark purple, and green nodal anthocyanins, respectively. The stem pubescence of 20 accessions was sparse, and seven accessions had intermediate stem pubescence. Branching habits were sparse, intermediate, and dense. Among the accessions, 16, nine, and two had sparse, intermediate, and dense branching habits, respectively.

The accessions were classified into three groups on the basis of leaf characters, such as leaf density, leaf color, leaf shape, and leaf pubescence of the youngest mature leaf. A total of 11 accessions had intermediate leaf density, 14 had sparse density, and two had dense leaf density. Similarly, 19 accessions had green leaves, six had light green leaves, and two had dark green leaves. Leaf shapes were ovate (13 accessions), lanceolate (12 accessions), or deltoid (two accessions). Among the accessions, 13 had white corollas, 13 had light yellow corollas, and only one had a purple corolla with white bases. A total of 18 accessions had intermediate flower position, five accessions had an erect flower position, and four had pendent flower positions. Corolla spot color, filament color, and anther color had few variations. All accessions lacked calyx pigmentation. Among the accessions, 19, five, and three had intermediate, entire, and dentate calyx margins, respectively. All accessions lacked calyx annular constriction. Fruit color at the intermediate stage was green in 21 accessions and purple in two accessions. One accession each had deep purple and white fruits. Among the accessions, 23 had red fruit color at maturity, and one each had white, pale orange yellow, yellow, and pale orange fruit color at maturity. Fruit shape was elongate in 21, triangular in three, blocky in two, and almost round in one. Fruit shape at the blossom end was pointed in 21 accessions, blunt in four, sunken in one, and sunken and pointed in one. Fruit blossom end appendages were absent in 25 and present in two. Some variations in fruit surface characteristics were observed: 16 accessions were semiwrinkled, seven were smooth, and four were wrinkled. Slight variation in fruit set was observed, with 16, six, and five accessions with intermediate, high, and low fruit sets, respectively.

Quantitative characteristics

Sixteen quantitative traits were used to evaluate the variations in the 27 chili landraces. The traits used were

Table 3. Variation in different accessions based on qualitative characters.

Plant characters	Descriptors	Fre-quency	Percent	Fruit characters	Descriptors	Fre-quency	Percent
Life cycle of the plant	Annual	23	85.2	Calyx pigmentation	Absent	27	100.0
	Perennial	04	14.8				
Stem color of young plant	Green	15	55.6	Calyx margin	Entire	05	18.5
	Green with purple stripes	12	44.4		Intermediate	19	70.4
					Dentate	03	
Nodal anthocyanin at plant maturity	Green	03	11.1	Calyx annular constriction	Absent	27	100.0
	Light purple	12	44.4		Present	00	00.0
	Purple	08	29.6				
	Dark purple	04	14.8				
Stem shape at maturity	Cylindrical	20	74.1	Anthocyanin spots and stripes	Absent	21	22.2
	Angled	07	25.9		Present	06	77.8
Stem pubescence of matured plants	Sparse	20	74.1	Fruit color at intermediate stage	White	01	03.7
	Intermediate	07	25.9		Green	23	85.2
					Purple	02	07.4
					Deep purple	01	03.7
Branching habit	Sparse	16	59.3	Fruit set	Low	05	18.5
	Intermediate	09	33.3		Intermediate	16	59.3
	Dense	02	07.4		High	06	22.2
				Fruit color at mature stage	White	01	03.7
					Pale orange yellow	01	03.7
Leaf characters					Pale orange	01	03.7
					Red	23	85.2
					Dark red	01	03.7
Leaf density	Sparse	11	40.7	Fruit shape	Elongate	21	77.8
	Intermediate	14	51.9		Almost round	01	03.7
	Dense	02	07.4		Triangular	03	11.1
					Blocky	02	07.4
Leaf color	Light green	06	22.2	Fruit shape at pedicel attachment	Obtuse	12	44.4
	Green	19	70.4		Truncate	13	48.1
	Dark green	02	07.4		Cordate	02	07.4
Leaf shape	Deltoid	02	07.4	Neck base	Absent	19	70.4
	Ovate	13	48.1		Present	08	29.6
	Lanceolate	12	44.4				
Leaf margin	Entire	26	96.3	Fruit shape at blossom end	Pointed	21	77.8
	Undulate	01	03.7		Blunt	04	14.8
					Sunken	01	3.70
					Sunken and pointed	01	3.70
Leaf pubescence for youngest mature leaf	Sparse	24	88.9	Fruit blossom-end appendages	Absent	25	92.6
	Intermediate	02	07.4		Present	02	07.4
	Dense	01	03.7				
				Fruit cross-sectional corrugation	Slightly corrugated	20	74.1
Inflorescence characters					Intermediate	06	22.2
					Corrugated	01	03.7
Flower position	Pendent	04	14.8	Fruit surface	Smooth	07	25.9
	Intermediate	18	66.7		Semi wrinkled	16	59.3
	Erect	05	18.5		Wrinkled	04	14.8
Corolla colour	White	13	48.1	Persistence of ripe fruit (pedicel with fruit)	Intermediate	05	18.5
	Light yellow	13	48.1		Persistent	22	81.5
	Purple with white base	01	03.7				
Corolla spot colour	Yellow	21	77.8	Persistence of ripe fruit (pedicel with stem)	Slight	01	3.70
	Green yellow	03	11.1		Intermediate	23	85.2
	Green	01	03.7		Persistent	03	11.1
		02	07.4				
Corolla shape	Rotate	27	100.0	Placenta length	<1/4 of fruit length	01	03.7
					>1/2 of fruit length	26	96.3
Filament color	White	22	81.5				
	Yellow	02	07.4				
	Light purple	02	07.4				
	Purple	01	03.7				
Anther color	Pale blue	21	77.8				
	Blue	05	18.5				
	Purple	01	03.7				

Table 4. Means, standard deviation (SD), minimum (min), maximum (max), and coefficient of variation (CV) for quantitative characters of 27 chili accessions.

Quantitative Characters	Mean ± SD	CV (%)	Min	Max
Plant height at 50% of the first fruit starts ripening	75.20 ± 18.29	24.31	59.7	125.0
Plant canopy width recorded after first harvest (cm)	70.00 ± 16.31	23.29	42.3	108.6
Stem length (height to first bifurcation (cm)	11.40 ± 03.54	30.95	4.0	9.0
Stem diameter (cm) middle part of first bifurcation	1.37 ± 00.47	34.51	0.7	2.2
Matured leaf length (cm)	9.16 ± 02.97	32.43	3.9	16.9
Matured leaf width (cm)	3.89 ± 01.80	46.29	2.0	11.9
Number of flowers per axil	1.74 ± 00.76	43.90	1.0	3.0
Corolla length	1.33 ± 00.55	41.60	1.0	3.0
Anther length (mm)	0.26 ± 00.08	31.73	0.1	0.5
Filament length (cm)	0.32 ± 00.13	43.12	0.1	0.6
Fruit length (cm)	7.66 ± 03.89	50.87	1.2	15.0
Fruit width (cm)	2.12 ± 01.16	54.99	0.2	5.6
Fruit weight (g)	10.80 ± 04.85	44.93	4.6	23.0
Fruit pedicel length (cm)	2.72 ± 00.88	32.42	0.1	4.2
Fruit wall thickness (mm)	0.39 ± 00.58	148.80	0.1	2.4
Number of locules	2.37 ± 00.49	20.76	2.0	3.0

plant height, plant canopy width, stem length, stem diameter, leaf length, and leaf width, number of flowers per axil, corolla length, anther length, filament length, fruit length, fruit width, fruit weight, fruit pedicel length, fruit wall thickness, and number of locules. Variation was observed among all the characteristics. The CV and minimum and maximum ranges were considered (Table 4). The plant growth parameters varied among accessions. Plant height (59.7–125 cm), plant canopy (42.3–108.6 cm), stem length (4–9 cm), stem diameter (0.7–2.2 cm), matured leaf length (4–9 cm), matured leaf width (2–11.9), number of flowers per axil (1–3), corolla length (1–3 mm), anther length (0.1–0.3 mm), filament length (0.1–0.6 cm), fruit length (1.2–15 cm), fruit width (0.2–5.6 cm), fruit weight (4.6–23 g), fruit pedicel length (0.1–4.2 cm), fruit wall thickness (0.1–2.4 cm), and number of locules (2–3) all exhibited variation. Among all the characters, fruit length and width showed high CV of 50.87% and 54.99%, respectively, and fruit wall thickness showed the highest CV of 148.80%.

Cluster analysis

As illustrated in Figure 3, the hierarchical clustering of 27 chili accessions on the basis of 47 morphological characteristics via the nearest-neighbor method defined the accessions into 10 clusters at a Euclidian distance of 5. Cluster one consisted of 11 accession lines that were characterized by slender, semiwrinkled, elongated fruits with pointed blossom ends.

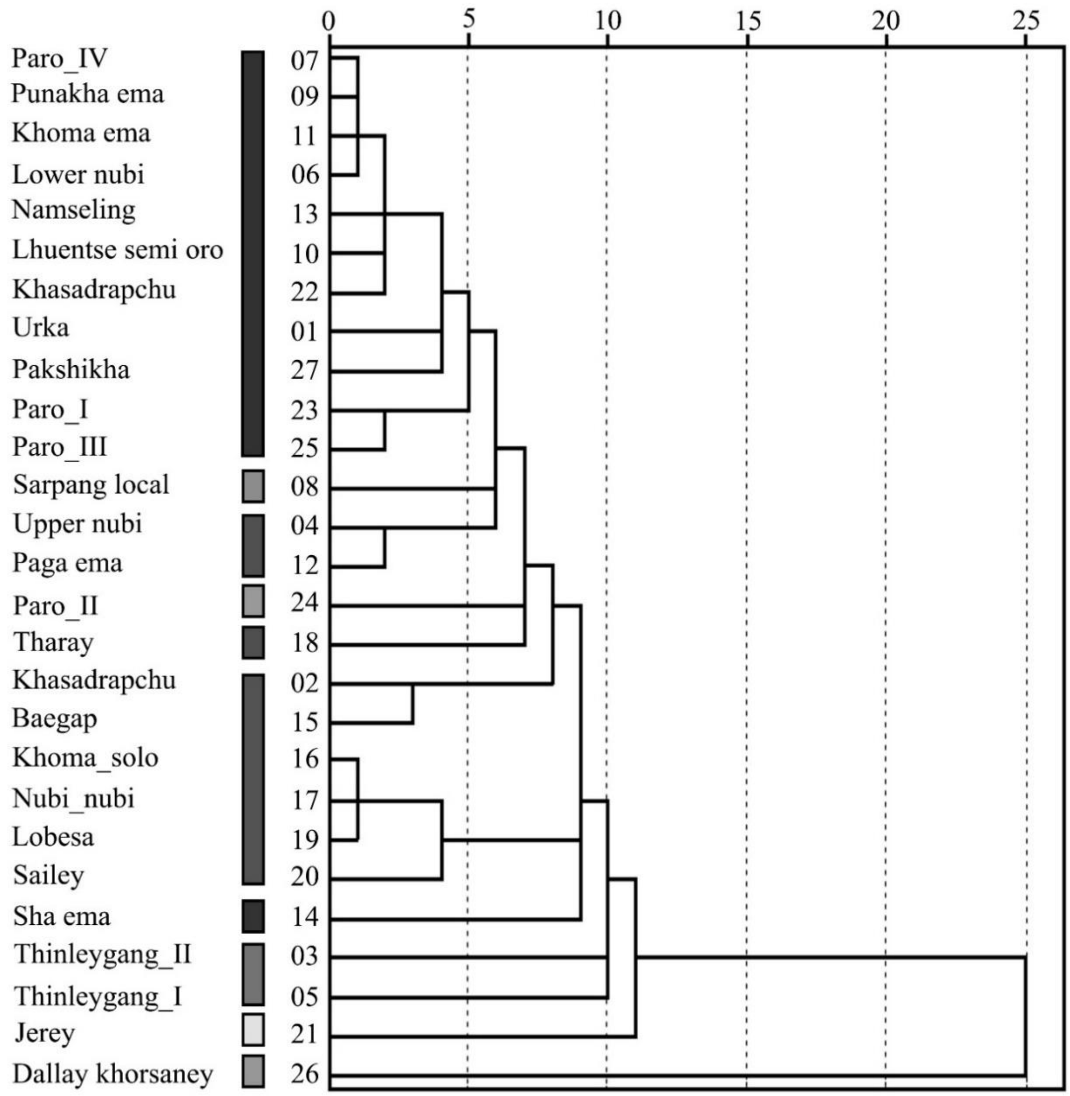

Figure 3. Dendrogram of 27 chili pepper accessions generated via the nearest-neighbor method based on morphological characteristics.

Cluster two consisted of 1 accession, namely, Sarpang local, which had short fruits with blunt ends. Upper Nubi and Paga ema were located in cluster three. These varieties had medium short fruits with pointed blossom ends and rotund pedicel ends. Paga ema and Paro ema, which had with long fruits with pointed blossom ends and high fruit diameter, were in cluster four. Tharay, which had erect fruits and a perennial growth habit, was in cluster five. Although the fruit growth habit of Sailey Khorsaney was similar to that of Tharay, it did not fall under the same cluster. Sailey was not located in the Jerey cluster given that Jerey fruits were also upright. Khasadrapchu ema, which had long, curved, wrinkled fruits, clustered with Baegap ema in the same cluster although Baegap ema had smooth fruit surfaces and did not become wrinkled when dried. Wrinkled dried fruits are preferred for *ema datshi* and fetches higher prices than other varieties. One accession, Sha ema, which was characterized by blocky fruits with blunt ends and dented shoulders, was in cluster seven. Sha ema used to be one of the most popular varieties in terms of taste. However, this variety is disappearing due to its high susceptibility to blight. Cluster eight contained two accession lines that were from the same geographical location, Thinleygang. Cluster nine contained Jerey, a member of *C. frutescens*, which was grown as an out-group, and cluster ten included Dallay Khorsaney.

PCA

The 47 morphological characteristics were subjected to PCA by using Promax oblique rotation. As shown in Figure 4, 14 components were selected as meaningful attributes with eigenvalues greater than 1. These components explained 83% of the total variation. The first principal component (PC 1) explained 8% of the total variation in the original data. The second and third components (PC 2–PC 3) each explained 7% of the variation. The third, fourth, and fifth components (PC 4–PC 6) accounted for 6% of the variation. The other principal components (PC 7–PC 14) contributed 5% of the variation. The total proportion of the variation explained by these 14 components was 83% (Table 5). The total variation explained and the correlations between the 14 components and the original morphological characteristics are shown in Table 5.

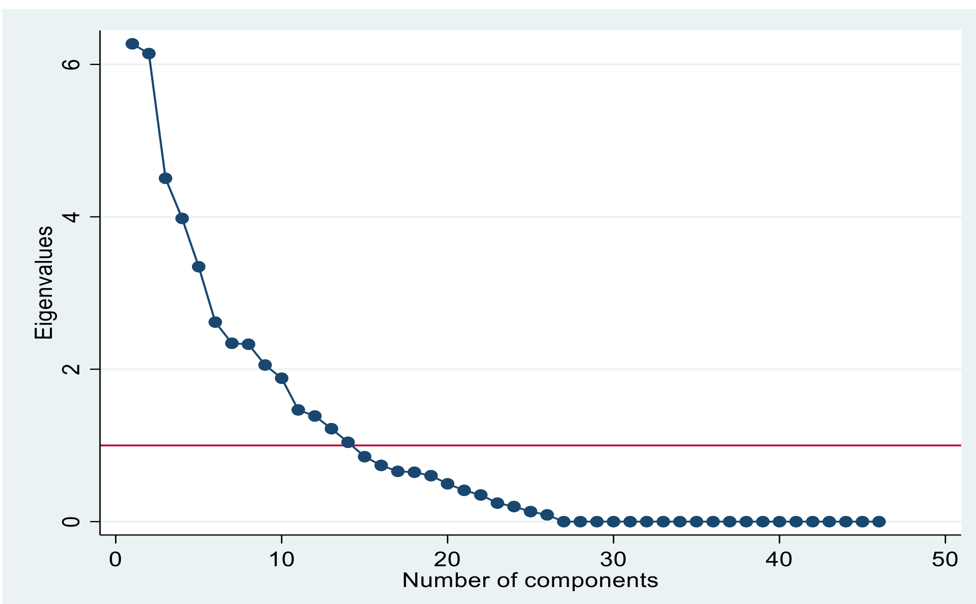

Figure 4. Scree plot of PCA.

Table 5. Variance explained by 14 principal components derived from 50 morphological characteristics of *C. annuum* genotypes and the weights of the original variables in each component.

	Principal Component Axis													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Eigen values	6.27	6.14	4.51	3.98	3.35	2.62	2.34	2.33	2.06	1.88	1.47	1.39	1.22	1.04
Explained proportion of variation (%)	0.08	0.07	0.07	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Cumulative proportion of variation (%)	0.08	0.16	0.23	0.29	0.35	0.41	0.47	0.52	0.58	0.63	0.68	0.73	0.78	0.83
	Correlations with original variable													
Life cycle	0.03	−0.01	0.11	−0.15	0.32	0.00	0.02	0.06	−0.14	−0.21	0.05	0.05	−0.05	−0.05
Stem color	0.41	−0.04	0.18	1.01	−0.13	0.08	−0.17	−0.02	0.07	−0.09	0.11	0.06	−0.02	0.03
Nodal anthocyanin	−0.12	−0.02	−0.08	0.02	0.05	0.11	−0.15	0.05	−0.01	0.01	0.08	0.12	0.02	0.02
Stem shape	1.19	0.03	−0.20	0.38	0.06	0.28	−0.04	0.02	−0.18	0.07	−0.08	0.06	0.08	0.12
Stem pubescence	0.27	0.26	0.06	−0.13	0.11	−0.10	−0.01	0.00	0.09	0.16	0.19	−0.09	0.08	0.18
Plant height	−0.23	−0.09	1.05	0.16	0.05	0.10	0.13	−0.11	−0.22	0.05	0.01	−0.03	−0.02	0.15
Plant girth	0.22	−0.07	0.05	0.08	−0.09	−0.15	0.18	−0.04	−0.11	−0.18	0.21	−0.06	−0.04	−0.05
Plant canopy width	0.02	0.09	0.09	−0.10	−0.08	0.04	0.27	0.22	0.01	0.06	0.09	0.15	0.21	0.16
Stem length	0.00	0.00	0.08	0.05	−0.04	−0.04	−0.04	−0.11	−0.03	0.01	0.10	0.09	−0.09	0.10
Stem diameter	0.03	0.16	−0.02	0.05	0.02	0.02	0.17	0.24	−0.14	0.03	−0.02	0.00	0.21	−0.25
Branching habit	0.07	−0.09	0.01	−0.09	0.17	−0.09	−0.22	−0.07	0.05	−0.02	0.04	−0.01	0.44	−0.06
Leaf density	0.10	−0.05	−0.03	−0.01	0.02	−0.07	0.09	−0.09	0.08	−0.09	0.08	0.09	0.89	0.12
Leaf colour	0.06	0.08	−0.10	−0.19	−0.05	0.09	0.14	0.11	−0.03	0.00	−0.03	0.03	−0.06	0.08
Leaf shape	−0.02	−0.19	0.05	0.02	0.23	0.03	0.10	0.16	0.27	0.13	−0.06	0.19	−0.09	−0.11
Lamina Margin	0.08	0.04	−0.03	−0.01	0.03	−0.02	0.19	0.04	0.00	0.14	0.03	0.06	−0.07	0.08
Leaf pubescence	0.04	0.20	0.44	0.07	−0.09	−0.07	0.01	0.02	0.26	−0.05	−0.23	0.01	−0.08	0.11
Matured leaf length	0.03	0.62	−0.08	−0.11	−0.01	0.06	0.01	−0.05	0.05	−0.12	0.03	−0.01	−0.05	0.09
Matured leaf width	0.04	0.49	−0.02	−0.04	−0.01	0.07	−0.08	0.01	0.07	−0.09	0.02	−0.05	−0.09	−0.07
No. of flowers per axil	−0.05	0.13	−0.22	−0.02	0.05	−0.18	−0.03	−0.09	0.04	0.03	0.09	0.21	−0.14	0.05
Flower position	0.05	0.00	0.03	−0.14	0.85	0.09	−0.24	0.15	−0.21	−0.12	−0.16	0.04	0.05	0.12

Table 5. (cont'd.)

	Correlations with original variable													
Corolla color	−0.17	0.04	0.14	−0.11	−0.14	0.30	−0.19	−0.03	0.14	−0.13	0.14	0.05	0.07	0.06
Corolla shape	0.16	0.03	−0.04	0.01	0.01	−0.07	−0.09	0.00	0.04	0.01	−0.10	−0.27	0.00	−0.04
Corolla length	−0.05	0.02	−0.01	0.15	−0.24	−0.12	−0.09	0.09	−0.11	0.07	−0.02	0.23	−0.04	0.26
Anther color	−0.02	0.06	−0.03	−0.23	−0.16	−0.01	−0.01	0.03	−0.22	−0.11	−0.18	0.02	0.02	0.07
Anther length	−0.24	0.07	0.02	0.12	0.21	0.04	−0.12	−0.08	−0.04	−0.04	−0.09	−0.16	−0.04	0.10
Filament color	−0.20	0.03	0.26	0.10	0.05	0.19	0.03	−0.03	0.04	0.09	0.00	−0.10	−0.01	−0.02
Filament length	0.11	0.01	0.09	0.04	−0.05	−0.06	0.00	−0.02	0.00	−0.14	0.13	0.02	−0.18	0.04
Calyx margin	0.03	0.05	−0.05	0.01	−0.12	−0.11	0.08	−0.04	0.08	−0.01	−0.10	0.01	0.06	−0.15
Calyx annular constriction	0.16	0.01	0.18	0.02	0.11	−0.15	−0.04	0.10	0.05	0.01	−0.10	−0.04	0.11	0.93
Anthocyanin spots/strips	0.13	0.08	0.02	−0.22	0.05	0.13	−0.04	−0.19	−0.03	−0.03	0.09	−0.03	0.00	−0.06
Fruit color (intermediate stage)	0.18	0.01	0.07	−0.25	−0.17	0.08	−0.23	−0.04	−0.12	0.37	−0.08	0.17	−0.04	−0.17
Fruit set	0.18	−0.19	−0.19	−0.04	−0.10	−0.07	0.03	0.08	0.02	−0.20	−0.11	0.00	−0.13	0.06
Fruit color (mature stage)	0.09	−0.12	0.04	−0.11	−0.12	−0.03	−0.08	0.04	−0.10	0.94	0.10	−0.17	−0.09	0.02
Fruit shape	0.03	−0.05	−0.12	−0.03	0.16	−0.01	−0.02	0.88	0.16	0.05	0.04	−0.06	−0.10	0.12
Fruit length	−0.09	0.01	−0.05	0.11	−0.16	−0.07	−0.07	−0.02	0.06	0.11	0.83	0.07	0.11	−0.08
Fruit width	0.00	−0.06	0.03	0.00	0.01	0.06	0.08	0.08	−0.09	−0.11	0.13	−0.02	0.02	0.01
Fruit weight	−0.03	−0.40	0.04	−0.40	0.02	0.12	−0.02	0.02	0.14	0.05	0.07	0.01	−0.01	0.15
Fruit shape at pedicel	−0.20	0.03	−0.19	0.06	−0.18	0.20	−0.05	0.13	0.99	−0.10	0.02	−0.02	0.08	0.05
Fruit wall thickness	−0.07	−0.07	0.01	0.06	−0.24	−0.10	−0.14	0.13	0.07	−0.06	−0.06	−0.30	0.08	−0.01
Fruit pedicel length	0.06	−0.05	0.23	0.00	−0.14	−0.04	−0.06	0.07	0.00	−0.04	−0.12	0.15	−0.01	−0.05
Neck base	−0.03	−0.02	0.14	−0.17	−0.20	0.08	0.97	−0.03	−0.04	−0.09	−0.09	−0.04	0.06	−0.05
Fruit appendages	0.00	0.05	0.07	0.05	−0.02	0.07	−0.11	0.44	−0.13	−0.03	0.52	−0.11	−0.09	−0.09
Fruit shape at blossom end	0.30	0.03	0.09	0.08	0.11	1.01	0.07	0.01	0.19	−0.02	−0.05	0.04	−0.08	−0.15
Fruit cross-section	−0.11	0.04	0.19	0.04	0.13	−0.34	−0.12	0.15	0.03	−0.04	0.03	0.12	0.04	−0.15
No. of locules	0.19	−0.08	0.02	0.04	−0.03	0.03	0.10	−0.02	0.04	0.09	−0.01	−0.17	0.13	0.09
Fruit surface	0.08	−0.04	−0.03	0.07	0.02	0.04	−0.05	−0.06	−0.01	−0.19	0.02	0.87	0.11	−0.05

DISCUSSION

Although morphological characteristics are influenced by the environment, morphological characterization is the simplest and one of the most important methods in the evaluation process. The characterization of genetic diversity is necessary to improve the utilization of genetic resources in breeding programs and to avoid the duplication of accessions in germplasm banks. Therefore, this study was performed as the first step in evaluating chili genetic diversity in Bhutan. Inflorescence characters, such as flower color, calyx constriction, and number of flowers per node, are among the most commonly used taxonomic descriptors in identification studies on Capsicum. White-flowered species include the domesticated species *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens L.*, and *C. pubescence* Ruiz and Pav. (Thul *et al.,* 2012). According to NBC (2015), the chili species cultivated in Bhutan include *C. annuum*, *C. frutescens*, C. *pubescens*, and *C. baccatum. C. pubescence* is distinguished from other species by its hairy leaves and black seeds (Bosland and Votava, 2012). However, all of the accessions evaluated in this study had the characteristics of *C. annuum* L., with 21 accessions possessing pale blue anthers, five blue, and one purple. The majority of the accessions had 1–2 flowers per axil, and all accessions lacked annular calyx constrictions and straw-colored seeds. Only Pakshika ema had purple anthers that are characteristic of *C. pubescens*. However, all the other characters of this accession were the same as those of *C. annuum*. Therefore, this accession was considered as a *C. annuum* variety. Dallay Khorsaney was previously described as *C. chinense* due its similar shape and high capsaicinoid content (Gurung *et al*., 2012). However, it was identified as a *C. annuum* accession in this study. The distinguishing characteristic of *C. chinense* Jacq. is the constriction at the base of the calyx (Smith and Heiser, 1951). Dallay Khorsaney lacked annular constriction. Bhutia *et al*. (2016) reported that Dallay Khorsaney grown in Sikkim is a *C. annuum* accession. Colney *et al*. (2018) also reported that their SSR and SNP data suggested that Dallay Khorsaney grown in North Eastern India is genetically closer to pungent *C. annuum* than to other accessions. The Dallay Khorsaney variety grown in Bhutan is round and slightly tapering, similar to the ones found in Nepal (Baral and Bosland, 2002) and in the northeastern Indian states of Sikkim and Darjeeing, thus confirming that this variety, which is very pungent and aromatic, is also a *C. annuum* variety.

The other species is *C. frutescens*, which used to be found growing in the forests of the southern Bhutan. It has small, conical, upright, spicy fruits with thin fruit walls, and it lacks calyx annular constriction between the calyx and pedicel (Carvalho *et al.*, 2014). Given its limited use, the cultivation of this species is also declining with only one or two bushes grown in kitchen gardens. This species exhibits limited variation in shape, size, and color, reflecting low genetic variability or restrictive selection due to its limited use. Baral and Boland (2004) reported that *C. chinense* and *C. frutescens* are two distinct species. However, Thul *et al.* (2012) reported that these two species are closely related on the basis of their shared flower characteristics and proximity at the genotype level.

Wangdi *et al*. (2019) reported that two varieties, namely Tsakaling and Indian Fat, have purple corollas that are the characteristics of *C. pubescens* Ruiez. & Pav. species indicating that *C. pubescens* is grown in Bhutan. However, these varieties are uncommon and were not evaluated in this study.

Diederichsen (2010) stated that a high CV indicates that an individual quantitative character can be used to distinguish several groups within the range from the minimum to the maximum value. Therefore, fruit wall thickness could be one of the quantitative characters used to distinguish chili landraces into groups given that its CV was 148.8%. According to Lannes *et al*. (2007), studies on *C. chinense* have revealed a positive relationship between fruit weight and fruit wall thickness. This relationship is important for the selection of varieties for sale on the fresh market given that fruits with thick pericarps show increased resistance to damage during postharvest handling. However, compared with fruits with thick pericarps, fruits with thin pericarps are more suitable for processing due to their higher content of soluble solids and the reduced energy inputs required for their dehydration considering that fruit pericarp thickness is negatively correlated with soluble solid content (Lannes *et al*., 2007). Fruit wall thickness is one of the characteristics of the chilies grown in Bhutan that determines their suitability for use in producing chili cheese. Fruit surface exhibited variation and was semiwrinkled, smooth, or wrinkled. Slight variation was observed for fruit set, with most varieties presenting intermediate fruit set. Chilies with thick walls and wrinkled skins are preferred for cooking cheese sticks because of their superior taste.

Thul *et al*. (2009) found maximum variance in fruit width and leaf width. This observation is in line with the results of this study. Fruit width ranged from 0.2 cm to 5.6 cm with a CV of 54.99%, and leaf width ranged from 2 cm to 11.9 cm with a CV of 46.29%. The similar result reported by Yumnam *et al*. (2012) suggests that leaf area can also be used as a descriptor for distinguishing among types of specimens. Variations could be the result of mutations and selections over the years (Baral and Bosland, 2002). Variation was also not distinctly geographical, indicating that landraces are being exchanged around the country.

The nearest-neighbor method was used in cluster analysis because it facilitated finding outliers. Jerey Khorsaney, a member of *C. frutescence*, was used as an out-group accession. Although Dallay Khorsaney is a *C. annuum* accession, it grouped separately into a distinct cluster mainly because of its fruit shape, which was round and slightly tapering, and leaf length and width, which were long and broad, respectively. Thinleygang I and II were in one cluster. This clustering pattern could be due to geographical variation. Sha ema, which was a blocky type with a maximum fruit weight of 23 g, was in a cluster by itself. Sha ema is traditionally the most popular variety grown in Bhutan. Although Urka bangala resembled Sha ema in its blocky shape, it was shorter, lighter, and had wrinkled fruit surfaces. Moreover, it was located in a different cluster. Khasadrapchu ema and

Baegap ema had similar characteristics except that Khasadraphcu ema had a semiwrinkled fruit surface and Baegap ema had a smooth surface. Moreover, Tharay Khorsaney was located in a different cluster. This clustering pattern could be ascribed to its fruit characteristics of uprightness with thin fruit walls. Lhuntshe semi oro, Khoma ema, Namseling ema, Lower nubi ema, Khasadrapchu ema, and Pakshika ema had semiwrinkled fruit surfaces and similar vegetative characteristics. Therefore, they were grouped in one cluster. Although Paro I and II had smooth surfaces, all of their other characteristics were similar to the characteristics of the previous cluster. According to Zewdie *et al*. (2004), given that the cultivated *C. annuum* var*. annuum* group show high regional and global diversity with a wealth of innumerable strains, landraces, and varieties, it challenges facile description and clustering into an inclusive and practicable classification. Therefore, a molecular-level study is important to allocate Bhutanese chili accessions into different groups.

PCA explained the proportion of the relative contribution of the various characters to the total variance of studied chili accessions. Approximately 83% of the total variation attained in PCA showed a sufficient percentage of variation. Pla (1986) suggested that at least 80% should be attained. Similarly, this result corroborates the findings of Matthew *et al*. (1994), who stated that the high number of principal components for *C. annuum* indicates high variation within the species. PC1 was contributed by the maximum loading of stem shape and stem pubescence, PC2 by mature leaf length, PC3 by plant height, PC4 by stem color, PC5 by flower position, PC6 by corolla color, PC7 by neck base, PC8 by fruit shape, PC9 by fruit shape at pedicel attachment, PC10 by fruit color at the intermediate stage, PC11 by fruit length, PC12 by the number of flowers per axil, PC13 by leaf density, and PC14 by calyx annular constriction. Therefore, qualitative and quantitative characters were responsible for the observed variation.

CONCLUSION

This study identified the main chili species grown in Bhutan as *C. annuum* accessions. High variations were observed among accessions of this species in terms of qualitative and quantitative characteristics. The accessions were grouped into 10 clusters on the basis of hierarchal clustering methods. Through PCA, 14 components were selected as meaningful attributes that accounted for 83% of the variation. Fruit characters showed high CV. Although morphological characters are important in the study of genetic diversity, their use for substantiating real genetic variation is challenging because of their mostly polygenic nature and their dependence on various environmental factors. Therefore, molecular characterization, which has not been done yet, is recommended to further confirm the genetic diversity of chili accessions in Bhutan.

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REFERENCES

AVRDC (2015). Suggested cultural practices for chili pepper. The Asian Vegetable Research Development Center.

Baral JB, Bosland PW (2002). Genetic diversity of a *Capsicum* germplasm collection from Nepal as determined by randomly amplified polymorphic DNA markers. *J. Am. Soc. Hort. Sci*. 127(3): 318-324.

Baral JB, Bosland PW (2004). Unraveling the species dilemma in *Capsicum frutescens* and *C. chinense* (Solanaceae): a multiple evidence approach using morphology, molecular analysis, and sexual compatibility. *J. Am. Soc. Hort. Sci*. 129: 826-832.

Berke T (2002). Pepper project in the processing of the 16th International pepper conference Tampico, Mexico. The Asian Vegetable Research Development Center.

Bertan I, Carvalho FIFD, Oliveira ACD (2007). Parental selection strategies in plant breeding programs. *J. Crop Sci. Biotechnol*. 10 (4): 211 - 222 R

Bhutia KL, Tombisana Meetei NG, Khanna VK (2016). In vitro regeneration of Dalle Khursani, an important chili cultivar of Sikkim, using various explants. *Agrotechnol*. 5(1). DOI: 10.4172/2168-9881.1000142

Bosland PW, Votava EJ (2000). Peppers: Vegetable and Spice Capsicums. UK and New York: CABI.

Bosland PW, Votava EJ (2012). Peppers: Vegetable and Spice Capsicums. 2nd Edition. CPI group.

Bozokalfa MK, Esiyok D, Turhan K (2009). Patterns of phenotypic variation in a germplams collection (*Capsicum annum* L.) from Turkey. *Span. J. Agric. Res.* 7: 83-95.

Carvalho SIC, Ragassi CF, Bianchetti LB, Reifschneider FJB, Buso GSC, FG. Faleiro (2014). Morphological and genetic relationships between wild and domesticated forms of peppers (*Capsicum frutescens* L. and *C. chinense* Jacquin). *Genet. Mol. Res*. 13(3): 7447-7464.

Dem C, Dem P, Chhetri GB (2008). Commodity chain analysis: Chili. Ministry of Agriculture, Royal University of Bhutan: Thimphu.

Diederichsen A (2010). Phenotypic diversity of Jerusalem artichoke (*Helianthus tuberosus* L.) germplasm preserved by the Canadian gene bank. *Helia* 3(3):1-16.

Ebert A, Kumar S, Tenkouano A, Afari-Sefa V (2013). Pepper (*Capsicum* spp.) germplasm dissemination by AVRDC. The world vegetable center: An Overview and Introspection. *Chronica Hort.* 53: 21.

Galetti PM (2018). Conservation genetics. Brenner's Encyclopedia of Genetics, 2013, Pages 165-168. https://doi.org/10.1016/B978-0-12-801238-3.65366-7.

Geleta LF, Labuschagne MT, Viljoen CD (2004). Relationship between heterosis and genetic distance based on morphological traits and AFLP markers in pepper. *Plant Breed*. 123: 467-473.

Guerra EP, Destro D, Miranda LA, Montalvan R (1999). Parent selection for intercrossing in food type soybean through multivariate genetic divergence. *Acta Sci*. 21(3): 429- 437.

Gurung T, Techawongstien S, Suriharn B, Techawongstien S (2012). Stability analysis of yield and capsaicinoids content in (*Capsicum* spp.) grown across six environments. Euphytica 87(187):11-18.

IPGRI (1995) Descriptors for *Capsicum* (*Capsicum* spp.). International Plant Genetic Resources Institute (IPGRI), Rome.

Lannes SD, Finger FL, Schuelter AR, Casali VWD (2007). Growth and quality of Brazilian accessions of *Capsicum chinense* fruits. *Scientia Hort.* 112: 266-270.

Matthew C, Lawoko CRO, Korte CJ, Smith D (1994). Application of canonical discriminant analysis, principal component analysis, and canonical correlation analysis as tools for evaluating differences in pasture botanical composition. *New Zealand J. Agric. Res.* 37:509-520.

National Biodiversity Center (2008). Plant genetic resources of Bhutan: Field crops. Ministry of Agriculture and Forest, Royal Government of Bhutan. Thimphu, Bhutan.

National Biodiversity Center (2015). The history of the introduction and adoption of important food crops in Bhutan Rice, maize and potato, National Biodiversity Centre Ministry of Agriculture and Forests Serbithang, Thimphu, Bhutan

Pla E (1986). Análisis multivariado: Método de componentes principales. 94 p. Secretaría General de la Organización de los Estados Americanos, Washington, DC, USA.

Pradhan P (1996). Bhutan country report to the FAO international technical conference on plant genetic resources. Leipzig, Germany.

Schreinemachers P, Ebert AW, Wu MH (2014). Costing the *ex situ* conservation of plant genetic resources at AVRDC The World Vegetable Center. *Genet Resour Crop Evol* 61: 757–773.

Smith PG, Heiser CB Jr (1951). Taxonomic and genetic studies on the cultivated peppers, *Capsicum annuum* l. and *C. Frutescens* L. *Am. J. Bot*. 38: 362–368.

Sudre´ CP, Gonc¸alves LSA, Rodrigues R, do Amaral Ju´nior AT, Riva-Souza EM, Bento CS (2010). Genetic variability in domesticated Capsicum spp as assessed by morphological and agronomic data in mixed statistical analysis. *Genet. Mol. Res*. 9: 283–294.

Thapa L (2012). Traditional chili pepper diversity and farmers perception on its decline. B.Sc. Dissertation.CNR. Royal University of Bhutan.

Thul ST, Darokar MP, Shasany AK, Khanuja SP (2012). Molecular profiling for genetic variability in *Capsicum* species based on ISSR and RAPD markers. *Mol. Biotechnol*. 51: 137–147.

Thul ST, Lal RK, Shasany AK, Darokar MP,Gupta AK, Gupta MM, Verma RK, Khanuja SP (2009). Estimation of phenotypic divergence in a collection of Capsicum species for yield-related traits. *Euphytica* DOI 10.1007/s10681-009-9882-y.

Wangdi T, Watcharawetsaringkharn S, Philadee J, Pooprompan P (2019). Evaluation of Morphological Characteristics of Bhutanese Landrace Chilies (*Capsicum* spp.). *Thai J. Sci Technol*. DOI: https://doi.org/10.14456/tjst. 2019.51

Yumnam JS, Tyagi W, Pandey A, Meetei NT, Rai M (2012). Evaluation of Genetic Diversity of Chili Landraces from North Eastern India Based on Morphology, SSR Markers and the Pun1 Locus. *Plant Mol. Biol. Rep.* 30(6): 1470-1479.

Yuzbasıoglu E, Ozcan S, Açik L (2006) Analysis of genetic relationships among Turkish cultivars and breeding lines of *Lens culinatis.* Mestile using RAPD markers. *Genet. Resour. Crop Evol*. 53(3): 507-514.

Zewdie, Y., N. Tong and P. Bosland. 2004. Establishing a core collection of Capsicum using a cluster analysis with enlightened selection of accessions. *Genet. Resour. Crop Evol.* 51: 147-151.