

Morphological variation of cinnamon (*Cinnamomum verum* Persl) germplasm in Matara District of Sri Lanka

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ABSTRACT

Forty seven representative cinnamon accessions were collected from Matara District of Sri Lanka to analyze the morphological variation of *Cinnamomum verum* germplasm. Morphological characters viz. Leaf length, Leaf width, Leaf length-width ratio, Petiole length, Leaf arrangement, Leaf shape, Leaf apex, Leaf base, Leaf texture, Upper surface leaf color, Flush color, Bark color, Bark surface, and Bark fragrant were recorded. Principal component analysis (PCA) using four quantitative morphological characteristics, indicated that the first two principal components (PCs) with Eigen values of more than one and accounted for 88.88% of the total variance. Cluster analysis classified 47 accessions into nine groups. The present study demonstrates a considerable diversity of morphological characters among the accessions that can be useful in germplasm management and future crop improvement programs.

Key words: *Cinnamomum verum* germplasm, morphological characters, Principal component analysis, Cluster analysis

INTRODUCTION

The genus *Cinnamomum* belongs to the family Lauraceae and consists of about 110 species of evergreen trees and shrubs (Purseglove *et al.*, 1969). Out of nine *Cinnamomum* species, *Cinnamomum verum* is one of the most important species in Sri Lanka which contributes to 70% of the world true cinnamon bark production (Abeysinghe *et al.*, 2009). The export volume of cinnamon and earning for 2012 was 14,435 metric tons and 16,654.7 million rupees respectively (Sri Lanka Custom, 2012). Cinnamon is endemic to Sri Lanka and it had been found in central hilly areas of Sri Lanka and also in Sinharaja and Knuckles forest reserves. The ideal environmental condition for cinnamon is available in wet zone of Sri Lanka. However commercial cinnamon cultivation is carried out in intermediate zones of mid and low country, where annual rainfall is more than 1750 mm. Cinnamon is mostly cultivated along the coastal belts of Kalutara, Galle, Hambantota at an elevation of about 250 m above mean sea level. The most suitable temperature is between 25°C- 32°C (Department of Export Agriculture, 2013). Cinnamon bark is mainly used as a spice for flavoring food product and leaf oil is used as flavor ingredients and also in cosmetics and pharmaceutical industries (Paranagama *et al.*, 2001). Different biological activities including anti-diabetic, anti-inflammatory, astringent and diuretic effects have

been popularized cinnamon in folk medicine (Lee *et al.*, 2010). In modern medicine, cinnamon is combined with other ingredients to treat diarrhea, internal hemorrhage, impotency, typhoid, halitosis, checking nausea and vomiting and for restoring normal skin color on the face (Warrier *et al.*, 1994).

According to Ravindran *et al.*, (2004) leaf shape of cinnamon varies from oval or elliptic to lanceolate-oval or narrowly elliptic, $3 \times 7 - 8 \times 25$ cm, leaf apex shortly or broadly acuminate and leaf base acutish or cuneate. A study by Wijesinghe and Gunarathna, (2001) showed correlation between leaf size and shape with yield in seven different types of true cinnamon. According to this observation trees with large round leaves and big leaves had high bark yield. Moreover bark oil (cinnamaldehyde %) quality is higher in the variety of inwardly curved leaves and high quality leaf oil was obtained from the small round leaves.

Cinnamon flower exhibits protogynous dichogamy and it is cross pollinated (Joseph, 1981). Thus, vegetative propagation is necessary for producing uniformly high yielding populations and for propagating elite lines (Rema *et al.*, 1997). A core collection is a representative subset of a large number of populations which intends to improve management

and use of a germplasm collection (Diwan *et al.*, 1995). It is also a powerful material for evaluation of germplasm, identification of trait-specific accessions, gene discovery, allele mining, genomic study, marker development, and molecular breeding (Qiu *et al.*, 2013). Cluster analysis followed by Principal component analysis had been used to cluster *Cinnamomum* spp. into groups and to show relationship among the species on the basis of morphological characters (Ravindran *et al.*, 1991).

Morphological variation of a crop indicates the genetic diversity and effect from environment. Both environmental and genetic effects contribute to phenotypic variation within and among populations (Allard and Bradshaw, 1964; Andrew *et al.*, 2010). Some molecular study has been conducted to evaluate genetic differences on *Cinnamomum* species (Ho and Hung, 2011; Joy and Maridass, 2008; Lin *et al.*, 1997; Kameyama, 2012; Soulange *et al.*, 2007; Lee *et al.*, 2010; Kojoma *et al.*, 2002; Sandigawad and Patil, 2011; Kuo *et al.*, 2010). A molecular research has been done on the genetic analysis of *Cinnamomum* species by sequencing *TrnL* intron region, intergenic spacer between *trnT-trnL*, *trnL-trnF*, *trnH-psbA* and nuclear ITS (Abeyasinghe *et al.*, 2009). Another work has been carried out to find a more reliable approach to identify *Cinnamomum* species correctly using RAPD and SRAP techniques. Some primers gave highly polymorphic banding patterns using these techniques. This preliminary study showed that using these molecular markers, it is possible to identify the *Cinnamomum* species (genus specific and species specific) and intra-species variations (Abeyasinghe *et al.*, 2014). Therefore, the present study is focused on the *Cinnamomum* germplasm which were collected from different locations of Matara district to analyze the morphological variation.

MATERIALS AND METHODS

Total forty seven accessions were collected according to their distinct morphological characters from these cinnamon growing areas particularly from Deiyandara, Ehala Athuraliya, Karapotu Gala, Palolpitiya, Ehalawitiyala and Kamburupitiya during September, 2014. Semi-hard 1/1.5 inch stem cutting with 1 or 2 leaves and active buds from every

accession had been planted in nursery for further studies. In the Laboratory the Leaf length (LL), Leaf width (LW), Leaf length-width ratio (LLWr), Petiole length (PL) of samples were measured while other morphological traits Leaf arrangement (LA), Leaf shape (LS), Leaf apex (LAP), Leaf base (LB), Leaf texture (LT), Upper surface leaf color (ULC), Flush color (FC), Bark color (BC), Bark surface (BS), and Bark fragrant (BF) were observed in the time of every field visit during September, 2014. Flush color had been observed following Munsell Color Chart (Munsell Color, 1977). Morphological analysis of collected samples was done considering both quantitative and qualitative characters.

Analysis of variance applying descriptive statistics such as mean, standard deviation, coefficient of variation and correlation coefficient for quantitative traits were calculated. Principal Component Analysis (PCA) was conducted in order to identify the patterns of morphological variation using IBM SPSS Statistics 20.0 software (version 20), IBM, USA. Clustering of genotypes into similar groups was carried out using Ward's hierarchical algorithm based on squared Euclidean distances.

RESULTS AND DISCUSSION

Fourteen morphological characters were recorded from 47 accessions. Among fourteen morphological characters, four quantitative characters of Leaf length (LL), Leaf width (LW), Leaf length-width ratio (LLWr), Petiole length (PL) and ten qualitative characters of Leaf arrangement (LA), Leaf shape (LS), Leaf apex (LAP), Leaf base (LB), Leaf texture (LT), Upper surface leaf color (ULC), Flush color (FC), Bark color (BC), Bark surface (BS), and Bark fragrant (BF) were recorded.

The correlation coefficient was observed between four different morphological traits (Table 1). There were positive significant linear relationships between the Leaf width and Leaf length (0.699), Petiole length and Leaf length (0.613) and Leaf width and Petiole length (0.574) at 0.01% significant level. The positive and significant relationships among the traits will provide plant breeders an understanding on manipulation of such traits.

Table 1. The correlation of quantitative traits according to Pearson's Correlation Coefficient evaluated in cinnamon germplasm collections.

Variable	LL	LW	LLWr	PL
LL	-	.699**	.367*	.613**
LW		-	-.396**	.574**
LLWr			-	.045
PL				-

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

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

The Principal Component Analysis showing the factor scores of each character among the cinnamon accessions, eigen values and percentage total variance accounted by four principal component (Table 2). The PCA was used to remove redundancy in the data set. The first two principal components (PC-1 and PC-2) accounted for most of the variability observed among the accessions and their eigen value is more than 1. The first two principal components accounted for 88.88% of the total variability where PC-1 explained 56.45% of the total variability was loaded on LL, LW and PL and the PC-2 accounted for 32.43% of the variation and was loaded on LLWr.

PCA is commonly used to analyze large data sets. It has been used to evaluate germplasm of rice (Sohrabi et al., 2012), olive (Cantini et al., 1999), vineyard peach (Nikolic et al., 2010), peach (Perez et al., 1993), loquat (Leguizamón et al., 2003; Badenes et al., 2000; Martinez-Calvo et al., 2008), and apricot (Yilmaz et al., 2012; Ruiz and Egea, 2008). Its main purpose is to extract the important information from the table, to represent it as a set of new orthogonal variables called principal components, and to show the pattern of similarity of the observations and of the variables as points in maps (Abdi and Williams, 2010).

Table 2. Eigen values of the correlation matrix and their contribution to total variation of cinnamon germplasm collections.

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.25	56.47	56.47	2.25	56.47	56.47	2.25	56.45	56.45
2	1.29	32.40	88.88	1.29	32.40	88.88	1.29	32.43	88.88
3	.43	10.92	99.80						
4	.008	.193	100.00						

The cluster analysis using Ward’s method classified the 47 cinnamon accessions in to nine clusters at rescaled distance of 3.0 (Fig. 1). Accessions from Cluster no. 8 showed highest CV% of LL (15.10%), LW (13.53%) and PL (28.28%), which indicated those accessions are highly variable in contrast to the other accessions (Table 3). On the other hand, CV% of LW (11.61%) and PL (17.30%)

of the accessions from cluster no. 9 also higher than the other accessions. Accessions belong to cluster no. 1 and cluster no. 5 showed variation in CV% of PL (11.73% and 22.12% respectively) comparing other accessions. All accessions in one cluster represent the respective cluster in terms of qualitative characters while the qualitative characters vary among clusters.

Table 3. Mean (M), Standard Deviation (SD) and Coefficient of Variation (CV%) of quantitative characters according to clusters.

Cluster number	LL			LW			LLWr			PL		
	M	SD	CV %	M	SD	CV%	M	SD	CV %	M	SD	CV%
1	14.01	1.22	8.73	6.51	0.36	5.55	2.15	0.12	5.71	2.06	0.24	11.73
2	18.70	0	0	9.13	0	0	2.05	0	0	2.10	0	0
3	12.90	0.63	4.91	7.68	0.03	0.45	1.68	0.09	5.15	1.90	0.17	9.12
4	19.48	0.59	3.03	7.44	0.68	9.14	2.63	0.17	6.43	2.37	0.25	10.63
5	11.37	0.91	7.97	5.73	0.50	8.67	1.99	0.09	4.61	1.33	0.29	22.12
6	7.43	0	0	3.40	0	0	2.19	0	0	1.20	0	0
7	12.64	1.04	8.22	4.73	0.33	7.09	2.67	0.08	2.87	1.36	0.13	9.38
8	15.45	2.33	15.10	5.75	0.78	13.53	2.68	0.04	1.60	2.00	0.57	28.28
9	13.37	1.28	9.60	5.71	0.66	11.61	2.35	0.09	3.66	1.67	0.29	17.30

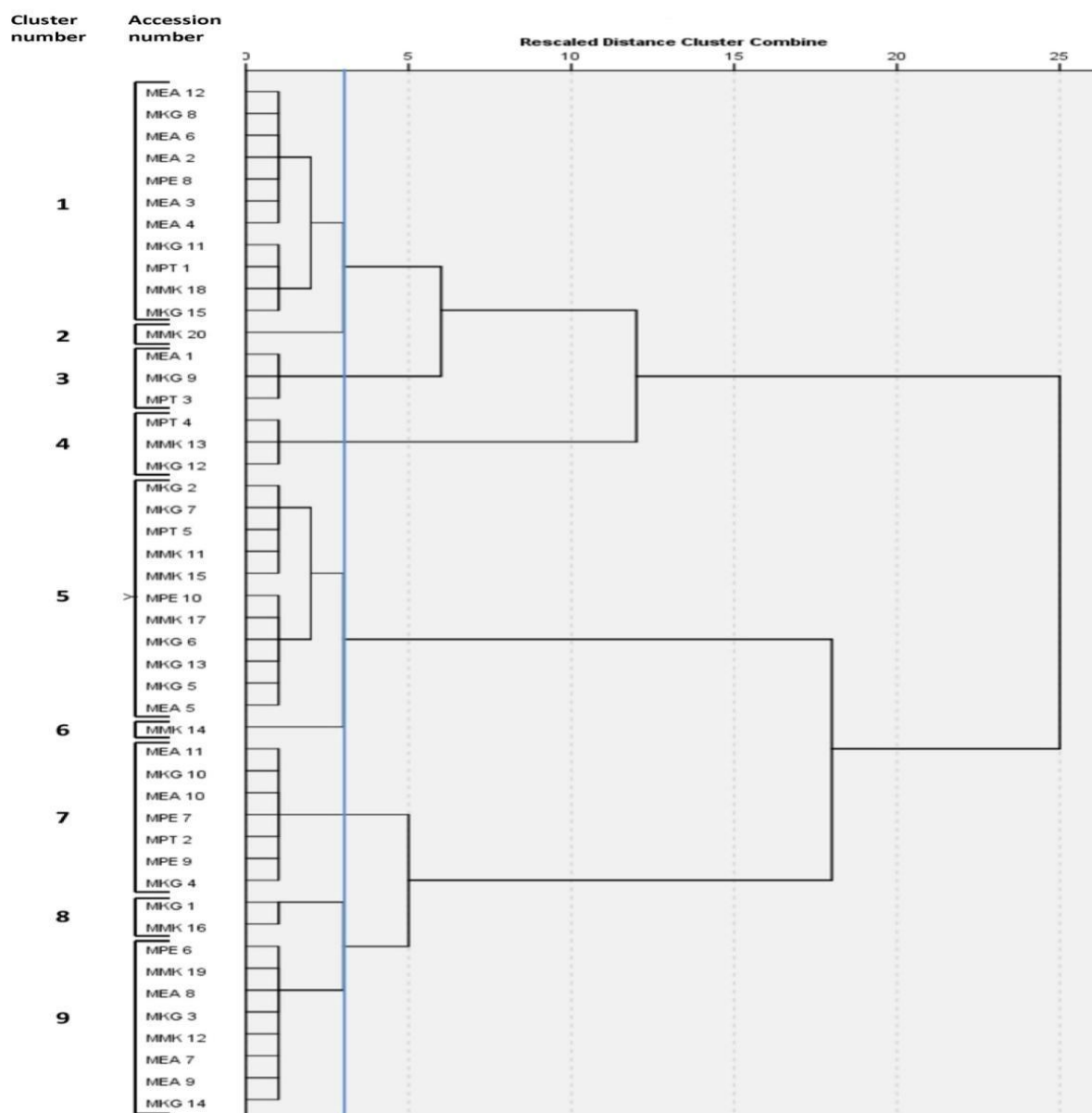


Figure 1: Dendrogram of cinnamon accessions derived through Ward's linkage Cluster Analysis based on four quantitative traits.

The qualitative characters of all the accessions were arranged in Table-5 following quantitative characters originated from nine clusters. Qualitative characters of LA, LS, LB and ULC of the accessions belong to cluster 4 and cluster 8 are similar to each other whereas, cluster 1, 5, 7 and 9 with large number of accessions showed considerable variation in qualitative traits. Flush color of cinnamon accessions showed a variation in the collection. There may be a relationship between their oil contents and flush color as previously reported by (Krishnamoorthy *et al.*, 1988). Accessions from all the nine clusters showed

variations in Leaf shape (Fig. 2). One accession (MPT-03) belongs to cluster no.03 have ovate Leaf shape which is different from the other accessions. In addition, accessions MEA-11 and MKG-01 grouped in to cluster no.07 and cluster no.08 respectively showed narrowly elliptic Leaf shape which is not similar to the other accessions. According to the two types of trait combination it has been clearly manifested that both types of traits has a substantial contribution in accession grouping. These variations ensured genetic differentiation and allele richness among the accessions.

Table 4. Qualitative traits with Type code in cinnamon germplasm.

S. No.	Qualitative traits	Pattern/ Color type	Type code
1	Leaf arrangement (LA)	Opposite	1
		Sub-opposite	2
		Opposite or sub-opposite	3
		Opposite to sub-opposite	4
2	Leaf shape (LS)	Elliptic	1
		Lanceolate	2
		Ovate	3
		Narrowly elliptic	4
		Elliptic to broadly elliptic	5
		Ovate-lanceolate	7
3	Leaf apex (LAP)	Ovate-oblong to ovate-lanceolate	11
		Acute	1
		Obtuse	2
		Acuminate	3
		Gradually acuminate	4
		Long acuminate	5
		Broadly acuminate	6
		Narrowly acuminate	7
		Blunt or subacute	10
		4	Leaf base(LB)
Obtuse	2		
Cuneate	3		
Shortly acute	5		
Rounded or subacute	6		
	6		
5	Leaf texture (LT)	Coriaceous	1
		Subcoriaceous	2
		Rigidly coriaceous	3
		Thinly to stiffly coriaceous	4
		Chartaceous to rigidly chartaceous	5
		Chartaceous	6
6	Upper surface leaf color (ULC)	Dark green	1
		Green	3
			3
7	Flush color (FC)	2.5R 7/6	2
		2.5R 6/8	4
		5GY 7/10	6
		2.5GY 8/10	7
		2.5GY 8/6	8
		2.5R 4/8	9
		2.5R 7/8	12
			12
8	Bark color (BC)	Brown	1
		Whitish brown	2
		Light brown	3
9	Bark surface (BS)	Slightly rough	1
		Rough	2
		Smooth	4
			4
10	Bark fragrant (BF)	Weak fragrant aroma	1
		Intermediate fragrant aroma	2
		Good fragrant aroma	3
		Strong fragrant aroma	4

Table 5. Variation of qualitative characters within clusters which were derived through Ward's linkage method.

Cluster number	Accession number	Qualitative characters									
		LA	LS	LAP	LB	LT	ULC	FC	BC	BS	BF
1	MEA 12	3	5	3	1	5	1	9	1	2	1
	MKG 8	4	11	4	5	5	1	7	3	2	2
	MEA 6	2	1	1	5	1	1	9	1	1	2
	MEA 2	3	5	6	5	6	3	9	3	4	1
	MPE 8	4	2	6	1	5	1	12	1	4	3
	MEA 3	4	7	7	5	5	1	12	1	1	2
	MEA 4	4	5	10	1	6	1	4	1	1	2
	MKG 11	1	1	1	1	3	1	12	2	1	1
	MPT 1	4	7	3	6	6	1	2	1	1	2
	MMK 18	4	2	1	1	6	1	12	3	4	1
	MKG 15	2	2	6	1	6	3	8	2	1	2
2	MMK 20	2	5	1	5	5	1	6	3	4	2
3	MEA 1	4	7	1	5	6	1	2	1	1	2
	MKG 9	4	5	10	5	6	1	9	1	4	2
	MPT 3	4	3	2	2	1	1	9	1	4	2
4	MPT 4	4	2	3	1	5	1	12	1	4	4
	MMK 13	4	2	5	1	6	1	6	1	4	4
	MKG 12	4	2	3	1	6	1	9	3	4	1
5	MKG 2	1	1	10	6	3	3	9	3	4	1
	MKG 7	1	7	10	6	2	1	6	3	4	1
	MPT 5	4	7	3	2	1	1	9	3	4	2
	MMK 11	2	5	1	1	1	3	6	3	4	3
	MMK 15	4	5	4	1	5	1	12	1	4	2
	MPE 10	2	1	3	6	6	1	12	3	4	2
	MMK 17	4	1	1	1	4	1	6	1	1	2
	MKG 6	1	1	4	6	1	3	7	1	1	1
	MKG 13	4	1	3	5	5	1	4	2	1	1
	MKG 5	1	7	10	6	3	1	4	1	4	2
MEA 5	4	7	3	6	5	1	7	2	2	2	
6	MMK 14	2	1	10	5	6	1	7	1	4	3
7	MEA 11	2	4	1	1	4	1	12	1	2	1
	MKG 10	4	4	10	5	6	1	9	1	4	2
	MEA 10	3	4	10	1	6	3	8	3	1	3
	MPE 7	4	1	7	1	5	1	7	1	4	4
	MPT 2	4	2	3	1	5	1	9	1	4	3
	MPE 9	2	4	3	3	6	1	7	1	4	2
	MKG 4	4	2	3	1	6	1	9	2	2	1
8	MKG 1	4	4	3	1	6	1	2	2	4	2
	MMK 16	4	4	5	1	6	1	4	1	1	1
9	MPE 6	4	1	3	1	6	1	12	1	1	2
	MMK 19	4	1	7	1	2	1	4	1	4	2
	MEA 8	4	2	4	1	3	1	9	1	2	1
	MKG 3	1	7	3	1	2	3	9	3	4	1
	MMK 12	2	1	4	1	6	1	9	3	4	2
	MEA 7	3	1	3	5	6	1	9	3	2	1
	MEA 9	3	5	4	5	5	1	7	3	2	2
	MKG 14	2	1	4	1	6	1	12	2	1	4

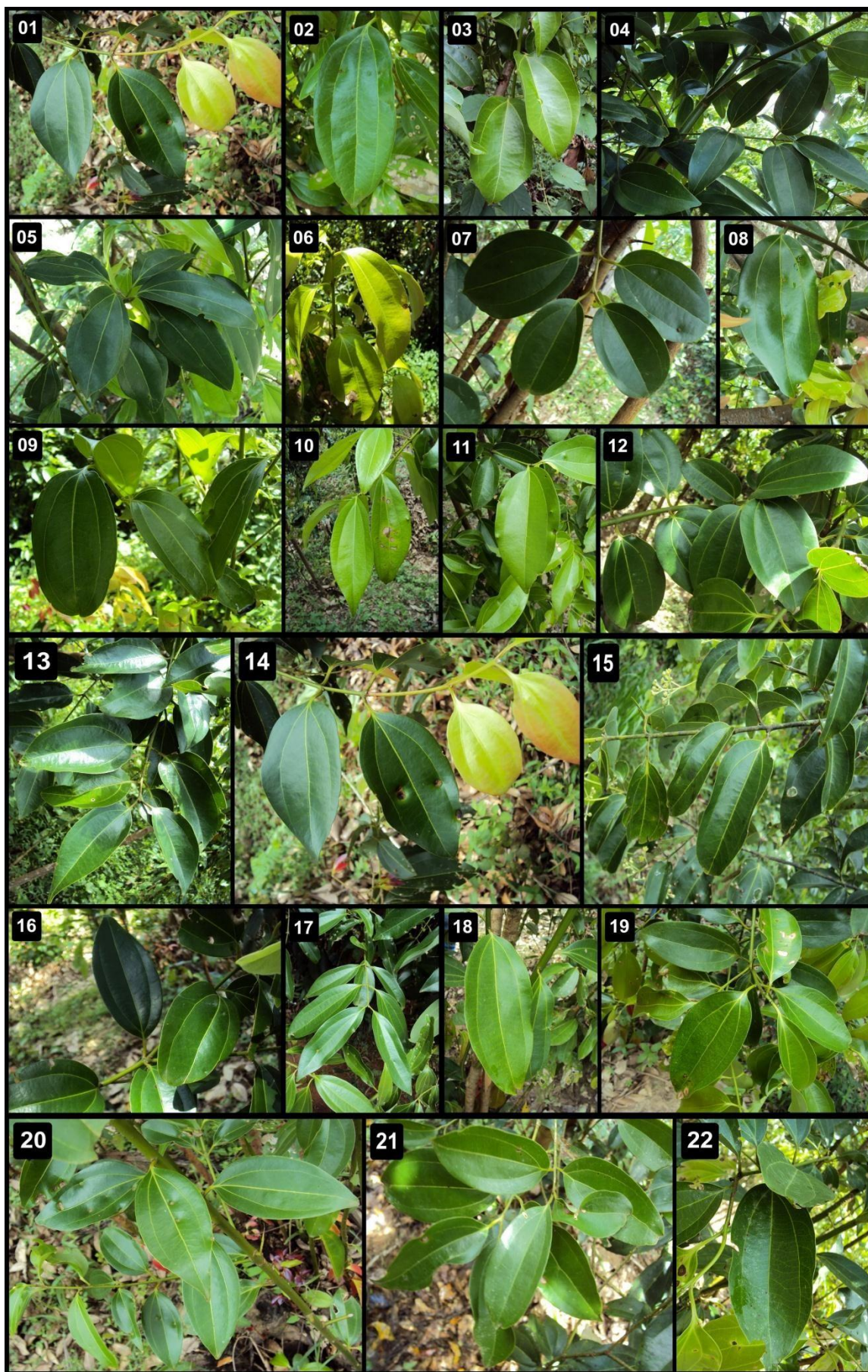


Figure 2: Variation in leaf shape within clusters; **Cluster-01:** 01(MEA-06) Elliptic, 02 (MMK-18) Lanceolate, 03 (MEA-02) Elliptic to broadly elliptic, 04 (MPT-01) Ovate-lanceolate, 05 (MKG-08) Ovate-oblong to ovate-lanceolate; **Cluster-02:** 06 (MMK-20) Elliptic to broadly elliptic; **Cluster-03:** 07 (MPT-03) Ovate, 08 (MKG-09) Elliptic to broadly elliptic, 09 (MEA-01) Ovate-lanceolate; **Cluster-04:** 10 (MMK-13) Lanceolate ; **Cluster-05:** 11 (MKG-06) Elliptic, 12 (MMK-11) Elliptic to broadly elliptic, 13 (MEA-05) Ovate-lanceolate; **Cluster-06:** 14 (MMK-14) Elliptic; **Cluster-07:** 15 (MPE-07) Elliptic, 16 (MPT-02) Lanceolate, 17 (MEA-11) Narrowly elliptic; **Cluster-08:** 18 (MKG-01) Narrowly elliptic; **Cluster-09:** 19 (MMK-12) Elliptic, 20 (MEA-08) Lanceolate, 21 (MEA-09) Elliptic to broadly elliptic, 22 (MKG-03) Ovate-lanceolate.

There is a variation in qualitative and quantitative characters among the cinnamon accessions of Matara district which were categorized into nine distinct clusters at rescale distance of 3. Accessions MMK14 and MMK 20 could be distinct

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