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Morphological interrelationship of selected economically important Capsicum spp in Sri Lanka

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Abstract – *The genus Capsicum belongs to the family Solanaceae has five domesticated species, C. annuum, C. baccatum, C. chinense, C. frutescens, and C. pubescens, and more than thirty wild species. Knowledge of the local Chilli (Capsicum spp) varieties and minor scale growing Capsicum species; specially the 'Kochchi' group as well as wild species, are not properly described, evaluated and documented. This constitutes has created a major research gap by not having a proper understanding specially genetic constituents of the minor scale growing species as well as wild species. As a basic solution to this problem, the present study was conducted to study the interspecies relationships using morphological characterization. A total of twenty-seven (27) accessions from the Plant Genetic Resources Center, Gannoruwa, and Peradeniya were morphologically characterized in the present investigation. They corresponded to eighteen (18) Capsicum annum; 'Miris' accessions, eleven (11) Capsicum frutescens; 'Kochchi'. All five plants grown from each accession were subjected to morphological characterization using the Manual for Capsicum morphological characterization developed by the Plant Genetic Resource Center in 1999. Morphological data were recorded for 38 characters corresponding to 27 qualitative and 11 quantitative traits. Morphological data were computed to calculate the means for each of the accessions. A phenogram was generated using the Numerical Taxonomic System, Mesquite software. The phonogram generated displayed morphological diversity among Capsicum accessions by separating the entire phylogeny into four (04) major clades, whereas with two individual clades, 1 and 2; C. annuum 557 and C. annuum 476. The third clade indicates interrelationships of nine Capsicum frutescens accessions namely 391, 762, 11641, 1070, 9089, 1083, 11642, and 11644. Capsicum annuum; 12829, 255, and 388 accessions nested in the second clade by closely associated with the Capsicum frutescens accessions indicating Capsicum annuum accession umber 12829 as the common ancestor. However, this relationship should be further studied through molecular phylogenetical analysis. Clade number four (04) is further divided into two clades separating one cluster only for Capsicum annuum accessions; 180, 5152, 1223, 819, 1782, 1781, 1780, and 1778. The present study does not reveal the morphological phylogeny entirely of the studied Capsicum accessions. Although morphological characterization is recognized as the starting point in the assessment of genetic diversity, morphological markers should be complemented with molecular markers for more advanced genetic diversity study in Capsicum.*

***Keywords**: Morphological characters, Capsicum annuum, Capsicum frutescens, phonogram*

1.0 INTRODUCTION

The genus *Capsicum* consists of all the 'chili', 'pepper' plants, and the confusing terminology 'chilli' is often used frequently and interchangeably with other names globally including 'chile', 'aji', and 'paprika' to refer to multiple species (Basu and De, 2003). The genus Capsicum has been cultivated since at least 6000 B.C in America. The genus has five domesticated species, *C. annuum, C. baccatum, C. chinense, C. frutescens*, and *C. pubescens*, and more than thirty wild species (Carrizo Garcia et al, 2013; Heiser and Pickersgill, 1969). The most commonly cultivated Capsicum species is *C. annuum*, which is domesticated in northern Latin America (Pickersgill, 1971; Kraft et al, 2014). *C. chinense* and *C. frutescens* are domesticated in tropical northern Amazonia, while *C. baccatum* and *C. pubescens* are more prevalent in Latin America and mid-elevation southern Andes, respectively (Pickersgill, 1971) The domesticated species can be classified by morphological traits: seed color, corolla yellow spot, number of flowers per axil, calyx annular constriction, and flower position (IBPGR, 1983).
In Sri Lanka we refer to all the Capsicum as '*Miris*' and differences identify with either the shape or the color of the pods. We do not have specific names for hundreds of species, cultivars or varieties we cultivate or see in our surroundings. The number of global species within the *Capsicum* genus has long been subject to debate, with various authors ascribing 25 species to the genus, 33 by Morrison in 1680, 27 by Tournefort in 1700, two by Linnaeus in 1753, and five by Smith and Heiser, 1951 (Basu and De, 2003). There are presently considered to be five domesticated species of *Capsicum* from approximately 25 recognized species in the genus, the primary distinguishing characteristics being flower and seed colour, the shape of the calyx, number of flowers per node, and their orientation; these five species are *C. annuum, C. frutescens, C. chinense, C. baccatum* and *C. pubescens* (Hawkes et al., 1979; Basu and De, 2003; Aguilar-Melendez et al., 2009). Cultivated *C. annuum* is thought to have been domesticated from wild populations of *C. annuum* var. *glabriusculum* in Mexico, possibly multiple times from geographically separate wild populations (Aguilar-Melendez et al., 2009).

Sri Lanka has a number of Capsicum species that are popular among consumers as well as unpopular species grown mainly at the village level. Most of these species are underutilized having their own aroma, pungency, taste, and flavor. Further, some of these varieties show resistance to the pests and diseases and are specially resistant to the current scenario of environmental and climatic changes. Among commonly grown species five domesticated species are *Capsicum annum* L, *C. frutescens* L, *C. chinense* Jacq., *C. baccatum* L., *C. pubescens* Ruiz and Pavon. *C. annuum* L. is the most commonly cultivated either for pungent fruited genotypes called Chilli or '*Miris*' or non-pungent fruited genotypes called sweet pepper (syn. capsicum, paprika, bell pepper etc.) (Sanjay et al., 2009). Some *Capsicum* spp (syn. Kochchi, Nai Miris, Hini Miris etc.) is having high pungency and different aroma as well as taste and flavour, preferred by most the Sri Lankans. However, minor scale growing these *Capsicum* spps. known as '*Kochchi*' is underutilized in Sri Lanka and most popular as the home garden crop.

1.1 Research rationale

The ability to characterize morphological diversity is indispensable for effective management and sustainable use of Capsicum genetic resources in breeding programmes. Primary characterization involves measuring simple plant characters that can be easily recorded through visual observations at different plant growth stages (leaf area, fruit shape, size and colour, plant prickliness, and hairiness). Secondary characterization deals with more complicated morphological traits of agronomic importance such as pest and disease resistance, fruit set, yield potential, and biochemical (glycoalkaloid or antioxidant) properties (Ayad, 1995). Therefore, morphological crop descriptors allow quick and easy discrimination between phenotypes. They are generally highly heritable traits that can be easily recorded through visual observations and that are equally expressed in all environments.

Knowledge of the local Chilli (*Capsicum* spp) varieties and minor scale growing Capsicum species; specially the '*Kochchi*' group as well as wild species, are not properly described, evaluated and

documented. This constitutes a major research gap since minor scale growing Capsicum species; such as '*Kochchi*' group is currently underutilized. Further, there is no proper understanding of the wild species of Capsicum. Enhancement and protection of the local species or varieties on the other hand require analysis of their characteristics, diversity and relationship with similar accessions. There is an urgent need for the active reintroduction of Capsicum genetic diversity resources into the current production system due to various reasons. First, the use of *Capsicum* spp effectively with improved varietal compositions among Sri Lankans is required to optimize their use and also protect the existing local species and cultivars from extinction. Secondarily, overall research must be diverted to raise improved varieties with the economical values specially genetically divert or adjusted improved varieties such as, to resist pests and diseases, changing climate, to acquire high yield, to develop new varieties with consumer referable pungency, taste, and aroma.

Understanding genetic relationships are possible using morphological characterization as well as molecular characterization of Capsicum. Further, understanding the genetic resources will also enable researchers to identify accessions with desirable traits, monitor their genetic stability and integrity and screen for duplicate accessions to minimize waste of resources and lower management costs. Morphological descriptors for *Capsicum annuum* L, *C. frutescens* L, *C. chinense* Jacq., *C. baccatum* L., *C. pubescens* Ruiz and Pavon. *C. annuum* L. has been developed by Plant Genetic Resource Center (PGRC), Gannoruwa which provides internationally accepted definitions for these descriptors and includes complete information on important quantitative and qualitative traits illustrated by figures and measured either in metric or arbitrary scale. To this end, the present research study was conducted to access the morphological diversity of Capsicum accessions under *in-situ* conservation at PGRC, Gannoruwa, Sri Lanka. Therefore, the objective of the research is to initiate morphological characterization of Capsicum genetic resources for a basic understanding of the genetical interrelationship among species and varieties which will be helpful for future evaluation, monitoring, and documentation.

2.0 MATERIALS AND METHODOLOGY

2.1 Planting Material

A total of twenty-seven (27) accessions from the Plant Genetic Resources Center, Gannoruwa, and Peradeniya were morphologically characterized in the present investigation. They corresponded to eighteen (18) *Capsicum annum; 'Miris'* accessions, eleven (11) *Capsicum frutescens*; '*Kochchi*'. These accessions are maintained as *in-situ* collections at the Plant Genetic Resource Center. Among the 27 Capsicum accessions, nine accessions were collected from the Intermediate Zone of Sri Lanka while the remaining accessions were collected from different agro-ecological regions in Sri Lanka as listed in Table 2.

2.2 Experimental site

The experiment was conducted at the Open University of Sri Lanka, Nawala, Nugegoda to ensure that all Capsicum accessions were at the same stage of growth for morphological characterization. The experiment site received a mean annual rainfall of <800 mm and had suitable agro-climatic conditions favorable for the growth and development of eggplant crops with successful expressions of all traits.

2.3 Establishment and maintaining pot plants

Nurseries were prepared inside the insect-proof plant houses. Seeds were sown on separate plastic trays 30 cm in length and 15 cm in width and 10 cm in depth separately. Before seed sowing, hot water treatment was performed by soaking seeds in a hot water bath at 50°C for 25 minutes followed by fungicide dressing with Captan to prevent any incidence of seed and soil borne diseases. Three weeks after seed sowing, the plants were transferred to the 1 feet diameter pots

filled with 1:1:1:1 – Top soil : sand : cow dung : coir dust potting mixture. One plant per accession with 5 replicates was grown such that 5 plants per each accession with a total number of 135 plants from all the 27 accessions were studied. Transplants, management, and cultural operations were carried out as per the recommendations of the Department of Agriculture. All cultural practices in terms of pest and disease control, applying fertilizer, and watering were followed according to the recommendations of the Department of Agriculture. One month after transplanting, the plants were covered with insect-proof net bags, 90 cm in width and 120 cm in height to avoid cross-pollination. Self-pollination was carried out to produce seeds for future research.

Table 01: Accessions received from Plant Genetic Resource Center

Accession Number	Common/Accession name	Scientific name	Collected location
000762	Sudukochchi	*Capsicum frutescens*	Mathale
01783	Malumiris	*Capsicum annum*	Kurunegala
01782	Malumiris	*Capsicum annum*	Kurunegala
01781	Malumiris	*Capsicum annum*	Kurunegala
01780	Malumiris	*Capsicum annum*	Kurunegala
01778	Malumiris	*Capsicum annum*	Kurunegala
09089	Kachchi	*Capsicum frutescens*	Rathnapura
10173	Miris	*Capsicum frutescens*	Sri Lanka
10170	Miris	*Capsicum frutescens*	Sri Lanka
11644	Kola kochchi	*Capsicum frutescens*	Sri Lanka
11642	Dam kochchi	*Capsicum frutescens*	Sri Lanka
11641	Kochchi	*Capsicum frutescens*	Sri Lanka
00255	Miris	*Capsicum annum*	Hambanthota
00476	Miris	*Capsicum annum*	Nuwara Eliya
12830	Malumiris	*Capsicum annum*	MI (FCRDI)
12829	Wannimiris	*Capsicum annum*	MI (FCRDI)
12935	Malumiris	*Capsicum annum*	Trincomalee
00388	Hen miris	*Capsicum annum*	Kurunegala
1223	Malumiris	*Capsicum annum*	Anuradhapura
1385	Malumiris	*Capsicum annum*	Badulla
5152	Malumiris	*Capsicum annum*	Gampola
557	Miris	*Capsicum annum*	Nuwara Eliya
00819	Miris	*Capsicum annum*	Galle
00180	Miris	*Capsicum annum*	Hambanthota
00193	Miris	*Capsicum annum*	Hambanthota
00391	Sudukochchi	*Capsicum frutescens*	Kurunegala
00840	Kochchi	*Capsicum frutescens*	Galle
1070	Kochchi	*Capsicum frutescens*	Kegalle
1083	Heenkochchi	*Capsicum frutescens*	Kegalle

2.4 Morphological Characterization

All five plants grown from each accession were subjected to morphological characterization using the Manual for Capsicum morphological characterization developed by the Plant Genetic Resource Center in 1999. Morphological data were recorded for 38 characters corresponding to 27 qualitative and 11 quantitative traits (Tables 2 and 3.). Each of the accession for these characteristics was determined for all 38 characters for all thirty-eight descriptor states were recorded.

The morphological traits recorded for the different Capsicum accessions were entered into an Excel datasheet and were prepared to document the above-mentioned groups of plant characters. Morphological data is computed to calculate the means for each of the accessions. A phenogram was generated using the Numerical Taxonomic System, Mesquite software (Za, 2014). Thirty-eight standardized qualitative and quantitative traits were subjected to generate the phenogram.

3.0 RESULTS AND DISCUSSION

The morphological differences between large-scale cultivated Capsicum (Chilli) are easily discerned. All underutilized forms of chilies have small, red, berry-like fruits with colors and sizes attractive to birds. Further, they had various fruit shapes. Some domesticated species have deciduous fruits, which, if not eaten by birds, fall to the ground while the seeds are still at peak viability, while some exhibit variable fruit and flower coloration (designed to appeal to the human

Table 2: Quantitative morphological descriptor states measured in an arbitrary scale; codes for traits and description of the scale used in morphological diversity study of Capsicum collections listed

Quantitative traits	Range (scale)
Plant height	1 - 5 (1 = short~ < 25, 2 = 25 - 45, 3 = 46 - 65, 4 = 66 - 85, 5 = very large ~ > 85)
Mature Leaf length	Not coded (Same stage as in 10 cm - Average of 10 leaves)
Mature Leaf Width	Not coded (Same stage as in 10 cm -Measured on the widest part of the leaf use same leaves as in 13)
Days to flowering	Not coded (50% of plants have at least one open flower)
Number of flowers per axil	1 = 1, 2 = 2, 3 = 3 / more, 4 = many flowers in bunches but each in individual axil, 5 = other (cultivars with two flowers in first axil and with one only in the other)
Fruit length	Not coded (Average fruit length of 10 ripe fruits of the second harvest)
Fruit width	Not coded (Average fruit width of 10 ripe fruits of the second harvest)
Fruit Weight	Not coded (Average fruit width of 10 ripe fruits of the second harvest)
Fruit wall thickness	Not coded (Average fruit width of 10 ripe fruits of the second harvest, measured at the point of maximum width to one decimal point)
1000 Seed Weight	Not coded (100 x 10)
Number of Seeds per fruit	1 - 3(1 = < 20, 2 = 20 – 50, 3 = > 50)

Table 3. Qualitative morphological descriptor states measured in an arbitrary scale; codes or traits and description of the scale used in morphological diversity study of Capsicum collections listed

Qualitative traits	Range (scale)
Hypocotyls Colour (recorded when terminal bud is 1 – 2 mm in size)	1 - 3 (1 = white, 2 = green, 3 = Purple)
Cotyledons leaf colour	1 - 9 (1 = light green, 2 = green, 3 = dark green, 4 = light purple, 5 = purple, 6 = dark purple, 7 = variegated, 8 = yellow, 9 = other)
Life Cycle	1 - 3 (1 = annual, 2 = biennial, 3 = perennial)

Stem Colour	1 - 4 (1 = green, 2 = green with purple stripers, 3 = purple, 4 = other)
Nodal Anthocyanin (Recorded at plant maturity in whole plant)	1 - 7 (1 = green, 3 = light purple, 5 = purple, 7 = dark purple)
Stem Pubescence (Recorded on mature plants, excluding the first two nodes below the shoot)	3 - 7 (3 = sparse, 5 = intermediate, 7 = dense)
Plant growth habit (Observed when 50% of the plants bear ripe fruits)	3 - 9 (3 = prostrate, 5 = intermediate, 7 = erect, 9 = other)
Branching habit	3 - 7 (3 = sparse, 5 = intermediate, 7 = dense)
Leaf colour (Recorded when in 50% of plants the first fruit has begun to ripen - 10 leaves on the main branches)	1 - 8 (1 = yellow, 2 = light green, 3 = green, 4 = dark green, 5 = light purple, 6 = purple,7 = variegated, 8 = other)
Leaf shape	1 - 3 (1 = deltoid, 2 = Ovate, 3 = lanceolate)
Leaf pubescence	3 - 7 (3 = sparse, 5 = intermediate,7 = dense)
Calyx margin	1 - 4 (1 = entire, 2 = intermediate, 3 = dentate, 4 = other)
Calyx annular constriction	0 - 1 (0 = absent, 1 = Present)
Anthocyanin spots or stripes on the fruit	0 - 1 (0 = absent, 1 = Present)
Fruit color at intermediate stage	1 - 8 (1 = white 8 = other)
Fruit Set	3 - 7 (3 = low, 5 = intermediate, 7 = high)
Fruit colour at mature stage	1 - 13 (1 = white, 6 = orange, 8 = red, 10 = purple, 12 = black, 13 = other)
Fruit shape	1 - 6 (1 = elongate, 2 = round,3 = triangular, 4 = campanulate, 5 = blocky, 6 = other)
Fruit shape at pedicel attachment	1 - 5 (1 = acute, 2 = obtuse, 3 = truncate, 4 = cordate, 5 = lobate)
Neck at base of fruit	0 - 1 (0 = absent, 1 = present)
Fruit shape at the blossom end	1 - 5 (1 = pointed, 2 = blunt, 3 = sunken, 4 = sunken and pointed, 5 = other
Fruit blossom end appendage	0 - 1 (0 = absent, 1 = Present)
Fruit cross-sectional corrugation (1/3 from pedicel end)	3 - 7 (3 = slightly corrugated, 5 = intermediate, 7 = corrugated)
Fruit surface	1 - 3 (1 = smooth, 2 = semi wrinkled, 3 = wrinkled)
Fruit pungency at maturity	0 - 6 (0 = not, 3 = low, 5 = intermediate, 6 = high)
Seed colour	1- 4 (1 = straw, 2 = brown,3 = black,4 = other
General uniformity of the accession	1 - 3 (1 = uniform, 2 = variable, 3 = highly variable)

eye); gigantism of the fruits, seeds, flowers, and leaves (Cochran, 1940; Eshbaugh, 1976); and retention of the fruit on the peduncle at maturity (Pickersgill, 1969; Eshbaugh, 1976).

When early taxonomists compared various Capsicum taxa, they noted that chilies were sorted into two distinct groups: one typified by small, red fruits and the other by large fruits. This classification effectively separated the wild and domesticated forms of Capsicum but bore no relevance to evolutionary relationships.

The thirty-eight quantitative and qualitative descriptor states characterized displayed a high level of morphological diversity among Capsicum accessions (Figure 01). The range of variation for the different quantitative descriptors revealed wide variability in all the quantitative descriptors studied. Numerical comparisons of morphological traits (Cochran 1940; Eshbaugh 1970; Jensen et al. 1979; Pickersgill et al. 1979) and cytogenetic analyses (Shopova 1966; Ballard et al. 1970; McLeod et al. 1979a, 1979b, 1982; Moscone et al. 1993) have been used to resolve relationships from the past. The numerical analyses typically included a limited number of species and focused primarily on the relationships of cultivated varieties to their wild progenitors. The numerical analyses of thirty-eight qualitative and quantitative morphological characters of the twenty-seven accessions resulted in the phonogram in Figure 01. The phonogram indicated four prominent clades with two individual clades, 1 and 2; *C. annuum* 557 and *C. annuum* 476. The third clade indicates interrelationships of nine *Capsicum frutescens* accessions namely 391, 762, 11641, 1070, 9089, 1083, 11642, and 11644. *Capsicum annuum;* 12829, 255, and 388 accessions nested in the second clade by closely associated with the *Capsicum frutescens* accessions indicating *Capsicum annuum* accession umber 12829 as the common ancestor. However, this relationship should be further studied through molecular phylogenetical analysis.

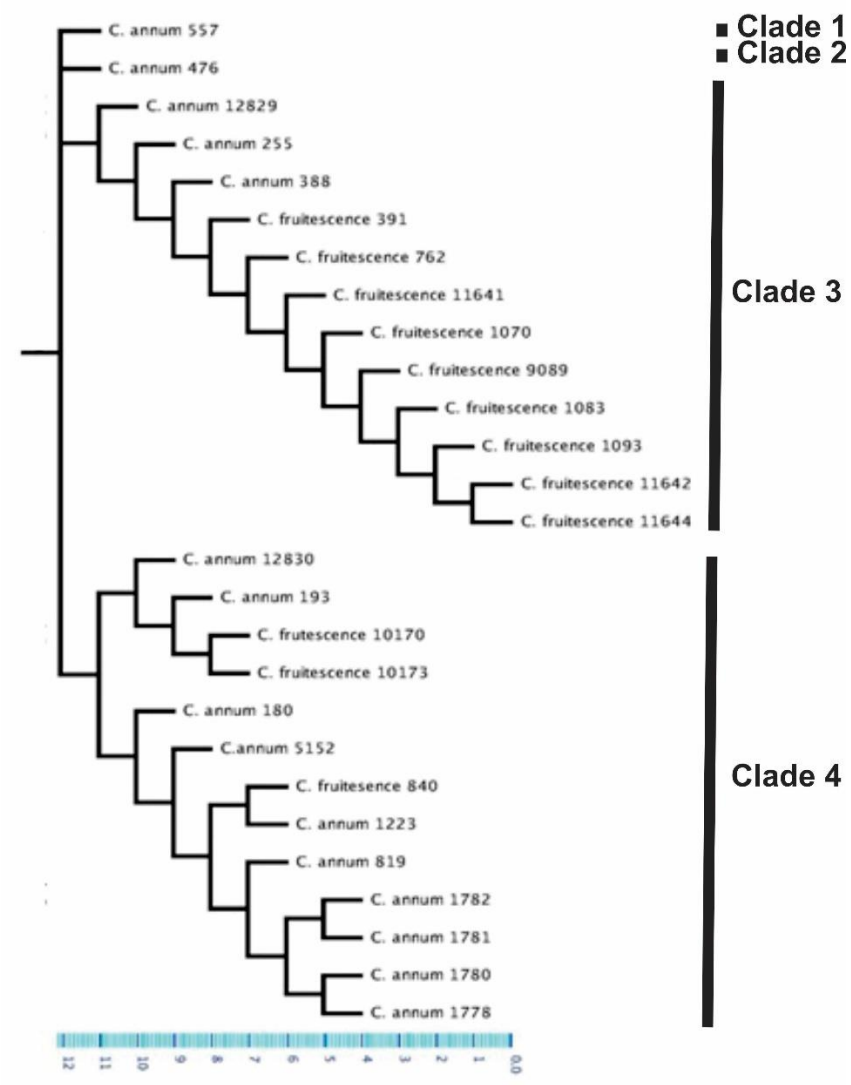

Fig. 01 - Phenogram of twenty-seven (27) Capsicum accessions

Clade number four (04) is further divided into two clades separating one cluster only for *Capsicum annuum* accessions; 180, 5152, 1223, 819, 1782, 1781, 1780, and 1778. As per the previous study

done by analyzing rDNA-ITS similar results were obtained by proving monophyly of the *C. annuum* with a 71% support value by Shiragaki et al., 2020. However, *Capsicum frutescens* accession number 840 may be misclassified, because the corolla color of 840 was greenish, while it is purple in general (Eshbaugh, 2012). The second division of the clade 4 again indicates the *Capsicum annuum* as the common ancestor of the morphologically similar *Capsicum frutescens* accessions; 10170 and 10173. These two accessions had 75% of morphological similarity to the studied qualitative and quantitative characteristics evaluated. The results of the present study are in partial agreement with the previous studies done with the molecular marker analysis (McLeod et al, 1983; Ince et al., 2010; Jeong et al, 2010; Silvar et al, 2016), at the same time we were unable to entirely different between the *C. annuum* and *C. frutescens.* The analysis using molecular markers or DNA barcoding requires many Capsicum species for species identification, and thus takes a lot of effort. On the other hand, phaenogamic presentation of the traits will not cater entirely resolve the phylogenetic relationships. Therefore, phylogenetic analysis using other reliable genetic markers is essential.

4.0 CONCLUSION

The phonogram generated from numerical comparisons of thirty-eight quantitative and qualitative descriptor states is characterized by displaying morphological diversity among Capsicum accessions by separating them into four (04) major clades. *C. annuum* accessions; 180, 5152, 1223, 819, 1782, 1781, 1780, 1778 had monophyletic relationships by nesting into one cluster. Other accession numbers did not have any consistency while placed in the tree firmly. Therefore, the present study does not reveal the morphological phylogeny entirely of the studied Capsicum accessions. Although morphological characterization is recognized as the starting point in the assessment of genetic diversity, morphological markers should be complemented with molecular markers for more advanced genetic diversity study in Capsicum.

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