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ORIGINAL ARTICLE

Eco-geographic and Genetic Variation among Populations of *Terminalia chebula* Retz. in Sri Lanka

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Abstract

Terminalia chebula Retz. (*Aralu* in Sinhala) is a perennial medicinal plant known for its vital medicinal properties and globally used in treating various health problems. Despite its significance, agronomic or ecological research on this species is limited compared to studies on its medicinal values. This research aimed to locate and identify the ecological distribution of *T. chebula* in different agro-ecological regions of Sri Lanka and to characterize different populations based on the morphological and genetic variation. Samples were collected from 125 *T. chebula* trees representing various populations, and 40 morphological characters were used for morphological characterization. Data were analyzed using principal component analysis and cluster analysis using the SAS statistical software. Genetic characterization was conducted through base sequencing at rbcL region of the genome. Results indicated that *T. chebula* populations in Sri Lanka can be clustered into four groups based on their morphological characters. Each group consists of plants from different locations, suggesting that grouping is not based on geographic or environmental parameters, a finding collaborated by genetic characterization. However, 80% of the *Nilgala* population grouped in one cluster and all the natural *T. chebula* plants are located only in the intermediate zone, indicated a relationship between environmental parameters and the species under natural conditions. Conversely, planted trees are found across all agro-climatic zones of Sri Lanka. Further, this study discussed the implications of these on the conservation, expansion of cultivation and effective utilization of *T. chebula*.

***Keywords**: Aralu, Ecological distribution, Genetic characterization, Morphological characterization, Nilgala population*

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1. Introduction

Terminalia chebula Retz. (*Aralu* in Sinhala) is a perennial medicinal plant belonging to the family *Combretaceae*. This species naturally occurs in India, Pakistan, Sri Lanka, Cambodia, Myanmar, Vietnam, China, Laos, Thailand, Bhutan, Taiwan, Nepal and Bangladesh (Kailash et al. 2022). Various parts of the plant possess vital medicinal properties and are extensively used in Ayurveda, Sidda, Unani, and traditional Chinese medicinal systems. Hence, *T. chebula* is known as "King of medicines" (Kailash et al. 2022). It is relatively rare in Sri Lanka when compared to other *Terminalia* species and widely used in local native medicine (Ayurveda Department 2002).

Terminalia chebula plants are fire-resistant due to their thick bark, which has a high concentration of polyphenolic compounds. These plants naturally grow mainly in savannah forests, patna lands and tropical dry mixed/evergreen forests in Sri Lanka where fire is a common phenomenon during the dry season (Jayaweera 1980). Fire probably helps in the germination of seeds by breaking seed dormancy (Purohit and Vyas 2005).

Fruits/seeds, immature seeds, and bark of roots in *T. chebula* are used for medicinal purposes. Immature seeds are called '*Bala Harithaki'* in Ayurveda (Ayurveda Department 2002). *Terminalia chebula* contains terflavin B, a type of tannin, while chebulinic acid and chebulagic acid are found in the fruits (Senthilkumar and Subramanian 2008). *Terminalia chebula* has astringent, purgative, stomachic and laxative properties (Ayurveda Department 2002). Finely powdered fruit is used as a dentifrice for carious teeth, bleeding and ulceration of the gums (Jayaweera 1980). *Terminalia chebula* is used in tanning leather and purification of petroleum (Thakur et al. 2008).

Therefore, *T. chebula* is identified as an economically important plant, though it is under exploited. Due to the vital medicinal properties, it is listed among the 50 extensively used medicinal plant species in Sri Lanka (Handa et al. 2006). However, there has been very little agronomic and ecological research conducted on this species, in contrast to the numerous studies on its medicinal value (Ayurveda Department 2002). The lack of information on agronomic and ecological aspects is a significant barrier to fully exploit the potential of *T. chebula* in Sri Lanka.

Terminalia chebula is reported to be a predominantly out-crossing species, which leads to significant morphological variations in the population (Thakur et al. 2008). Consequently, the plant exhibits considerable morphological variation in its leaves and fruits (Ayurveda Department 2002).

The overall objective of the research was to perform morphological and genetic characterization of *T. chebula* populations in Sri Lanka. The specific objectives of the research project were to: (i) locate and identify the ecological distribution of *T. chebula* in different agroecological regions of Sri Lanka; (ii) characterize *T. chebula* populations in Sri Lanka using morphological variation of the species; and (iii) identify relationships between morphological variation and the genetic makeup of the species.

2. Materials and Methods

2.1 Sampling locations

Locations of *T. chebula* plants and populations were identified using literature sources (Jayaweera 1982; Philcox 1997), and information given by officials of the Departments of Forest and Wildlife Conservation, Indigenous Medicine and the Bandaranaike Memorial Ayurvedic Research Institute, Ayurvedic practitioners and the general public. Subsequently, sampling sites were determined based on distribution of populations in different agro-ecological regions, and the relative importance of the sites. Sampled sites and trees were classified as natural or planted based on the characteristics of their locations and populations (Fig. 1).

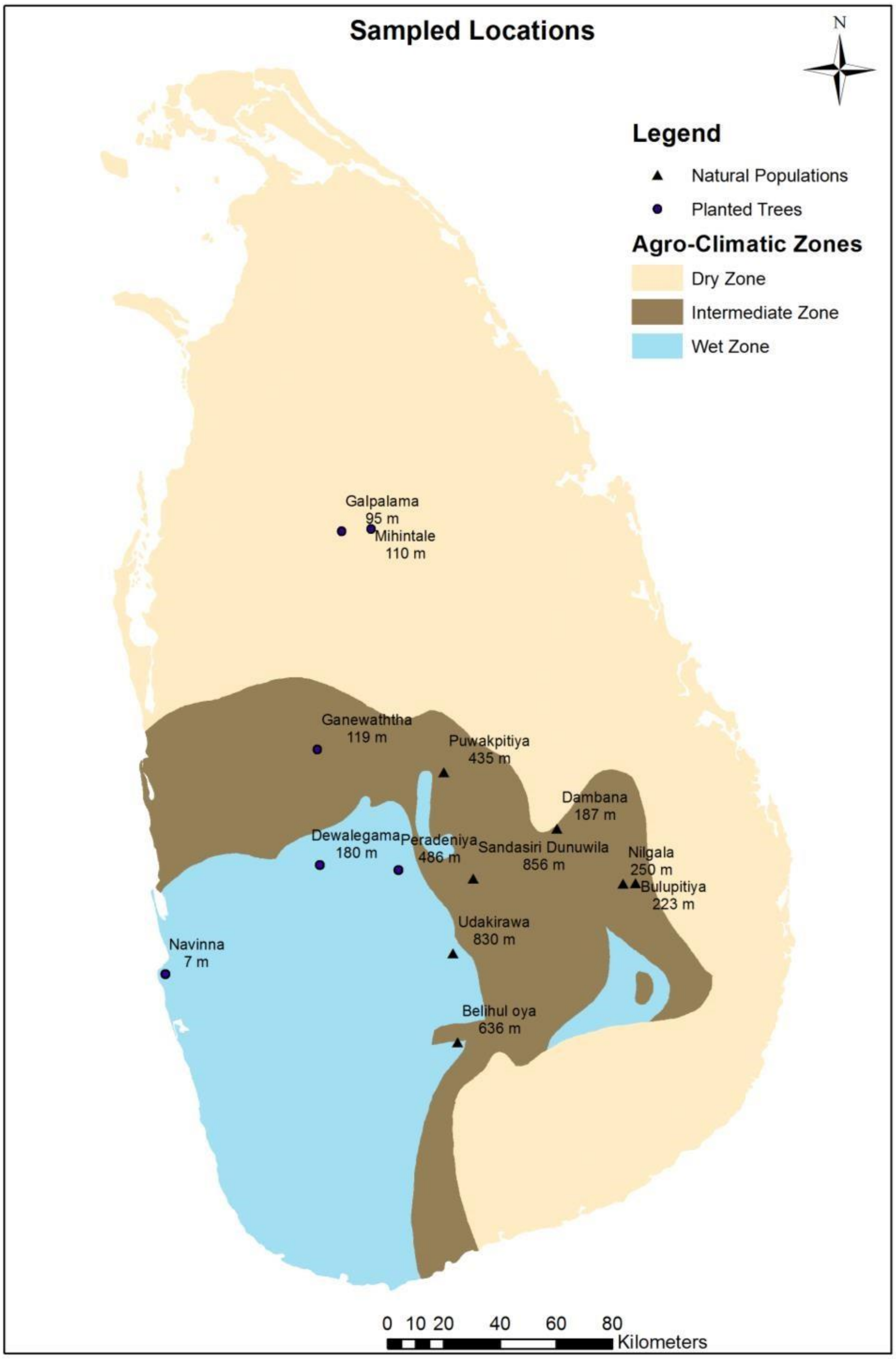


Figure 1. Locations of Sampled Populations (Places with elevations)

The data collection from leaf, flower and fruit samples, as well as data entry and analysis were done at the Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka, Puliyankulama, Anuradhapura, Sri Lanka. Genetic analysis to determine variations among samples was performed at the Genetech Molecular Diagnostics and School of Gene Technology, Colombo, Sri Lanka, where 30 leaf samples representing all the studied *T. chebula* populations tested.

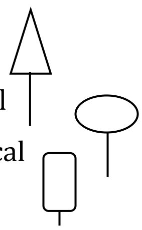
2.2 Sampling Procedure

In natural populations, 10 to30 trees per site were sampled. In order to identify within and between population variations, a tree cluster of 5 to 6 trees was selected. A minimum distance of 1 km was maintained between clusters. All sampled individual trees were observed for identified characters, recorded and samples of other plant parts were collected. All trees from cultivated sites were observed and sampled. In total, 125 trees were sampled from seven natural populations and six planted locations. From each sampled tree, 25 leaves, 5 to 25 flowers, and 5 to 25 fruits were collected for morphological character analysis. Rainfall patterns, relative humidity (RH%), temperature and soil type in relevant agro-ecological region were taken as secondary data from Punyawardena (2008).

2.3 Variables Used for Morphological Characterization

The variables used for morphological characterization were categorized into the following groups: tree characters (Table 1), bark characters (Table 2), leaf characters (Table 3), flower characters (Table 4) and fruit characters (Table 5). These categories were then used for cluster analysis and principal component analysis.

Table 1. Tree characters of *Terminalia chebula,* methods of measurement and related details

No	Tree Character	Units	Measuring instrument	Method of measurement	Scale used
1	Tree height	m	Clinometer	-	-
2	DBH	cm	Measuring tape	Divide the circumference by π	-
3	Tree age	years	Secondary data	Data not available for most of trees	-
4	Tree shape	—	—	Observation	1 - Conical 2 - Spherical 3 - Cylindrical 
5	Tree vigour	—	—	Observation	1 - Weak 2 - Moderate 3 - Strong (how trees adapt to the particular environment compared to surrounding trees)

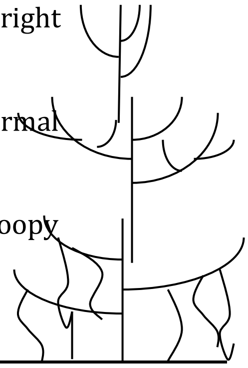
6	Branching density	—	—	Observation	1 - Sparse (easily can see through the canopy) 2 - Medium (can see through canopy via small openings) 3 - Dense (cannot see through canopy at all)
7	Branching pattern	—	—	Observation	1 - Upright 2 - Normal 3 - Droopy 

Table 2. Bark characters of *Terminalia chebula* (IBPGR, 1980) and methods of measurement

No	Bark Character	Units	Measuring instrument	Method of measurement	Scale used
1	Colour	—	Munsell colour charts	Comparison	1 - White 2 –Brown 3 - Black
2	Thickness	mm	Vernier caliper	—	—
3	Surface texture	—	—	Observation	1 - Smooth (no splits on the bark) 2 - Moderate (few splits here and there on the bark), 3 - Rough (splits are all over the bark)

Table 3. Leaf characters of *Terminalia chebula* (IBPGR, 1980) and methods of measurement

No	Leaf Character	Units	Measuring instrument	Method of measurement	Scale used
1	Leaf arrangement	—	—	Observation	1- Opposite 2 - In between 3 - Alternate 4 - Spiral
2	Leaf colour	—	Munsell colour charts	Comparison	1 - Light Green 2 - Green 3 - Dark Green
3	Leaf orientation	degrees	Protractor	Using leaf angle	Clockwise angle to stalk
4	Leaf petiole length	mm	Foot ruler	—	—
5	Leaf area	cm^2	Leaf area meter	—	—
6	Leaf length	cm	Leaf area meter	—	—
7	Leaf width	cm	Leaf area meter	—	—
8	Leaf Perimeter	cm	Leaf area meter	—	—
9	Leaf Thickness	mm	Vernier caliper	—	—
10	Chlorophyll	SPAD value	SPAD meter	—	—
11	Leaf shape	—	—	Observation	1 - Oval 2 - Oblong 3 - Lanceolate 4 - Oblanceolate 5 - Elliptic
12	Leaf margin	—	—	Observation	[a]1 - Entire 2 - Undulate 3 - Finely serrate

13	Leaf base shape	—	—	Observation	1 - Acute 2 - Rounded 3 - Oblique 4 - Cordate
14	Leaf apex shape	—	—	Observation	1 - Acute 2 - Acuminate 3 - Apiculate 4 - Obtuse 5 - Emerginate
15	Leaf aroma	—	—	Observation	1 - Weak 2 - Moderate 3 - Strong (Observed after rubbing few leaves from a tree and all were done by one person)
16	Leaf venation	—	—	Observation	1 - Arcuate

Table 4. Flower characters of *Terminalia chebula* (IBPGR, 1980) and methods of measurement

No	Flower Character	Units	Measuring instrument	Method of measurement	Scale used
1	Colour	—	munsel colour charts	Comparison	1 - Light yellow 2 - Yellow 3 - Greenish yellow
2	Types of flowers	—	—	Observation of parts	1 - Male 2 Hermoprodite 3 - Female
3	Flower arrangement	—	—	Observation	1 - Single 2 - Clustered
4	Male parts	#	—	Count	—
5	Female parts	#	—	Count	—

Table 5. Fruit characters of *Terminalia chebula* and methods of measurement

No	Fruit Character	Units	Measuring instrument	Method of measurement	Scale used
1	Fruit weight	g	Electronic balance	—	—
2	Fruit shape	—	—	Observation	1 to 7 as named in Ayurveda
3	No of ridges	#	—	Count	-
4	Fruit colour	—	Munsel colour charts	Comparison	1 - Light green 2 – Green 3 - Purple green 4 – Purple 5 - Light brown 6- Brown
5	Fruit diameter	mm	Vernier caliper	—	—
6	Cross section shape	—	—	Observation	1 - Round 2 - Elliptic 3 - Other (Other variable shapes)
7	Longitudinal section shape	—	—	Observation	1 - Round 2 - Elliptic 3 - Other (Other variable shapes)
8	Fruit clustering	—	—	Observation	1 - Single 2-Clustered
9	Fruit surface texture	—	—	Observation	1 - Smooth (Shiny and smooth, no ridge observed) 2 - Moderate (Not shiny and no clear ridges) 3 - Rough (Clear ridges and rough surface)

The morphological data and environmental parameters (Table 6) assessed during the study were identified, measured, and recorded using values for parametric variables or codes for non-parametric variables, as recommended by the IBPGR (1980).

Table 6. Environmental or geographical parameters and methods of measurement

No	Environmental parameters	Units	Measuring instrument	Method of measurement	Scale used
1	Site topography	—	—	Observation	1 – Flat 2-Undulated 3 - Sloppy
2	Elevation	m (AMSL)	GPS Receiver	—	—
3	GPS coordinates	Lat. (N^0) Longi. (E^0)	GPS Receiver	—	—
4	Type of population	—	—	Observation	1 -Monoculture 2 - Mixed
5	Associated vegetation	—	—	Observation	Associated species Dominant species
6	Temperature	^0C	(Thermometer)	Secondary data	-
7	Rainfall	$mmyr^{-1}$	(Rain gauge)	Secondary data	-
8	RH	%	(Hygrometer)	Secondary data	-

Leaf and fruit characters were measured and recorded using collected samples. Tree and bark characters, along with five environmental parameters, were recorded at each sampling site/tree through direct observation. The remaining environmental parameters were obtained from secondary data sources.

2.4 Data Analysis

The data were analyzed using multivariate techniques (Sneath and Sokal 1973), especially Principal component analysis (PCA) and Cluster analysis. Principal components were obtained using covariances for the PCA. Additionally, environmental factors and climatic data such as site topography, elevation, type of population, associated vegetation, temperature, rainfall, and relative humidity (RH) were correlated with morphological parameters (and sometimes with the principle components) to explain the final dendrogram developed from cluster analysis.

Data analysis was conducted using SAS statistical Software (SAS 2004). The PCA results were presented in terms of eigen values and cluster analysis was used to develop the dendrogram. The dendrogram was obtained by hierarchical clustering of the first three principal components using the Ward linkage method (Ward 1963). During the analysis, some of the morphological characters were omitted due to reasons given in Table 7.

Table 7. Morphological characters removed from the analysis and reasons for the removal

Characters removed	**Reasons for removal**
Tree age	Higher number of missing values
Flower Colour, Types of flowers, Flower arrangement	No variability among trees
Leaf Arrangement, Leaf Venation	Lower variability among trees
Leaf margin, leaf apex shape, tree vigour	Low significance in PCA

2.5 Genetic Characterization

DNA was extracted from 0.3 g of fresh leaf material using modified CTAB method with the addition of 2% PVP40. All samples were purified using Invitrogen Plant DNA extraction kit (Invitrogen USA) according to the manufacturer's protocol to avoid problems of PCR inhibition. The extracted DNA was amplified using PCR according to David Erickson's standard protocol.

Subsequently, DNA sequencing was done at the **'rbcL'** region (CBOL Plant Working Group, 2009). A phylogenetic tree was the constructed using "BioEedit" software, based on the electrogrames representing the base sequences at rbcL region of each barcoded sample.

3. Results and Discussion

3.1 Ecological Distribution of Terminalia chebula in Sri Lanka

Terminalia chebula trees are naturally distributed in several parts of the Intermediate Zone of Sri Lanka. However, planted trees can be found in all the agro-climatic zones. The locations of sampled and non-sampled natural populations, as well as planted trees of *T. chebula,* their agro-ecological regions and related site information were plotted in a map of Sri Lanka as shown in Fig. 2 using their GPS coordinates.

This distribution indicated that environmental conditions of the Intermediate Zone of Sri Lanka are particularly suitable for growth and natural reproduction of *T. chebula* plants, as evidenced by the significantly higher population density in this zone (Fig. 2). However, the plants can thrive in other areas under human interventions on germination. Natural regeneration was not observed under any of the studied planted tree, although some of them produce fruits and viable seeds.

According to Sharma and Thakur (2016) *T. chebula* is distributed up to an altitude of 1600 m in India, but not in arid zone. It is found in mixed dry deciduous forests and is often located in tropical and subtropical areas, predominantly in hilly regions, except in arid zone (Singh et al. 2019). Similarly, Kailash et al (2022) suggested that the central and south India are highly suitable for *T. chebula*. These areas share climatic conditions similar to those of the Intermediate Zone of Sri Lanka. The mean annual temperature, temperature seasonality, and isothermality appeared to be the most important variables determining the distribution of this species, which is directly influenced by climate change (Kailash et al. 2022). Furthermore, the global distribution of this species is also limited due to its sensitivity to climate (Kailash et al. 2022).

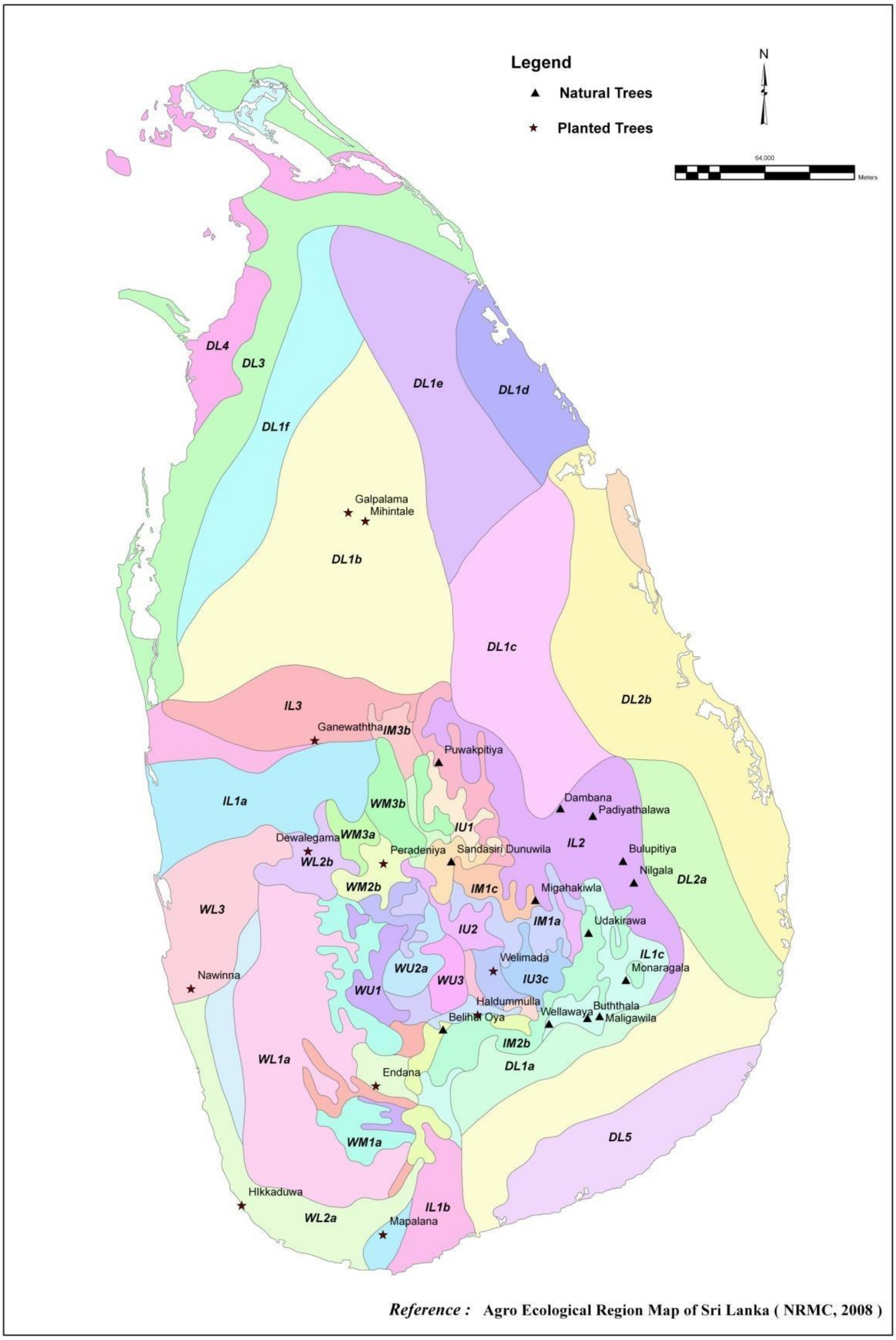


Figure 2. Ecological Distribution of *Terminalia chebula* populations in Sri Lanka

*Sources for non-sampled locations are Jayaweera (1982); Officers of Departments of Forest and Wildlife Conservation and Indigenous Medicine and Herbarium samples in National Herbarium at the Department of National Botanic Gardens, Peradeniya.

3.2 Morphological Variation of Terminalia chebula

No observable variations in flower characters were found (Table 8). In contrast, Variations were observed in stem/trunk characters, leaf characters and fruit characters of *T. chebula* (Table 9 and Fig. 3) during the study. There was no significant (*p>0.01*) within-tree variation in fruit characters; however, significant variation (p<0.01) was noted in fruit characters such as fruit shape and fruit size between trees (Table 9 and Fig. 3). Both between-tree variations and within-tree variations were significant (p<0.01) regarding leaf characters.

Table 8. Flower characters of *T. chebula.*

Flower Character	Value/Category	Remarks
Colour	Yellowish white	
Type of flower	Hermaphrodite	No variation in floral
Flower arrangement	Clustered	characteristics among
Number of stamens	10	samples.
Number of ovaries	01	

Figure 3. Variation in fruits obtained from different *Terminalia chebula* trees in intermediate zone

Dar and Agnihotri (2017) reported significant variations in morphological and physiological traits such as fruit weight, fruit length and diameter, seed length and diameter and seed germination of *T. chebula* fruits collected from 12 different locations across four states in India. These findings align with the between-tree variation in fruits as illustrated in Table 9 and Fig. 3.

Table 9. Fruit characters of *Terminalia chebula* that shows between-tree variations

Fruit Character	Mean	Standard Deviation	Max	Min
Weight (g)	6.3	7.67	13.12	1.07
Diameter (mm)	13.2	10.14	39.1	1.73
Longitudinal section (mm)	18.0	13.23	49.5	2.52
Perimeter (mm)	72.6	13.97	120	42
No of Ridges	3.2	2.16	8	2
Fruit Character	**Category 1 %**	**Category 2 %**	**Category 3 %**	**Category 4 %**
Fruit shape (Name given in Ayurveda)	1 - Vijaya 20%	2 - Putana 18%	3 - Rohini 23%	4 - Jivanti 0%
	5 - Chetaki 18%	6 - Abhaya 9%	7 - Amrutha 12%	
Fruit colour	Light green 65%	Green 3%	Purple green 12%	Purple 20%
Fruit clustering	Single 21%	Clustered 79%		
Surface texture	Smooth 41%	Moderate 47%	Rough 12%	
Cross section shape	Round 70%	Elliptic 21%	Other 9%	
Longitudinal section shape	Round 27%	Elliptic 70%	Other 3%	

According to Thakur et al. (2008) fruit and seed traits exhibit significant variation between trees from which samples were collected. Authors found a positive correlation between fruit diameter and fruit weight. However, none of these studies mentioned about within-tree variation in fruit characters or variations in leaf and flower characters, which were evaluated in present study. Navhale et al, (2011) reported that morphological variations are greater than genotypical variation in *T. chebula*. Furthermore, the heritability of fruit characters such as total soluble solids (TSS) weight of dry fruit, weight of fruit pulp, total sugars and acidity percent is higher, and those characters could be used to select superior trees for breeding purposes (Navhale et al. 2011).

3.3 Terminalia chebula Tree Clusters by Morphological Variation

The first three Principal Components (PC) obtained using all studied morphological characters explained 87.8% of the accumulated variation in *T. chebula* populations (Table10), which is sufficient to represent the overall variation of the total population. The eigen vector indicated that leaf area was the key variable for PC 1, chlorophyll content, fruit diameter and fruit longitudinal section were the major contributors to PC 2 and key variables for PC 3 were leaf perimeter and DBH.

Table 10. Eigen values, percentage of total population variance explained by principal components

PCA	Eigen Value	Percentage	Cumulative Percentage	Chi Square	DF	Prob> Chi Square
1	1360.7	59.9	59.9	4,972.69	594	< 0.0001
2	401.0	17,7	77.6	4,108.61	560	< 0.0001
3	231.5	10.2	87.8	3,615.61	527	< 0.0001
4	100.4	4.4	92.2	3,115.25	495	< 0.0001
5	64.1	2.8	95.0	2,782.14	464	< 0.0001
6	39.7	1.7	96.8	2,462.33	434	< 0.0001

The dendrogram obtained by hierarchical clustering of the first three principal components (Table 10) exhibited four distinct groups at a linkage distance of 1.26 (Fig. 4) in the population, excluding two outliers. Unique characters of each cluster are given in Table 11. Leaf area was the most significant contributor to first principal component, indicating that clustering is highly based on leaf characters of the plant. Leaf characters exhibited significant within-tree variation and between-tree variations.

The greater contribution of fruit characters, namely fruit diameter and fruit longitudinal section, to the principal components suggested a relationship between clusters and fruit characters. This phenomenon indicated that it is possible to group *T. chebula* trees according to fruit shapes. According to Ayurveda (Ayurvedic Department, 2002), there are seven types of *T. chebula* trees based on fruit shapes, that matches with this finding as six types of them were identified during the present study. Additionally, significant variations in fruit diameter, fruit length and fruit weight were observed in samples collected from different *T. chebula* trees in India (Thakur et al. 2008; Dar and Agnihotri 2017), supporting between-tree variation, which is mostly genetically driven (Navhale et al. 2011).

In the dendrogram (Fig. 4), each cluster consisted of plants from different locations, suggesting that grouping was not based on geographic or environmental parameters. However, 80% of the Nilgala population grouped into one cluster (Cluster 4), and all the natural *T. chebula* plants are located only in the intermediate zone, indicating a relationship between environmental parameters and the species under natural conditions. The reason for two groups (sub-clusters) observed within cluster 4 was the identification of two clear seed categories within the cluster. Plant 22 and 25 were two outliers in the dendrogram (Fig. 4), which might represent other groups/ clusters that do not have sufficient number of individuals within our sample.

Fire is a possible occurrence in Nilgala-like intermediate zone forest areas where *T. chebula* has a competitive advantage being a fire-resistant species. Fire also helps seed germination by breaking dormancy, favoring the natural regeneration of the species. Kailash et al. (2022) highlighted that, *T. chebula* is highly sensitive to climate change, and its presence is governed by existing environmental conditions of the location of population.

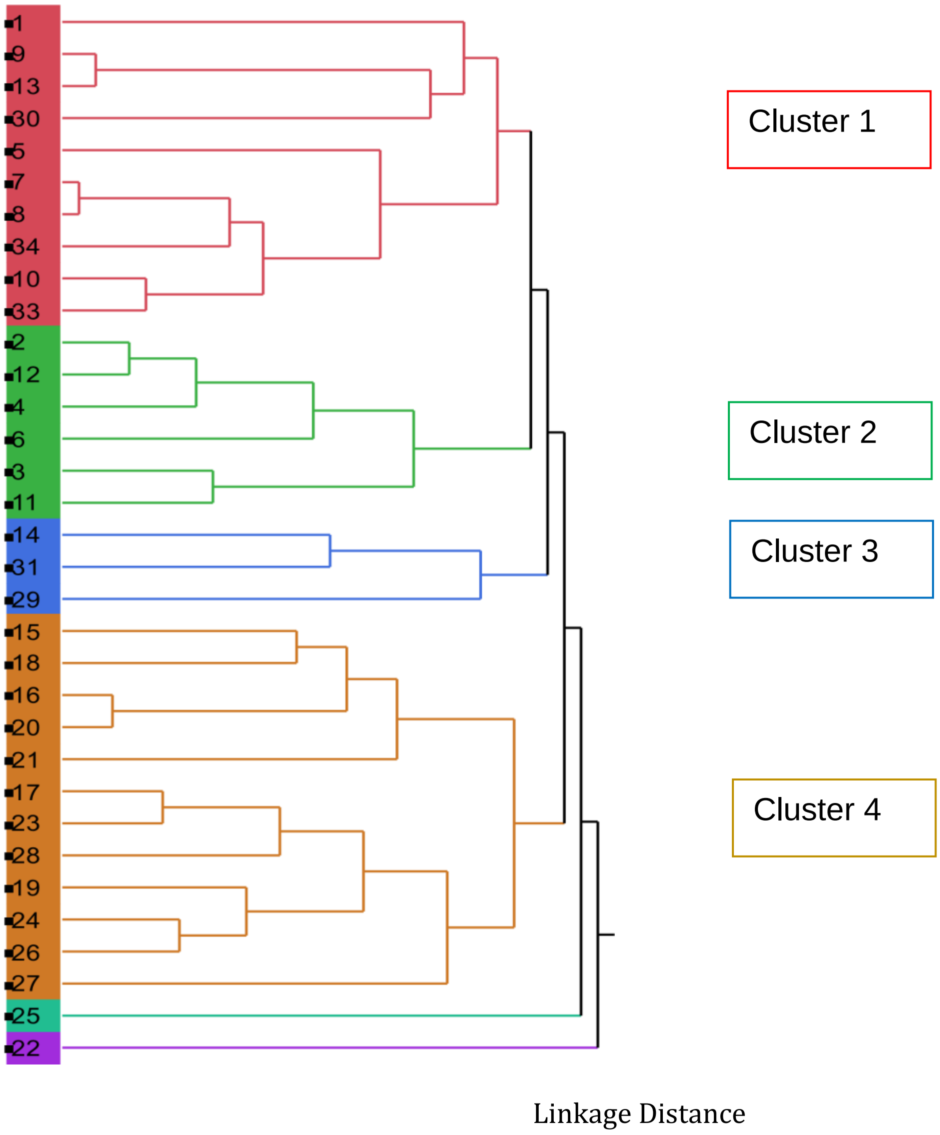


Figure 4. Dendrogram illustrating the 04 clusters obtained using first three principle components

Table 11. Unique characters of the clusters in dendrogram

Cluster	Unique Characters
Cluster 1	Fruits are relatively large (Average fruit weight = 5.1 g)
Cluster 2	Leaves are similar in size (Ave. leaf area = 27 cm^2), Same fruit shape (3 - Rohni)
Cluster 3	Leaves are very large (59.1 cm^2), Fruits are large (Ave. weight:5.3 g)
Cluster 4	Tree vigour is high, leaf orientation is nearly same (52^{o} –66^{o}), Leaf shape is similar (1 - Oval), All the plants are from Nilgala.

3.4 Genetic Characterization

Phylogenetic tree (Fig. 5) of genetic analysis was obtained from only six samples out of 30 *T. chebula* leaf samples, due to difficulties in extracting DNA caused by high concentration of phenolic substances. The results of genetic analysis (Fig. 5) indicated that grouping according to genetic material is not related to the geographic location of the tree or whether it is natural or planted. Lower genetic variation between samples from Ganewaththa (T501) and Nilgala (T411, T412 and T415) suggested that trees in Ganewaththa may have established using planting materials from Nilgala population. This result is further supported by Cluster 4 of the dendrogram (Fig. 4), which was obtained from clustering of morphological parameters. T144, from Sandasiri Dunuwila population, is not clustered with the Nilagala population. However, T401 is an outlier from the Nilagala population.

The RAPD analysis proved useful in characterizing and differentiating *T. chebula* accessions, revealing large genetic variation among the twelve studied accessions. As a result of the RAPD analysis, the 12 accessions were divided into two clear clusters (Ranjini et al. 2015). Barcoding at rbcL region was used to distinguish inter-species genetic diversity in African *Combretaceae* (Onefelly and Stanys 2019).

Navhale et al. (2011) reported that the estimates of phenotypic variations were higher than the genotypic variations for most characters, indicating that these characters were influenced by environmental factors. The heritability was high for total soluble solids followed by weight of dry fruit, weight of fruit pulp, total sugars and acidity percent (Navhale et al. 2011).

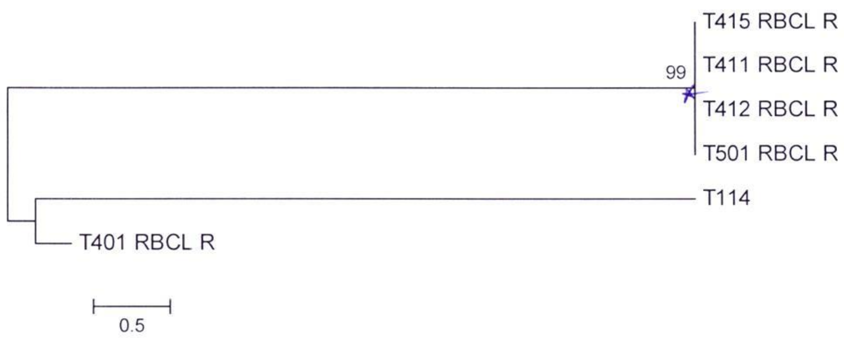


Figure 5. Phylogenetic tree obtained analyzing base sequences of samples

3.5 Implications on Conservation and Use of Species

Terminalia chebula plants are more frequently available in Nilgala and Dambana forest reserves, where the largest populations in Sri Lanka were observed. These natural habitats are crucial for *in-situ* conservation of the species. Additionally, the identified variation within the species should also be conserved within natural habitats due to its implications for commercial utilization.

Given its economic importance, commercial cultivation could be promoted as an *ex-situ* conservation strategy. This could be achieved by providing planting materials to general public free of charge, addressing the problem of seed germination under planted trees (Dar and Agnihotri 2017). Furthermore, commercial cultivation of the species could reduce the threat to natural populations by minimizing seed collection and its impact on natural regeneration.

Four clusters identified during the study through morphological characterization provide a useful criterion to streamline the medicinal usage of the plant or its parts. However, this criterion along is not sufficient making decisions on conservation and utilization. Clustering should be strengthened through comprehensive genetic and chemical constituent analysis, taking the samples from each and every cluster as well as from natural and planted trees. Such future research is necessary to provide recommendation on the commercial usage of intra-species variation.

These findings would be beneficial for drug manufacturers and medical practitioners of various medicinal systems for the betterment of their patients. Additionally, the use of the root bark for pruritic illnesses and urticaria could be promoted by preparing or formulating new drugs after chemical analysis of the bark of roots.

4. Conclusions

Terminalia chebula trees are naturally found in the Intermediate Zone of Sri Lanka, with planted trees seen in all agro-climatic zones. The intermediate zone's environmental conditions favor the species' growth and reproduction, unlike the wet and dry zones. *Terminalia chebula* populations can be grouped into four clusters based on morphological characters, mainly leaf area and fruit shape variations. Genetic analysis shows no link between genetic variation and geographic location or whether the trees are natural or planted. Additionally, genetic variation did not match morphological variation. Fruit characters vary between trees but are consistent within a tree, indicating genetic control. This aligns with Ayurveda's categorization of seven *T. chebula* types based on seed morphology.

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