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RESEARCH ARTICLE

CHARACTERIZATION AND DIVERSITY ANALYSIS OF UNDERUTILIZED *Capsicum chinense* L. ACCESSIONS IN SRI LANKA

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Abstract

Chili is one of the most needed spices worldwide, and consumers use it in different quantities to suit their taste and aroma across continents. Among more than thirty *Capsicum* species, five species are domesticated, and *Capsicum annum*, *Capsicum chinense*, and *Capsicum frutescence* are mainly cultivated for their unique pungency. While *Capsicum annum* is widely cultivated and being improved in different directions, *C. frutescens and C. chinense* are underutilized, and their potential for commercialization and new variety development has not been thoroughly evaluated or documented in Sri Lanka. This study conducted a comparative assessment of twenty *C. chinense* accessions for thirty-one qualitative and quantitative characteristics at two consecutive growth seasons. *TJ112*, *TJ123*, *TJ59,* and *TJ105* recorded the best per plant yield of 231.53 g, 219.3 g, 178.36 g, and 156.63 g, respectively. *TJ123* recorded the largest fruits with maximum fruit circumference, fruit length, seeds per fruit, fruit wall thickness, and weight. The weight per fruit, corolla length, and fruit wall thickness significantly contributed to the yield increment, and these intercorrelated traits are the yield determinants of *C. chinense*. Two principal components derived from the quantitative traits explained 71.04% of cumulative variance. Hierarchical agglomerative clustering formed four distinct clusters, while cluster IV comprised the best-yielding accessions. These are potential accessions for producing high-yielding *C. chinense* varieties. Future studies should focus on evaluating their capsaicin content, aroma, other chemical properties, and environmental resilience. Expanding the collection of *C. chinense* accessions will enhance the chances of selecting better accessions.

Keywords: *Capsicum chinense*, Cluster analysis, Genetic diversity, Morphological traits, Principal component analysis

INTRODUCTION

Chili, a species of genus *Capsicum*, is a dominant spice in the culinary and agricultural sectors worldwide. Renowned for their distinctive pungency and diverse flavour profiles, chili species are integral to various cuisines. It contributes significantly to economies through its extensive use in food processing. In addition to being a typical natural food colouring, *Capsicum* is also utilized as an ingredient in medications and as an extract in pesticides, animal repellents, and self-defence sprays (Bosland 2016). Pungent species of *Capsicum* are used as spices, and others are used as vegetables.

Within the genus *Capsicum*, diverse 38 species exist (Trifruiti *et al.,* 2021). The considerable diversity of *Capsicum*, which results in variation, makes it a model crop (Park *et al.,* 2011). The domesticated five species on *Capsicum* are: *C. annum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens (Ko et al., 2022). Capsicum annum* is the most widely cultivated and commercially significant among 38 *Capsicum* species (Trifruiti *et al.,* 2021). *Capsicum frutescens* and *Capsicum chinense* exhibit remarkable diversity in taste, colour, shape, and heat levels, making them invaluable to culinary traditions and agricultural practices.

C. annuum may have been domesticated in one or both regions of Mexico: northeastern Mexico and central-east Mexico (Kraft *et al.,* 2014). *Capsicum pubescens* is primarily cultivated in the mid-highland regions of

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South-Central America and exists solely as a cultivated plant (Palombo *et al*., 2024), with no discovery of any ancestral wild populations to date (Barboza *et al*., 2022). *C. baccatum* accessions were grouped based on geographic distribution in South America, with one group primarily from the western regions and the other from the eastern regions, overlapping in Bolivia, suggesting multiple domestication sites (Albrecht *et al.,* 2012). *C. frutescens* may have originated in Brazil or Bolivia (Satyawan *et al.* 2023). The Yucatán Peninsula in Mexico has been declared as the centre of origin of *C. Chinense*, and Yucatana produces the best quality and the highest amount of *C. chinense* (López Castilla *et al.,* 2019).

According to Verma *et al.* (2024), the temperature ranges of niche areas for *C. annuum, C. chinense, and C. frutescens* were 11.0°C–20.7°C, 12.2°C–28.6°C, and 11.3°C–33.1°C, respectively, showing a significant variation in traits with high heritability. Furthermore, they have reported the impact of temperature on the agromorphological traits of those three species. However, In Sri Lanka, the adaptability ranges of each species differ considering the average temperature fluctuations across the island.

Based on data derived from the statistical agency of the Food and Agriculture Organization of the United Nations (FAO), a record 36,972.49 million kilograms of chili were produced worldwide in 2022, cultivated on 2,020,816 hectares (Hortidaily, 2022). Sri Lankans consume an average of 2.2 kg of dry chili per person annually, requiring around 53,000 tons to meet the demand. Additionally, approximately 15,000 kg of seeds are needed to achieve such demand. Furthermore, in 2022, the importation of seed chili was around 5,300 kg (Hewage *et al.,* 2022). In 2022, the green Chili cultivation area during the *Yala* season was 5,264.5 hectares, while in the *Maha* season, it was 6,830.5 hectares, totaling 12,095.0 hectares-*Yala* accounted for 27,316.3 MT for production, and *Maha* contributed 35,718.3 MT, with a total output of 63,034.6 MT (Department of Statistics, 2024). Hence, chili accounts for a considerable share of Sri Lanka domestic economy.

Sri Lankan consumers prefer high pungency in Chili. Capsaicinoids in chili trigger vanilloid receptors, causing the sensation of spiciness (Zhu *et al.,* 2023). The phenolic compounds, carotenoids, and capsaicinoids in various chili varieties have been examined (Alonso-Villegas *et al*., 2023), and the capsaicinoid-glucoside has been reported in a range of 6.93 to 9.85 mg/kg of fresh weight basis in different chili varieties (Elkhedir *et al.,* 2024). The data obtained from different species of *Capsicum* (*C. annuum, C. baccatum,* and *C. chinense*) has shown significant differences in the volatile organic compounds, with each species displaying distinct dispersion variations (Taiti *et al*., 2023). Being ingested by mammals, pungent capsicum causes a distinctive burning sensation due to Capsaicinoids, an alkaloid generated in fruit vehicles (Bosland *et al*., 2015). Since the burning sensation is recognized as heat in the mammalian brain, the intensity of the pungency is expressed as the heat index (Huang *et al.,* 2013).

Capsaicin is used for peripheral neuropathic pain (Santos *et al.,* 2024), cancer treatments (Salles and Faria, 2024; Şanlıer *et al.,* 2024), as an antioxidant and antibacterial substances other than used as a usual food colourant and food additive (Gonçalves *et al.,* 2024). The *Habanero*, and *Scotch bonnet*, both *C. chinense* varieties that originated in the Caribbean islands, are the world's hottest chili (Kurian and Starks, 2002), with capsaicin contents of 47.632 and 23.096 mg/g, respectively (Gahungu *et al.,* 2011). Further, *Scotch Bonnet*'s Scoville Heat value (SHV) has been reported as 142931, which falls between the limits of high pungent 100.000-350.000 SHV (Gahungu *et al.,* 2011). The *Habanero* variety has reported a pungency ranging from 200,000 to 350,000 (Thomas *et al.,* 1998). On the Scoville scale, Bell peppers have been rated between 0-100 SHV with 16.000.000 SHV (Sanatombi and Sharma, 2008). These values indicate that *C. chinense* is comprised of the world's hottest varieties, and hot chilies are valued for their production of hot chili oil and source (Cocan *et al.,* 2021). However, commercial cultivation of *C. chinense* and *C. frutescens* is minimal.

Smaller scale farmers in Sri Lanka have only recently started growing a hybrid variety of *C. chinense*, *Scotch Bonnet*, imported from Malaysia.

Sri Lanka is a biodiversity hotspot, and various *C. chinense* and *C. frutescens* accessions are available in home gardens. This species is known among Sri Lankan consumers for its intense hot taste and distinctive flavours, often described as fruity and aromatic, and is highly prized in specific culinary contexts. Despite its potential, *C. chinense* remains underutilized in commercial agriculture in Sri Lanka. However, considering the high demand for *C. annum*, *C. chinense* is a good substitution for the reduction of demand for *C. annum* since *C. chinense* small amount is enough to reach the capsaicin contents that are needed to satisfy the taste. Further, fewer pest and disease incidents, drought tolerance, and perennial growth habits of *C. chinense* are plus points for commercializing the crop in Sri Lanka (Garruna-Hernandez *et al*., 2014; Nieto-Garibay *et al*., 2022).

The present study focused on the diversity of *C. chinense* accessions collected from different locations in Sri Lanka. The collected accessions have not been evaluated previously for morphological diversity. By examining the phenotypic diversity of *C. chinense* accessions, this study aimed to explore valuable traits that could contribute to the development of new, resilient *C. chinense* varieties suited to both local and global markets in future. This will facilitate the initial information to select the best parents to produce hybrid varieties to reduce the recent importation of *Scotch bonnets*. The potential of preserving and utilizing *C. chinense* accessions to broaden the genetic diversity is emphasized based on the observed diversity. Furthermore, this study constructed a key to distinguish *C. chinense* accessions that can be utilized for selecting materials for varietal improvements and commercial cultivation. *C. chinense* will be a good substitution for *C. annum* contributing to sustainability and food security. Menike *et al.* (2018) have revealed the genetic and morphological diversity of some different selected *C. chinense* and *C. frutescens* accessions.

MATERIALS AND METHODS

The experiment was conducted in at Faculty of Agriculture, university of Ruhuna, Sri Lanka—twenty traditional *C. chinese* accessions (given numbers: *TJ22, TJ105, TJ112, TJ131, TJ59, TJ106, TJ116, TJ134, TJ64, TJ107, TJ120, TJ135, TJ 68, TJ108, TJ123, TJ151, TJ103, TJ110, TJ124, TJ160*) were collected from Plant Genetic Resource Centre, Gannoruwa, Agriculture Research Stations, and different locations in Sri Lanka for the experiment.

Seeds were sown in nursery pots (8 X 5 cm) filled with a mix of topsoil and compost. After three weeks, the seedlings were transplanted into larger pots (25 X 30 cm) containing a 3:1 ratio of topsoil and compost. The pots were arranged in a Randomized Complete Block Design with four replicates, each consisting of ten pots (*Supplementary Figure 1*). The experiment was conducted over two *Yala* seasons. Each pot was spaced 60 x 45 cm apart, and weed management, irrigation, and plant caring were done as needed.

The data were collected on the following traits: Hypocotyl colour, cotyledon colour, leaf colour, leaf pubescence, mature leaf length (cm), mature leaf width (cm), days to flowering, flower position, corolla colour, corolla length, corolla spot colour, anther colour, stigma excretion, initial plant height (cm), stem colour, nodal anthocyanin, stem pubescence, plant height (cm), branching habit, fruit length, fruit girth circumference, number of seeds, fruit shapes, blossom end shapes, cross corrugation, width per fruit (g), fruit wall thickness (mm), yield (g) according to the *Capsicum* descriptor published by IPGRI (1995).

Data analysis

Quantitative data were analysed using IBM SPSS 22 statistical software (SPSS Inc., 2013). Correlation analysis, principal component, and cluster analysis were performed to evaluate the diversity of traditional *C. chinense* accessions, and a hierarchical dendrogram was constructed to identify the similarities and differences of the accessions. Qualitative data were included in a table (Table 1) and a plate (Supplementary Figure *2*). A key to distinguish different *C. chinense* accessions were constructed using morphological data.

RESULTS AND DISCUSSION

According to nineteen qualitative characteristics, five different corolla colours, five anther colours, six fruit shapes and four fruit blossom end shapes were observed in the accessions (Table 1).

The tremendous variability in the fruit shapes is a prominent characteristic of *C. chinense* (Carvalho *et al*., 2003, Lannes *et al.,* 2007). However, changes in qualitative characteristics in two different growth seasons of *C. chinense* has been reported by Bozokalfa and Eşiyok (2011). Such variation was not observed in the studied accessions across the two seasons in the present study. Distribution of blossom end shapes among the *C. chinense* accessions: pointed 35%, blunt 20%, sunken 5%, sunken and pointed 30%, and triangular 5%. The fruit shapes among the *C. chinense* accessions were elongated (15%), almost round (5%), triangular (20%), campanulate (25%), blocky (5%), and round (5%). Thirty-seven agronomic traits evaluated in Brazilian

Table 1: Qualitative characteristics of studied *C. chinense* accessions

Name	Leaf color	Leaf shape	Flower posi-tion	Corolla color	Corolla spot colour	Anther colour	Stem color	Growth habit	Fruit shape	Blos-som end shape
TJ22	Green	Lanceolate	Erect	White	Green	White	Green with PS	Intermediate	Round	Blunt
TJ59	L green	Deltoid	Intermediate	Yellow	Green yellow	Yellow	Green with PS	Erect	Triangular	Pointed
TJ64	Green	Deltoid	Pendant	White	Green	Pale blue	Green	Intermediate	Triangular	Blunt
TJ68	Green	Deltoid	Pendant	Light yellow	Green	Blue	Green	Intermediate	Triangular	Blunt
TJ103	L green	Deltoid	Erect	Yellow -green	Green	Pale blue	Green	Intermediate	Elongated	Blunt
TJ106	L green	Deltoid	Intermediate	Light yellow	Green yellow	Yellow	Green	Intermediate	Blocky	Sunken and pointer
TJ107	Green	Deltoid	Pendant	Yellow -green	Green yellow	Yellow	Green	Intermediate	Blocky	Sunken and pointer
TJ108	Green	Deltoid	Intermediate	Yellow -green	Green	Pale blue	Purple	Intermediate	Campanulate	Trian-gular
TJ110	Green	Deltoid	Intermediate	White	Green	Blue	Green	Erect	Pointed	Sunken and pointed
TJ112	Green	Deltoid	Intermediate	Yellow -green	Green yellow	Yellow	Green	Intermediate	Pointed	Sunken
TJ116	Green	Ovate	Pendant	Light yellow	Green	Pale blue	Green	Intermediate	Campanulate	Pointed
TJ120	Green	Ovate	Erect	White & PB	Green	Blue	Green	Intermediate	Triangular	Sunken and pointed
TJ123	L green	Deltoid	Intermediate	White & PB	Green	Blue	Green	Intermediate	Blocky	Pointed
TJ124	Green	Deltoid	Pendant	White	Green	Purple	Purple	Intermediate	Campanulate	Pointed
TJ131	Green	Lanceolate	Erect	Yellow	Green	Purple	Green	Prostrate	Campanulate	Pointed
TJ134	Green	Deltoid	Intermediate	Yellow -green	Green yellow	Yellow	Green	Erect	Blocky	Pointed
TJ135	Green	Lanceolate	Intermediate	White	Green	Blue	Green	Erect	Blocky	Pointed
TJ160	D green	Ovate	Erect	Light yellow	Green	Blue	Green	Erect	Campanulate	Sunken and pointed
TJ105	Green	Lanceolate	Erect	White	Green yellow	Yellow	Purple	Prostrate	Elongated	Pointed
TJ151	Green	Ovate	Intermediate	White	Green	Purple	Green	Erect	Elongated	Sunken and pointer

L green: Light green, D green: Dark green, White & PB: White and purple base, green with PS: Green with purple strip

C. chinense accessions revealed the maximum diversity in fruit colours and shapes (Alvares Bianchi, 2020). In a germplasm collection of 264 Caribbean *C. chinense* accessions, fruit colour exhibited the most diverse qualitative fruit trait (Bharath *et al.,* 2013). The plant growth habit was classified as prostrate in 10% of the accessions, intermediate in 60%, erect in 30% and compact plant type was not reported in the studied accessions. Intermediate branching habit was prominent, and the nodal anthocyanin was prominent in *TJ105*. The presence of anthocyanins in the node was identified as the trait contributing the most to the qualitative variability in eleven accessions studied by Muñoz-Ramírez *et al.* (2020). Inserted stigmas were prominent and found in 85% of the accessions. The stigma distributed other colours as white, yellow, pale blue, blue and purple (Table 1). The pericarp colour was red in all the accessions. However, the intensity of the redness changed with the time during ripening. The immature pericarp colour slightly changed in intensities of green in all accessions (*Supplementary figure 2*). Orange and yellow pericarp of *C. chinense* were reported by Luitel *et al.* (2020) and brown and black pericarp in *C. chinense* has been reported by Padilha *et al*. (2016). Orange and brown cultivars tend to have higher levels of esters, contributing to their fruity aroma notes, compared to red cultivars (Pino *et al.,* 2007). Nine pericarp colours, four shapes, and a wide range of variation in fruit size and weight in *C. chinense* has been reported by Fonseca *et al.* (2008). Hypocotyl colour changed from white to pale yellow and cotyledon leaf colours was green in all accessions other than one purple.

The planting materials were conserved for future purposes, and a key was constructed to distinguish the *C. chinense* accessions using most prominent characteristics. The constructed key can be used to assess the agronomic characteristics of the accessions, including their yield potential, before cultivation.

Capsicum chinense

1. Leaves are green colour
2. Leaf shape is ovate
3. Corolla has a purple base……………………………………………………………TJ120
3’. Corolla doesn’t have a purple base; only white colour or light yellow
4. Plant growth habit is erect………………………………………………………….TJ151
4’. Plant growth habit is not erect; It’s intermediate…………………………….TJ116
2’. Leaf shape is not ovate; exhibit a Lanceolate or Deltoid
5. Leaf shape is deltoid
6. Corolla spot colour is green-yellow
7. Anther colour is pale blue…………………………………………………TJ108
7’. Anther colour is not pale blue; the yellow colour
8. Blossom end shape is sunken ……………………………………….TJ112
8’. The blossom end shape is not sunken; it's pointed……… ……..TJ134
6’. Corolla spot colour is not green-yellow; it is green colour
9. Plant growth habit is intermediate
10. Fruit shape is triangle
11. Anther colour is purl blue………………………………………TJ064
11’Anther colour isn’t purl blue; it is blue………………………TJ068
10. Fruit shape isn’t triangle; blocky or campanulate
12. Fruit shape is blocky……………………………………………TJ107
12’. Fruit shape is not blocky; campanulate:……………………….TJ124
9’. Plant growth habit is not intermediate; they are erect manner.
13. Blossom end shape is sunken and pointer……………………..TJ135
13’Blossom end shape is pointed……………………………………..TJ110
5’. Leaf shape is not deltoid; lanceolate
14. Corolla colour is white
15. Blossom end shape is blunt…………………………………………TJ022
15’. Blossom end shape is not blunt; it is pointed…………………TJ105
14’. Corolla is not white; it is yellow………… ………………………………TJ131
1’. Leaves are not green colour: exhibit light green or dark green colours
16. Fruit shape is elongated …………………… ……………………………………TJ103
16’. Fruit shape is not elongated; triangular, blocky or campanulate
17. Corolla colour is light yellow
18. Anther colour is blue…………………………………………………………TJ160
18’. Anther colour isn’t blue; yellow colour……………………………… ….TJ106
17’. Corolla colour isn’t light yellow; it is yellow or white with a purple base
19. Corolla spot color is green ………………………………………………TJ123
19’. Corolla spot colour isn’t green; it is green yellow…………………….TJ059

Quantitative traits studied in *C. chinense* accessions.

TJ112 recorded the highest per-plant yield of 231.53 g. Similarly, *TJ123* recorded a significantly similar per-plant yield of 219.3 g, comparable to *TJ112*. Further, *TJ59* yielded the third-highest per-plant yield of 178.36 g and the highest seeds per plant (63 g), significantly similar to *TJ123*. Therefore, *TJ112, TJ123*, and *TJ59* were the top-yielding accessions among the studied *C. chinense* accessions (Table 2).

Table 2: Quantitative characteristics of the studied *C. chinense* accessions

	IPH (cm)	MPH (cm)	MLW (cm)	MLL (cm)	50% FD (Days)	YLD (g/ plant)	PGC (cm)	PL (cm)	Seeds per fruit	CL (cm)	FWT (mm)	FW (g)
TJ22	9.64^{b}	48.99^{c}	5.53	10.4	68^{a}	68.03^{gh}	6.54^{e}	3.31c	38^{ef}	0.62^{fgh}	1.57^{cd}	2.32^{ef}
TJ59	9.13^{bc}	69.06^{a}	9.25	15.38	54^{ef}	**178.36^{b}**	9.16^{d}	5.54b	**63^{a}**	0.84^{bc}	1.66^{bc}	**5.13^{b}**
TJ64	17.38^{a}	49.8^{c}	6.8	12.62	41^{h}	124.76^{ef}	6.48^{e}	3.8c	52^{b}	0.63^{fgh}	1.47^{de}	2.94^{de}
TJ68	13.06^{a}	53.51^{bc}	6.96	14.63	42^{gh}	131.2^{e}	5.57^{fg}	3.65c	53^{b}	0.64^{fg}	1.19^{ef}	2.39^{d}
TJ103	8.16^{c}	57.87^{b}	7.4b	14.18	58^{d}	140.89^{de}	6.32^{ef}	6.24b	46c	0.63^{fgh}	1.46^{de}	$2.9d^{e}$
TJ106	$9.29b^{c}$	50.44^{c}	7.93	13.86	58^{d}	175.75^{bc}	5.04^{fgh}	2.66c	35fg	0.61^{gh}	1.17^{fg}	1.83^{fgh}
TJ107	10.13^{b}	39.23^{d}	7.42	13.12	58^{d}	115.77^{f}	7.34^{cd}	1.71c	40de	0.71^{de}	1.03^{gh}	2.27^{fg}
TJ108	5.99^{d}	41.64^{d}	6.67	11.95	57^{de}	124.85^{ef}	7.23^{cd}	2.3c	40de	0.6^{gh}	1.09^{fgh}	2.34^{e}
TJ110	5.59^{d}	50.7^{c}	8.9	14.16	59^{cd}	157.68^{cd}	6.74^{de}	3.41c	28gh	0.64^{fg}	1.18^{fg}	2.17^{fg}
TJ112	12.24^{a}	54.61^{b}	8.65	15.25	58^{d}	**231.53^{a}**	5.33^{fgh}	6.09^{c}	41^{d}	0.62^{fgh}	1.54^{cd}	**3.61^{cd}**
TJ116	6.82^{d}	53.07^{bc}	6.31	11.13	57^{de}	150.41^{d}	6.83^{d}	4.2^{c}	23^{h}	0.65^{ef}	1.52^{d}	3.39^{d}
TJ120	7.8^{c}	51.89^{bc}	5.84	10.24	52^{f}	68.03^{gh}	2.58^{h}	2.81^{c}	35^{fg}	0.52^{h}	0.51^{h}	1.53^{gh}
TJ123	9.89^{b}	46.61^{cd}	6.19	11.24	54^{ef}	**219.3^{ab}**	**13.68^{a}**	**3.77^{c}**	**62^{a}**	**0.87^{a}**	**2.05^{a}**	**10.17^{a}**
TJ124	8.72^{bc}	59.2^{ab}	11.36	16.71	61^{bc}	81.53^{g}	$8.86d^{c}$	3.53^{c}	45^{c}	0.63^{fgh}	1.17^{fg}	3.42^{d}
TJ131	8.16^{c}	37.93^{d}	7.28	12.37	41^{h}	63.63^{h}	2.95^{eh}	1.77^{c}	**58^{a}**	0.51^{h}	1.03^{gh}	1.12^{h}
TJ134	8.49^{c}	61.58^{a}	6.98	11.13	56^{e}	98.62^{fg}	10.16^{ab}	3.63^{c}	41^{d}	0.77^{cd}	1.73^{b}	3.62^{cd}
TJ135	9.75^{b}	66.57^{a}	6.89	12.29	60^{c}	148.46^{d}	9.25^{d}	5.52^{b}	46^{c}	0.7^{de}	1.09^{fgh}	4.59^{bc}
TJ160	8.79^{bc}	54.92^{b}	11.14	17.09	46^{g}	**173.31^{c}**	**10.29^{ab}**	4.29^{c}	40^{de}	**0.86^{b}**	**1.8^{ab}**	**7.07^{ab}**
TJ105	6.75^{d}	51.53^{bc}	8.22	11.87	51^{f}	**156.63^{cd}**	5.03^{fgh}	**6.95^{a}**	28^{gh}	0.78^{cd}	**1.88^{a}**	**5.7^{b}**
TJ151	6.77^{d}	68.61^{a}	6.31	12.4	58^{d}	137.74^{de}	6.68^{de}	5.42^{b}	47^{bc}	0.67^{e}	1.68^{bc}	4.52^{bc}

IPH: Initial plant height (cm), MPH: MPH: Mature plant height (cm), MLW: Mature leaf width (cm), MLL: Mature leaf length (CM), 50%FD: Days taken to 50% flowering, YLD: Yield per plant (g/plant), PGC: Fruit girth circumference, PL: Fruit length (cm), CL: Corolla length (cm), FWT: fruit wall thickness (cm), FW: Fruit weight (g).

Interestingly, *TJ123* had the highest values (or) for several traits: fruit girth circumference (13.68 cm), fruit length (3.77 cm), seeds per fruit (62-significantly highest value), corolla length (0.87 cm), fruit wall thickness (2.05 mm), and average fruit weight (10.17 g). *TJ112* and *TJ59* recorded average fruit weights of 3.61 g and 5.13 g, respectively. *TJ160* recorded the second-highest fruit weight (7.70 g), which was not significantly different from *TJ123*, the heaviest fruit, and the third heaviest fruit bearers, *TJ59* (5.13 g) and TJ105 (5.7 g).

TJ105 had the most significant fruit wall thickness (1.88 mm) and the most extended average fruit length (6.9 cm). *TJ160*, the fourth-best yield producer, had a fruit girth circumference statistically similar to *TJ123* and a wall thickness significantly identical to the thickest fruit wall of *TJ123* (Table 2).

In *C. chinense* accessions studied by Alvares Bianchi (2020) in Brazil, the days to flowering ranged from 62 to 92, fruit weight ranged from 1.04 to 18.61 g, fruit length ranged from 7.85 to 8.49 cm, and pericarp thickness varied from 1.38 to 3.08 mm. In summary, in the present study, the average yield per plant ranged from 63.63 to 231.53 g, with fruit girth varying between 2.58 and 13.68 cm. Corolla lengths ranged from 0.51 cm (*TJ131*) to 0.87 cm (*TJ123*), while fruit wall thickness varied from 1 to 2.05 mm. The most miniature fruit, recorded at 1.12 g (*TJ131*), contrasted with the average weight of the largest fruit, which was 10.17 g (*TJ123*). The descriptor used in the present study encompassed a total of thirty-one characters; in a similar type of morphological diversity studies, fifteen (Costa *et al*. 2016), fifty-three (Lima *et al.* 2017), thirty-nine characters (Orobiyi *et al*., 2017) traits were investigated.

Fruit senescence was observed in *C. chinense* accessions once they reached maturity, and the fruit retention time at the maturity stage varied in the study accessions. This must be examined in future research studies.

Correlation analysis identification of yield determinants

The per-plant yield was significantly and positively correlated with weight per fruit (r=0.612), corolla length (r=0.591), and fruit wall thickness (r=0.492). Further, weight per fruit was correlated with fruit girth circumference (r=0.769), corolla length (r=0.865), and fruit wall thickness (r=0.791). Fruit wall thickness was associated with fruit girth (r=0.539) and corolla length (r=0.703). Corolla length was correlated with fruit girth circumference (r=0.836). These traits can be considered yield determinants of *C. chinense* (Table 3). On the other hand, the number of primary branches negatively affected yield, but not significantly. However, excessive vegetative growth may compete for photosynthetic products.

Table 3: Correlation analysis of studied eight agronomic traits

	IPH	50% FD	Yield	PGC	SPP	CL	NPB	FWT
Initial plant height (cm)								
50% flowering (number of days)	- .271							
Yield (g)	.106	.055						
Fruit girth circumference (cm)	.029	.147	.396					
Number of seeds per fruit	.436	-.405	.063	.317				
Corolla length (cm)	.039	.119	**.591****	**.836****	.295			
Number of primary branches	.279	-.285	-.029	**-.494***	-.065	-.340		
Fruit wall thickness (mm)	.014	.128	**.492***	**.539***	.123	**.703****	-.189	
Weight per fruit (g)	.000	-.012	**.612****	**.769****	.298	**.865****	-.333	**.791****

IPH: Initial plant height (cm), FD: Days to 50% flowering, PGC: Fruit girth circumference (cm), SPP: number of seeds per fruit, CL: Corolla length (cm), NPB: number of primary branches, FWT: Fruit wall thickness (mm)

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

According to the principal component analysis, the eigenvalues for each principal component (PC) ranged from 3.69 to 0.093 (Table 4). Two principal components had eigenvalues more than one, accounting for 71.03% of the total variance, with PC1 explaining 52.65% and PC2 explaining 18.37% of the variance, respectively. Genetic diversity evaluation using PCA has been conducted in several studies on *Capsicum* species. Seventy-five genotypes were studied by Taş Balkaya (2021), and six PCs explained 70.99% of the total variation. Nanayakkara *et al.* (2017) have conducted a similar genetic analysis on twenty-three *C. chinense* accessions, using principal component analysis to explain the variance of quantitative traits. Their results explained 98.2% of the total variance, with PC1 associated with mature leaf length, mature leaf width, and days to fruiting, while PC2 was associated with fruit wall thickness. Further, PCA explained 73.48% of the total variation in a collection of Northeast Indian *C. chinense* germplasm (Baruah *et al.,* 2023).

Table 4: Eigenvalue, percentage of variance and cumulative variance are explained by the principal components.

Principal component	Total	% of Variance	Cumulative %
1	**3.690**	**52.717**	52.717
2	**1.282**	**18.319**	**71.036**
3	.745	10.645	81.681
4	.625	8.928	90.610
5	.450	6.425	97.035
6	.115	1.644	98.679
7	.093	1.321	100.00

Based on the rotated component matrix, the first principal component (PC1) was associated with weight per fruit (g), corolla length (cm), fruit girth circumference (cm), fruit wall thickness (mm), and yield (g per plant). The second principal component (PC2) was primarily associated with the number of days to 50% flowering (Table 5).

Table 5: Rotated component matrix extracted from principal components

Trait	Principal component	
	1	2
Initial plant height (cm)	.075	-.795
50% flowering (number of days)	.100	**.793**
Yield (g)	**.707**	-.103
Fruit girth circumference (cm)	**.838**	.090
Corolla length (cm)	**.942**	.044
Fruit wall thickness (mm)	**.826**	.068
Weight per fruit (g)	**.948**	-.021

In the present study, using Ward's linkage method, two principal components extracted from the seven studied traits were used for clustering genotypes. This clustering algorithm successfully classified the twenty *C. chinense* accessions into five morphologically distinct clusters (Table 4) at a cluster distance of eight (Figure 1).

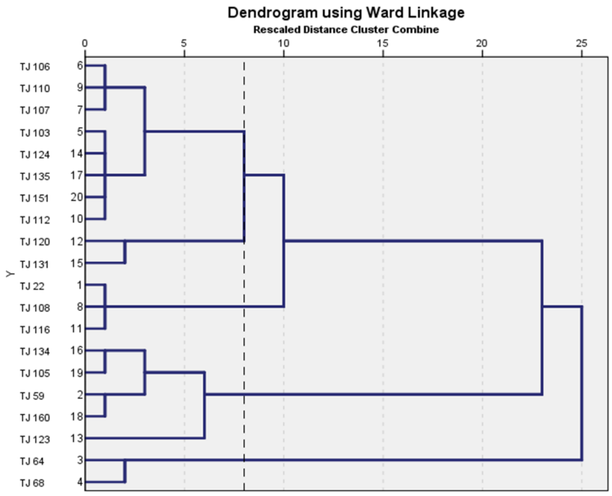

Figure 1: Hierarchical agglomerative clustering among the accessions: a dendrogram of genetic distance made using PCA data employing the Ward criterion method showing the diversity among traditional *C. chinense* accessions based on seven quantitative traits. The dashed line indicates the rescaled cluster distance where clusters were identified.

According to the hierarchical cluster analysis, five significant clusters were identified at a cluster distance of eight (Figure 1, vertical dotted line), with Clusters I, II, III, IV, and V containing 8, 2, 3, 5, and 2 accessions, respectively (Figure 2).

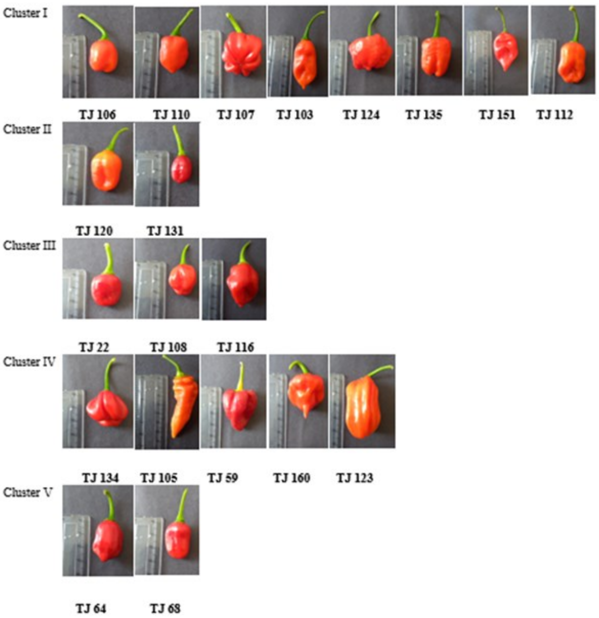

Figure 2: Fruits of *C. chinense* accessions in four Hierarchical agglomerative clusters.

The first cluster included eight *C. chinense* accessions, further divided into two subgroups. Two accessions were grouped into cluster II, and those recorded the lowest yield (63.63-68.03 g), a thin fruit wall (1-1.03 mm) and a short corolla length (0.51-0.52 cm). Cluster IV contained five accessions with the best four yield recording accessions (*TJ112, TJ123, TJ59, TJ160,* and *TJ105*) and *TJ134*, which was not among the best five yielding accessions but one of the two accessions that recorded the greatest fruit girth circumference (10.16 cm). *TJ64* and *TJ68* stood out as the most distinct from the other *C. chinense* accessions, Cluster V, and *TJ64* and *TJ68* were the tallest plants (17.38 cm, 13.06 cm) with the least number of days taken to 50% flowering 41-42 days). Identification of genetically distant parental accessions can be done based on the dendrogram. This emphasizes the importance of evaluating the genetic variability existing in the local genotypes for yield and yield-attributing traits.

A two-dimensional scatter plot shows the dispersion of *C. chinense* in three quadrants, confirming its broad variation (Figure 3).

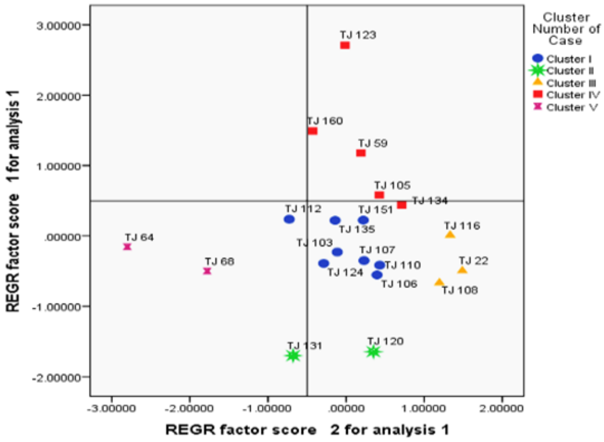

Figure 3: Two-dimensional (2D) scatter plot diagram in studied *C. chinense* accessions

Implications of the study

The study provides valuable information about the agronomic and morphological diversity of traditional *C. chinense* accessions in Sri Lanka. By evaluating a set of qualitative and quantitative traits, the research facilitates the identification of unique characteristics among accessions, which is important for germplasm conservation and breeding programs. The use of a *Capsicum* descriptor-based approach provides standardized characterization, enhancing the reliability of trait comparisons. The constructed morphological key serves as a practical tool for distinguishing different *C. chinese* accessions aid researchers and breeders in selecting accessions with desirable traits for further improvement. The correlation, principal component, and cluster analyses describe significant trait associations, which can inform breeding strategies aimed at enhancing yield, fruit quality, and adaptability. Furthermore, the observed variations in traits such as flowering time, plant architecture, and fruit characteristics offer valuable information for optimizing cultivation practices and improving productivity. Hence, this study contributes to the sustainable utilization and genetic enhancement of *C. chinense* in Sri Lanka, supporting future breeding and commercial cultivation efforts.

CONCLUSIONS

Understanding and preserving the diversity of *Capsicum* species, especially *Capsicum chinense*, is important for the future of sustainable agriculture and food security. By exploring the untapped potential of this backyard crop, we can contribute to developing new varieties that meet the challenges of a changing environment while enriching the culinary landscape. Our study on the diversity of *C. chinense* accessions in Sri Lanka is a step towards recognizing and utilizing the genetic diversity present in *C. chinense*. This study has successfully identified high-yielding accessions of *C. chinense*, with *TJ112, TJ123, TJ59*, and *TJ105* showing the most promising results regarding per plant yield and fruit characteristics. The significant correlation of traits such as weight per fruit, corolla length, and fruit wall thickness with yield emphasizes their importance as yield determinants. The hierarchical clustering and principal component analysis distinguished the accessions, with Cluster IV as the group with the best yield potential. These findings offer a foundation for future breeding programs to improve *C. chinense*. To further enhance the breeding efforts, subsequent research should be carried out into capsaicin content, aroma, other chemical properties, and fruit retention strength at maturity. Expanding the collection of *C. chinense* accessions will also be important for identifying and developing superior varieties with desirable traits. This comprehensive approach will significantly contribute to the commercialization and broader utilization of *C. chinense*, ultimately benefiting the agricultural and spice industry.

AUTHOR CONTRIBUTION

NYAR carried out the study, analyzed data, and wrote the first draft. HAPAS and ALR supervised the study. ALR improved the manuscript.

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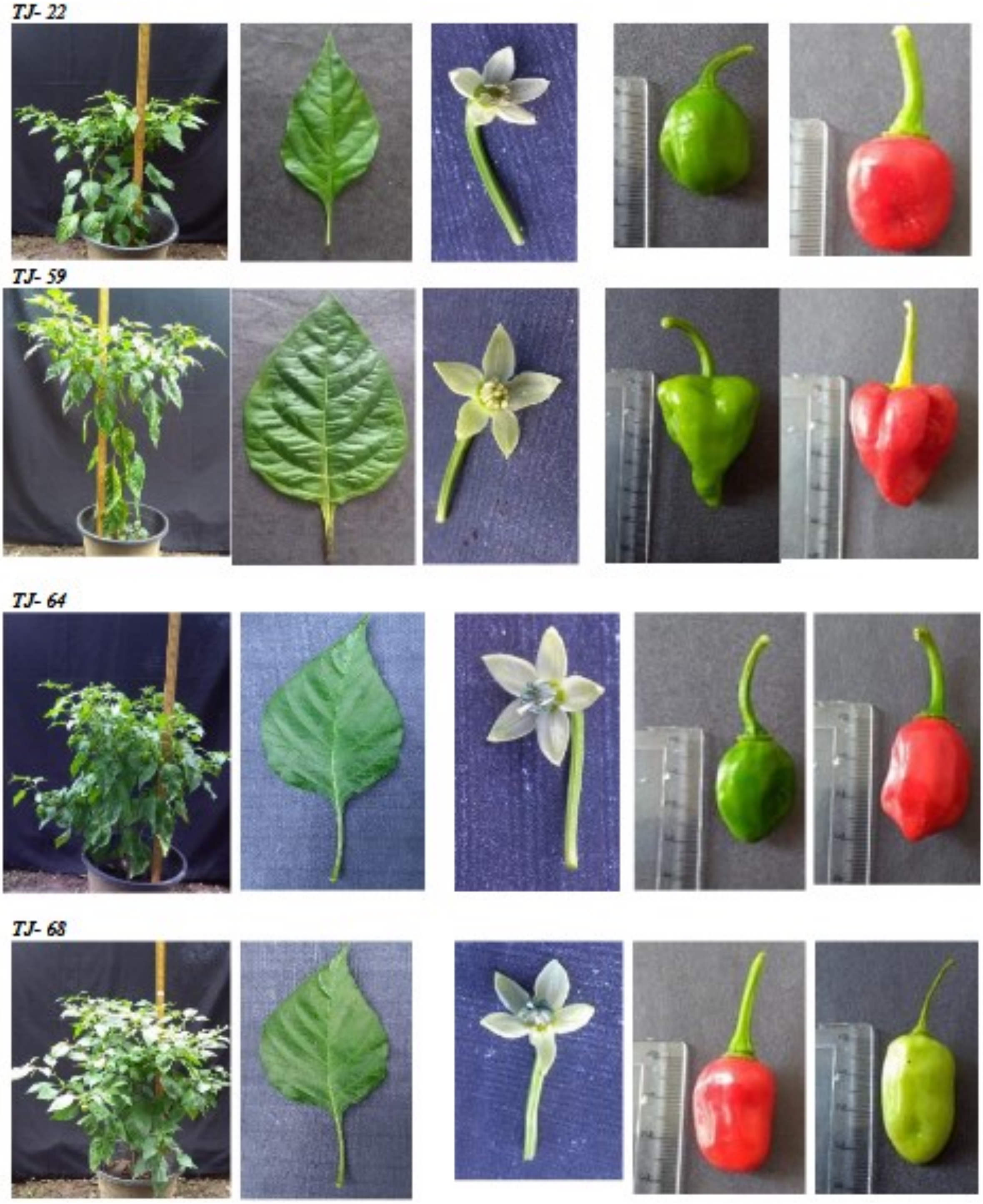
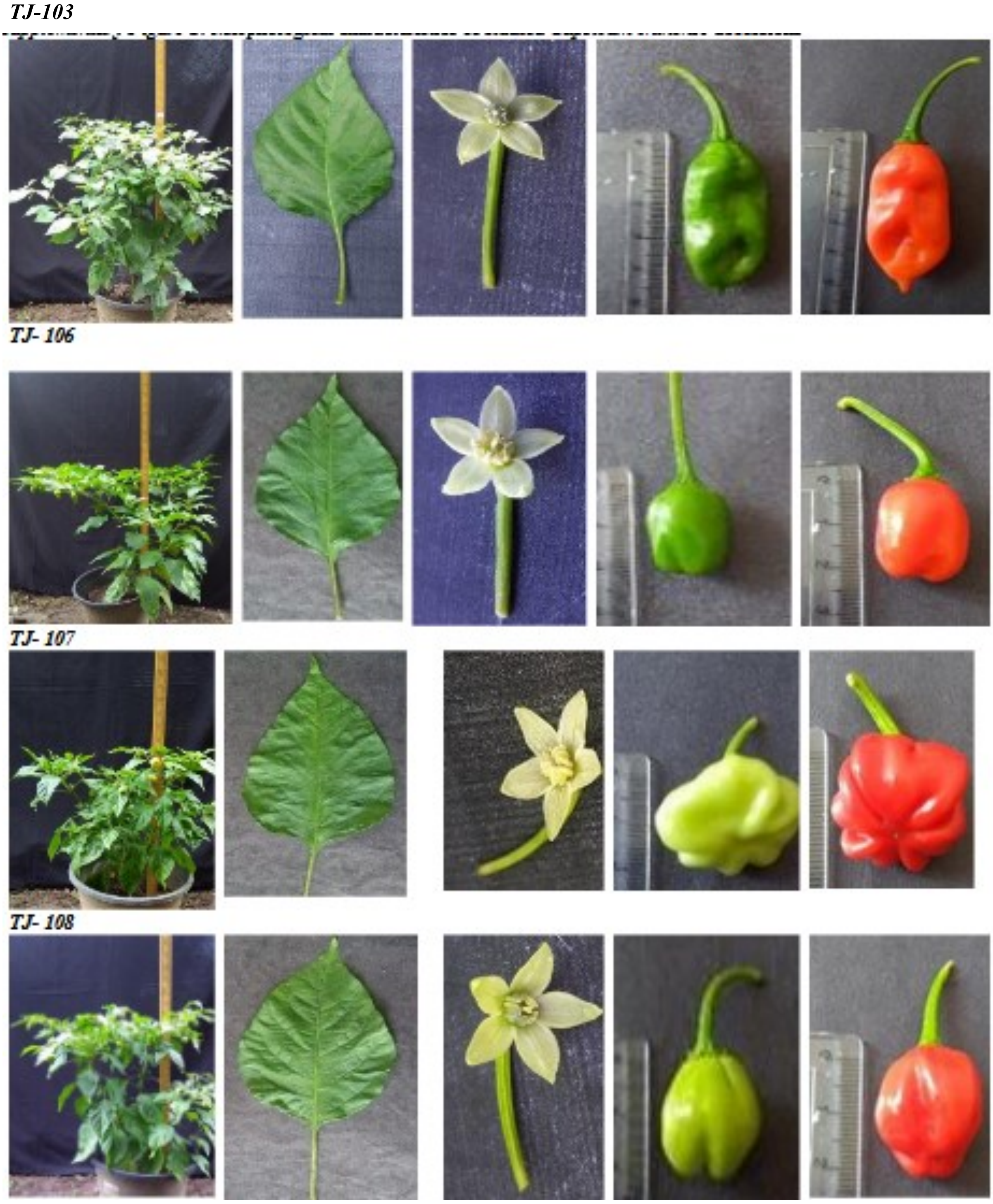
Supplementary Figure 1**: Pot cultivation of *C. chinense* accessions in the upper panel. Collection of pods and flowers of *C. chinense* in the lower panel**



Supplementary Figure 2**: Morphological characteristics of studied *****Capsicum chinense*** **accessions**



Supplementary Figure 2 continued

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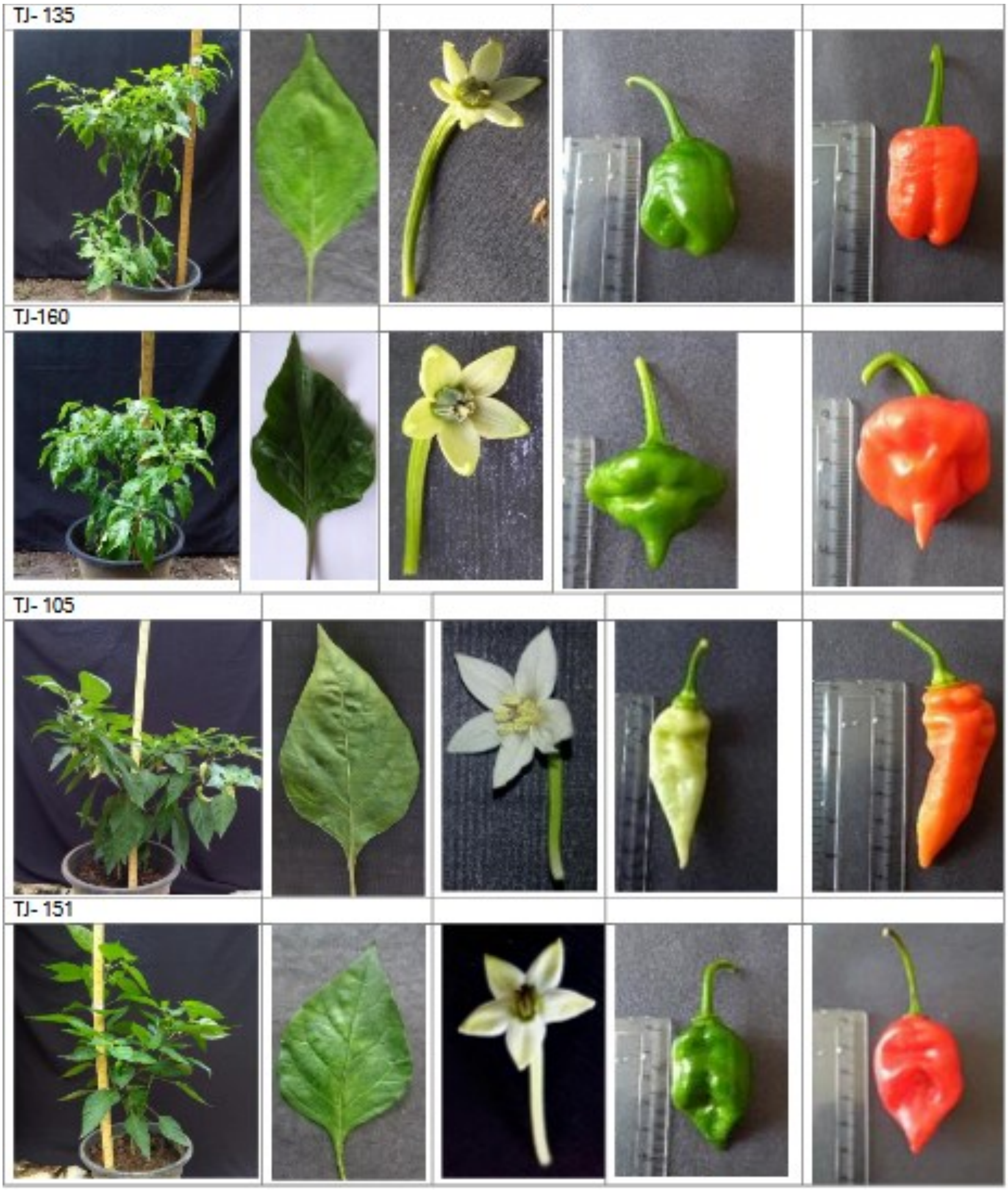
Supplementary Figure 2 continued



Supplementary Figure 2 continued