

Evaluation of Morphological Characteristics of Bhutanese Landrace Chilies (*Capsicum* spp.)

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Abstract

Chili plays an integral part in a different culture. It is cultivated as commercial and in backyards for spices, vegetables, ornamental and medicinal purposes. In Bhutan, chili (*Capsicum* spp.) is an important crop and is cultivated across the country. Twenty chili varieties cultivated in Bhutan were collected from different chili growing regions to evaluate morphological characteristics at Maejo University, Chiang Mai, Thailand. The experiment was conducted under 50% shade net in a completely randomized design with five replications. The results showed a significant difference among the varieties, the earliest flowering was recorded from 23 to 42 days in 15 varieties and late flowering in 67 days in 'Dhallay' variety. The mean plant height, canopy, and stem diameter were 76.3 ± 18.2 cm, 42.7 ± 7.6 cm and 7.5 ± 3.1 mm, respectively, while the mean fruit length, width and weight were 9.8 ± 4.4 cm, 2.1 ± 0.7 cm, and 11.1 ± 9.6 g, respectively. The varieties 'Tsakaling' and 'Indian Fat' had purple corolla color which indicates *Capsicum pubescens* Ruiz. & Pav. species. Geographical group analysis showed that varieties introduced from Japan had different characters from those of the Bhutanese landrace varieties except for leaf width, plant height, plant canopy and stem diameter. The results of morphological characteristics from this study will have potential application in chili genetic diversity conservation and plant breeding program.

Keywords: Chili; landrace; morphology; Bhutan

1. Introduction

Chili (*Capsicum* spp.) is an important crop that is cultivated throughout the world as a well known commercial crop. It is commonly known by pepper, chili and chile depending on its

location. Chilies have become an integral part of different cultures in the world. People around the globe cultivate this crop for spices, vegetables, ornamental and medicinal purposes (Sanatombi *et al.*, 2010; Xiao-min *et al.*, 2016). The pungent

varieties are mostly used as spices while non-pungent varieties are used as vegetables.

The crop was first cultivated by the people of tropical America and then spread to the other regions of the world (Pickersgill, 1997). Chili is also a vital cash crop grown by the farmers from the developing countries and it dominates the current global spice trade (Lin *et al.*, 2013). Its cultivation is concentrated in the warm temperate, sub-tropical to tropical countries.

In the genus *Capsicum*, there are 25 wild species and five cultivated species, *Capsicum annuum* L., *Capsicum frutescens* L., *Capsicum chinense* Jacq., *Capsicum baccatum* L., and *Capsicum pubescens* Ruiz. & Pav. (Pickersgill, 1997). Amongst the five cultivated species *Capsicum annuum* L. is widely cultivated (Lin *et al.*, 2013; Pickersgill, 1997).

In Bhutan chili is consumed both as spice and vegetable. Bhutanese diet consists of chili as an essential part and every meal contains chillies in various forms such as fresh green, dried and powdered (Ueda and Samdup, 2009)

The crop is cultivated in all districts of the country. Farmers in major chili growing areas earn good cash income from the sale of chili. The potential return per unit area of chili is high which is achieved in a relatively short period (Dorji *et al.*, 2009). Chili crop production in 2017 was recorded for 13,606 metric tonnes from the cultivation area of 7,571 acres with an average yield of 1,797 kg/acre and it was recorded as the highest cultivated vegetable in terms of the area beside potato (Ministry of Agriculture and

Forests, 2017).

Chili species that are cultivated in the country are *Capsicum annuum* L., *Capsicum frutescens* L., *Capsicum pubescens* Ruiz. & Pav. and *Capsicum baccatum* L. (National Biodiversity Centre, 2015). Three varieties of chillies have been released (Ghimiray and Katwal, 2013) until now. However various landrace varieties are cultivated by the farmers in different parts of the country under different environment conditions beside the released varieties. The most popular landrace varieties are 'Sha Ema' in Wangdue phodrang, 'Nubi Ema' in Trongsa, 'Kangpara Ema' in Trashigang and 'Pakshikha Ema' in Chukha. Studies show that there are 18 accessions of *Capsicum annuum* L. collected from different parts of the country as a plant genetic resource (Ghimiray, 2005).

The landrace varieties are cultivated from the seeds stored from the previous season. However, proper seed production methods are not practiced probably leading to cross-pollination among the varieties. Although chili is considered autogamous studies have found cross-pollination from 7-90% (Bozokalfa *et al.*, 2009) which could alter both phenotypic and genotypic characters of the landrace varieties. These landrace varieties provide an important source of variation for plant breeding. Ghimiray and Katwal (2013) suggested that traditional landrace varieties be collected for conservation and characterization of its diversity and utilization for further crop improvement activities. Considering the importance of chili crop in the

Bhutanese cuisine, its economic returns and future improvement program, this study was conducted with an objective to evaluate the morphological characters of Bhutanese chili varieties collected from various location of the

country. Morphological characteristics like fruit shape, fruit weight, flower colour, plant height, and leaf types were used to identify the characteristics of the germplasm.

Table 1 List of chili varieties collected from Bhutan for the study

Variety	Place and seed source	Elevation (masl)
Sha Ema	Wangduephodrang (Farmer)	1,310
Baegap	Wangduephodrang (Farmer)	1,310
Nubi	Trongsa (ARDC,Wengkhar)	2,200
Tamchu	Paro (Farmer)	2,250
Pakshikha	Pakshikha (ARDC, Wengkhar)	1,503
Namseling	Thimphu (Farmer)	2,350
Khasarapchu	Thimphu (Farmer)	2,350
Kabji	Thimphu (Farmer)	1,310
Bumthang	Bumthang (Farmer)	2,800
Tsakaling	Mongar (ARDC,Wengkhar)	1,650
Bangtsho	Bangtso (ARDC,Wengkhar)	2,180
Khalong	Khalong (ARDC,Wengkhar)	2,100
Urka	Trashiyangtse (ARDC, Wengkhar)	1,850
Dhallay	Mongar (ARDC, Wengkhar)	1,650
Indian	Mongar (ARDC, Wengkhar)	1,650
Indian fat	Mongar (ARDC, Wengkhar)	1,650
Indian short	Mongar (ARDC, Wengkhar)	1,650
Ornamental	Mongar (ARDC, Wengkhar)	1,650
Hog Intestine	Mongar (ARDC, Wengkhar)	1,650
Big Horn	Mongar (ARDC, Wengkhar)	1,650

2. Materials and methods

2.1 Planting materials

Chili seeds were collected from various parts of the country. A total of 20 varieties were collected from the eight chili growing regions. Seven varieties were collected from the farmers

and 13 varieties were collected from Agriculture Research and Development Centre (ARDC), Wengkhar, as shown in Table 1.

2.2 Study location

The experiment was carried out at Ornamental Horticulture field at Maejo

University, Chiang Mai, Thailand, located at 18°53'35" N and 99°0'42" E at an elevation of 280 meters above the sea level (masl).

2.3 Experimental design and data collection

The nursery was raised on 13 February 2018 in the plug tray inside the greenhouse. Completely randomized design (CRD) with five replications was carried out inside the net house (50% shade net). 100 plastic pots were filled with 1.5 kg soil mixture equally and then arranged (five rows and 20 columns) inside the net house. The plastic pots were then numbered from 1 to 100 in sequence. Fertilizers (NPK 15:15:15) of 0.45 g each were applied to all plastic pots. Seedlings were transplanted on 21 March 2018 after 36 days of sowing.

Morphological traits were recorded using the International Plant Genetic Resources Institute descriptors for *Capsicum* (1995). Plant height and canopy were measured using 100 cm scale while the fruit, leaf and stem diameter were measured by a digital vernier caliper (Coral stainless hardened). Fruits were weighted using a digital weighing balance (Sartorius BP 110 S). Plant growth habit, flower colour, fruit colour, and shapes were recorded visually.

The varieties were categorized into four groups based on the elevation of the place that seeds were collected. Group one consists of varieties that were collected from low elevation (less than 1,500 masl) 'Baegap', 'Sha' and 'Kabji'. Group two included varieties that were collected from high elevation (more than

2,000 masl) 'Nubi', 'Tamchu', 'Namseling', 'Khasarapchu', 'Bumthang', 'Bangtsho' and 'Khalong'. Group three included varieties that were collected from mid-elevation (between 1,500 to 2,000 masl) 'Pakshikha', 'Tsakaling', 'Urka', 'Dhallay', 'Indian', 'Indian Fat', 'Indian Short' and 'Ornamental'. Group four consists of those varieties that were introduced from Japan ('Hog Intestine' and 'Big Horn').

2.4 Statistical analysis

Statistical analysis was performed using R program software. Analyses of variance (ANOVA) and mean separation were done using Duncan Multiple Range Test.

3. Results and discussion

3.1 Characteristics of 20 chili varieties

Mean characteristics and ranges of 20 varieties were recorded for plant height, plant canopy, stem diameter, leaf length, leaf width, flowering days, fruit length, fruit width and fruit weight as shown in Table 2. The varieties showed variation which indicates characters of different species such as plant height, flowering day and fruit characteristics.

3.2 Plant characteristics

Plant height results showed that the tallest plant 'Dhallay' variety and the shortest 'Tsakaling' variety had 114.8 cm and 26.2 cm heights respectively. Plant canopy results showed the widest 'Big Horn' variety which had 61.0 cm and lowest 'Tsakaling' variety with 19.6 cm. Stem diameter result showed that the 'Indian' variety had the longest diameter of 19.6 mm while 'Tsakaling' variety had the lowest

diameter of 5.3 mm as shown in Table 3. Similar results on 'Dhally' variety were reported by Jha *et al.* (2017) and Agriculture Research and Development Centre (2016) that reported on the 'Sha Ema' and 'Baegap' varieties.

Stem colour in all varieties was green except for 'Tsakaling' and 'Indian fat' variety

which had purple stem colour. Plant growth habit was erect in 15 varieties, prostrate in 'Tsakaling' and intermediate in 'Indian', 'Indian fat', 'Indian short' and 'Ornamental' varieties. Branching habits were observed sparsely in 14 varieties, intermediate in six varieties and dense in 'Ornamental' variety as shown in Table 4.

Table 2 Mean±SD, minimum and maximum characteristics of 20 chili varieties collected from Bhutan

Characteristics	Mean±SD	Minimum	Maximum
Plant Height (cm)	76.3±18.2	26.2	114.8
Plant Canopy (cm)	42.7±7.6	19.6	61.0
Stem Diameter (mm)	7.4±3.1	5.3	19.6
Leaf Length (mm)	108.2±21.5	71.6	165.7
Leaf Width (mm)	54.3±17.2	34.2	120.0
Flowering Days	38.6±12.9	23.0	67.0
Fruit Length (cm)	9.8±4.4	1.9	188.0
Fruit Width (cm)	2.1±0.7	1.1	3.9
Fruit Weight (g)	11.1±9.6	1.8	42.9

3.3 Leaf characteristics

The longest leaf of 141.1 mm was observed in 'Hog Intestine' variety while the shortest one of 71.6 mm in 'Baegap' variety was shown. The widest leaf width was recorded 120.0 mm in 'Dhally' and the least 34.2 mm in 'Baegap' variety was evident as shown in Table 3. The leaf shapes were deltoid in 'Dhally' and 'Tsakaling' variety, lanceolate in 'Indian', 'Indian fat', 'Indian Short' and 'Ornamental' varieties while 14 varieties had oval shape (data not shown).

3.4 Flower characteristics

Flowering days of the varieties had a highly significant difference ($p < 0.01$) among the varieties. Earliest flowering was recorded in nine

varieties 'Sha Ema', 'Baegap', 'Nubi', 'Tamchu', 'Pakshikha', 'Namseling', 'Khasarapchu', 'Kabji', 'Bumthang', 'Bangtsho', 'Khalong', 'Urka', 'Indian short', 'Hog Intestine' and 'Big Horn' varieties from 23 to 42 days and late flowering in 'Dhally' at 67 days after the transplantation was recorded as shown in Table 3. The anther colour in 18 varieties was blue to purple except in two varieties ('Tsakaling' and 'Indian Fat') that had a purple colour. Corolla colour was purple in two varieties 'Tsakaling' and 'Indian Fat' while 18 varieties had white colour as shown in Figure 1 and 2. The purple colour of these two varieties was matched with the corolla colour of the *Capsicum pubescens* Ruiez. & Pav. species as

described by Organisation for Economic Co-operation and Development (2006) and indicates that these two varieties are in this species.

The number of flowers in axil in all varieties was one each. The flower position was erect in five varieties ('Pakshikha', 'Indian', 'Indian fat', 'Indian short' and 'Ornamental') and intermediate in 15 varieties.

3.5 Fruit characteristics

Fruit observation results showed the 18.8 cm longest fruit in 'Hog Intestine' variety while the 1.9 cm shortest one was found in 'Dhallay' variety. The 3.9 cm and 3.5 cm widest fruits were recorded in two varieties and the 1.1 cm least width was observed in 'Ornamental'. Fruit weight result showed the 42.9 g highest

Table 3 Plant and fruit characteristics of 20 chili varieties collected from Bhutan

Variety	LL (mm)	LW (mm)	PH (cm)	PC (cm)	SD (mm)	FD	FL (cm)	FW (cm)	FWt (g)
Sha Ema	85.1 ^{de}	38.3 ^{de}	70.4 ^{bcd}	38.8 ^b	7.0 ^{cde}	37 ^{cde}	9.9 ^{fg}	1.9 ^c	8.0 ^{def}
Baegap	71.6 ^e	34.2 ^e	70.6 ^{bcd}	36.8 ^b	5.5 ^e	39 ^{b-e}	11.1 ^{ef}	2.2 ^c	10.1 ^{cde}
Nubi	100.0 ^{cde}	54.0 ^{cde}	78.4 ^{a-d}	44.2 ^{ab}	6.1 ^d	32 ^e	12.1 ^{de}	2.0 ^c	9.4 ^{c-f}
Tamchu	101.6 ^{cde}	51.7 ^{b-e}	74.4 ^{bcd}	44.6 ^{ab}	6.1 ^{de}	38 ^{cde}	9.3 ^{gh}	2.1 ^c	9.9 ^{cde}
Pakshikha	102.8 ^{cde}	51.2 ^{b-e}	85.0 ^{a-d}	52.4 ^{ab}	6.7 ^{cde}	31 ^e	10.8 ^{efg}	1.4 ^d	5.0 ^{fgh}
Namseling	107.6 ^{cd}	53.3 ^{b-e}	75.6 ^{bcd}	47.0 ^{ab}	7.2 ^{cde}	33 ^e	12.9 ^{cd}	2.1 ^c	11.9 ^{cd}
Khasarapchu	93.1 ^{de}	45.9 ^{de}	84 ^{a-d}	43.6 ^{ab}	6.20 ^{de}	39 ^{b-e}	11.6 ^{de}	2.2 ^c	11.8 ^{cd}
Kabji	104.5 ^{cde}	53.6 ^{bcd}	60.2 ^d	35.8 ^b	5.65 ^e	29 ^e	15.1 ^b	2.9 ^b	21.3 ^b
Bumthang	98.3 ^{cde}	49.3 ^{b-e}	66.2 ^d	42.8 ^{ab}	6.3 ^{de}	23 ^e	13.1 ^{cd}	2.0 ^c	11.8 ^{cd}
Tsakaling	95.0 ^{de}	62.3 ^{bc}	26.2 ^d	19.6 ^c	5.3 ^e	55 ^{abc}	-	-	-
Bangtsho	96.2 ^{de}	43.9 ^{cde}	77.4 ^{bcd}	44.2 ^{ab}	6.1 ^{de}	29 ^e	8.2 ^h	2.0 ^c	8.4 ^{def}
Khalong	91.06 ^{de}	45.6 ^{cde}	69.4 ^{bcd}	44.6 ^{ab}	6.4 ^{cde}	29 ^e	8.2 ^h	2.1 ^c	6.7 ^{efg}
Urka	98.7 ^{cde}	49.7 ^{b-e}	70.4 ^{bcd}	41.2 ^b	6.29 ^{de}	35 ^{de}	5.6 ^{ij}	3.5 ^a	13.6 ^c
Dhallay	165.7 ^a	120.0 ^a	114.8 ^a	40.2 ^b	9.35 ^{ab}	67 ^a	1.9 ^l	1.5 ^d	2.0 ^h
Indian	115.6 ^{bcd}	50.2 ^{b-e}	103.0 ^{ab}	42.8 ^{ab}	19.6 ^a	58 ^{ab}	5.9 ⁱ	1.2 ^d	2.9 ^{ih}
Indian fat	115.1 ^{b-d}	50.1 ^{b-e}	76.4 ^{bcd}	42.4 ^b	8.2 ^{bc}	56 ^{abc}	4.2 ^{jk}	1.4 ^d	3.2 ^{gh}
Indian short	132.4 ^{bc}	60.8 ^{bc}	101.8 ^{abc}	43.4 ^{ab}	7.9 ^{bcd}	42 ^{b-e}	-	-	-
Ornamental	131.6 ^{bc}	49.4 ^{b-e}	87.0 ^{a-d}	43.4 ^{ab}	9.0 ^{ab}	54 ^{a-d}	3.64 ^k	1.1 ^d	1.8 ^h
Hog Intestine	141.1 ^{ab}	65.8 ^b	68.0 ^d	44.6 ^{ab}	7.1 ^{cde}	23 ^e	18.8 ^a	3.9 ^a	42.9 ^a
Big Horn	116.7 ^{bcd}	57.8 ^{bc}	67.0 ^d	61.0 ^a	6.9 ^{cde}	23 ^e	14.0 ^{bc}	2.7 ^b	19.5 ^b

Means having same letters on the same column were not significantly different at 0.05 probability level using the Duncan Multiple Range test. (LL = leaf length, LW = leaf width, PH = plant height, PC = plant canopy, SD = stem diameter, FD = flowering days after transplantation, FL = fruit length, FW = fruit width, and FWt = fruit weight)

Table 4 Plant growth habit and stem colour of 20 chili varieties collected from Bhutan

Variety	Stem colour	Plant growth habit	Branching habit
Sha Ema	Green	Erect	Intermediate
Baegap	Green	Erect	Sparse
Nubi	Green	Erect	Sparse
Tamchu	Green	Erect	Sparse
Pakshikha	Green	Erect	Sparse
Namseling	Green	Erect	Sparse
Khasarapchu	Green	Erect	Sparse
Kabji	Green	Erect	Sparse
Bumthang	Green	Erect	Intermediate
Tsakaling	Purple	Prostrate	Sparse
Bangtsho	Green	Erect	Sparse
Khalong	Green	Erect	Sparse
Urka	Green	Erect	Intermediate
Dhallay	Green	Erect	Sparse
Indian	Green	Intermediate	Intermediate
Indian fat	Purple	Intermediate	Intermediate
Indian short	Green	Intermediate	Sparse
Ornamental	Green	Intermediate	Dense
Hog Intestine	Green	Erect	Sparse
Big Horn	Green	Erect	Sparse

weight in 'Hog Intestine' variety and the 1.8 g lowest weight was found in 'Ornamental' variety as shown in Table 3.

Green fruit colour at immature stage and red colour at the mature stage were observed in 18 varieties, brown-red were found in 'Namseling' variety and yellowish-red were observed in 'Hog Intestine' variety. The fruit shapes of 18 varieties were elongate, round to oval in 'Dhallay' variety and blocky in 'Urka' variety. Bianchi *et al.* (2016) had a similar study in 30 accessions of pepper collected from Brazil

for morphological characters, which had a mean of 2.34 cm fruit length and 1.60 cm fruit diameter. Villota-Cerón *et al.* (2012) described the morphological characters of *Capsicum* spp. in Colombia. It was found that *C. annuum* had elongate and triangle shaped fruit, *C. baccatum* L. had elongate fruit shape, *C. chinense* L. had triangle to bell-shaped fruits, *C. frutescens* L. as well as *C. pubescens* Ruiz. & Pav. had triangle-shaped fruit. Thapa (2012) reported that the fruits of 'Pakshikha' variety grew upright with a slender shape.

The two varieties failed to set fruit although their flowers were purple in colour which indicates as *Capsicum pubescens* Ruiz. & Pav. species (Organisation for Economic Co-operation and Development, 2006). Csilléry (2006) stated that *Capsicum pubescens* Ruiz. & Pav. was cultivated in the Andes Mountains and had the best cold tolerance. Yamamoto *et al.* (2013) found that *Capsicum pubescens* Ruiz. & Pav. in Indonesia was cultivated in West and East Java above 1,400 masl. Tuy and Kenji (2015) stated that chili grew and yielded best at the temperature of 21-33 °C.

3.6 Group comparison among the varieties

Group analysis was carried out based on the source of seeds collected from low (group 1), mid (group 3), high elevation (group 2) and two varieties ('Hog Intestine' and 'Big Horn') that were introduced from Japan (group 4). Leaf length analysis showed a significant difference ($p < 0.05$) among group 1 and groups 2, 3, 4 but no significant difference ($p > 0.05$) between group 2 and group 3, group 3 and group 4. Plant canopy results showed no significant difference ($p > 0.05$) among the groups. Flowering days results showed a significant difference ($p < 0.05$) among group 3 and other groups but no significant difference ($p > 0.05$) among group 1, 2 and 4. Fruit length showed there was a significant difference ($p < 0.05$) among groups except for group 1 and 2. Fruit width results indicated that there were significant difference ($p < 0.05$) among group 4 and group 2, 3 but no significant difference ($p > 0.05$) between group 4

and 1, among groups 1, 2, 3. Fruit weight results show a significant difference ($p < 0.05$) among group 4 and others but no significance ($p > 0.05$) with other groups as shown in Table 5. There was no significant difference among the group ($p > 0.05$) in leaf width, plant height, plant canopy and stem diameter among the groups.

The variation in the varieties among the groups could be as *Capsicum* spp. exhibits a great variation in shapes, sizes, and colours (Walsh and Hoot, 2001). Other possible factors attributing variation were elevations of the source of plant materials. The group consists of varieties cultivated from 1,310 masl to 2,800 masl and one group with two introduced varieties (group 4).

4. Conclusion

Significant differences ($p < 0.05$) were observed among the 20 varieties in terms of plant height, canopy and fruit characteristics. Group-wise comparisons among the low, mid, high altitudes from the seed sources and Japanese varieties, there were a significant difference ($p < 0.05$) in leaf length, flowering day, fruit length, fruit width, and fruit weight. Two varieties 'Tsakaling' and 'Indian fat' failed to set fruit as these two varieties were grown in the cold regions.

The morphological characteristics and diversity evaluated for the 20 chili varieties will have potential application for current and in future in crop selection, genetic diversity conservation, and crop improvement programs.

Table 5 Group-wise comparison among chili varieties collected from different geographic regions in Bhutan

Group	LL	LW	PH	PC	SD	FD	FL	FW	FWt
1 (low elevation)	87.1c	42.1	67.1	37.1	6.0	34.6b	12.1b	2.3ab	13.1b
2 (high elevation)	98.3b	49.2	75.1	44.4	6.3	31.7b	10.8b	2.1b	10.0b
3 (mid elevation)	119.7ab	61.7	83.1	40.6	7.0	49.6a	5.3c	1.7b	4.7b
4 (Japan origin)	128.9a	61.8	67.5	52.8	7.8	23.0b	16.4a	3.3a	31.2a
p-value	0.02	0.27	0.52	0.98	0.06	0.002	0.0006	0.04	0.0009

Means having same letters on the same column were not significantly different at 0.05 probability level using the Duncan Multiple Range test. (LL = leaf length, LW = leaf width, PH = plant height, PC = plant canopy, SD = stem diameter, FD = flowering days after transplantation, FL = fruit length, FW = fruit width, and FWt = fruit weight)

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