Analysis of Leaf Characteristics of Local Moringa (*Moringa oleifera* Lam.) Germplasms in Sri Lanka

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Abstract

Moringa (Moringa oleifera Lam.) is one of the most popular vegetable crop species cultivated throughout the tropics with a high medicinal and nutritive value. However, there were no evidences regarding crop improvement, breeding or germplasm evaluation programmers reported for this valuable crop. Hence, this study was conducted in Grain Legume and Oil Crop Research and Development Center, Angunakolapelessa to identify the best Moringa *accessions with desirable morphological characteristics. Twenty accessions representing 4 districts which were maintained in field plots with 3 replications per accessions, were arranged in a Randomized Complete Block Design (RCBD). At 90 days after field planting, the 4 leaf morphological characters were recorded and hierarchical cluster dendrogram was generated to reflect their relatedness using cluster analysis. Our results revealed that the leaf dry matter content ranged between 22.1% and 26.4%. The maximum value of 26.4% was recorded in the M94 accession, which was collected from the Kurunegala district. Mean tripinnate leaf length ranged between 62.5±0.5 mm and 82.0± 4.68mm whilst the tripinnate leaf width ranged between 52.3 mm and 69.3 mm. Accordingly, the highest leaf dimensions were recorded in accession no. M14 and M97 collected from Batticaloa and Jaffna districts respectively. According to the dendrogram, accessions M14 and M97 were resulted as the most distinctive accessions in the study. Conversely, M14, M94 and M97 were clustered in one group depicting that the moringa leaf traits subjected to vary according to the geographical location within the country.*

Keywords: Moringa, Accessions, leaf characters, dry matter

I. INTRODUCTION

Moringa (*Moringa oleifera* Lam.) is belonging to the family Moringaceae (Paikra and Gidwani, 2017). It is a common vegetable species cultivated throughout the tropics, and a fast-growing perennial plant with high medicinal value (Sarkar, 2021). It is commonly referred as “Horseradish tree”, or “Radish tree “or Drumstick tree” (Obuobi, 2012). The fruits (pods) are long and slender which look like "drumsticks" and hence, it is denoted as with the popular name.

Moringa is rich in nutrition owing to the presence of a variety of essential phytochemicals in its leaves, pods and seeds. Specifically, seeds and leaves of the plant contain unsaturated fatty acid where oleic is prominent among the saturated fatty acids (Mahmood *et al.,* 2010) According to Khawaja *et al.* (2010), the Moringa is claimed to be the most nutrient-rich plant yet discovered. The leaves are rich in vitamins A, C, B1 (thiamin), B2 (riboflavin), B3 (niacin), B6 and Folate. Moreover rich in magnesium, iron, calcium, phosphorus, and zinc (Sarkar, 2021). The extracts from the leaves are used to treat malnutrition, augment breast milk in lactating mothers (Fahey, 2005). It is used as potential antioxidant, anticancer, anti-inflammatory, antidiabetic and antimicrobial agent (Ijarotimi *et al*., 2013).

The moringa plant is usually very slender, with an average height of about 10 meters. The tree has softwood and produces yellowish-white fragrant flowers (Chand *et al*., 2012). The leaves are bipinnate or more commonly tripinnate, grow up to 45 cm long, alternate and spirally arranged on the twigs. The leaflets are finely hairy, green. The fruits are pendulous, linear, three-sided pods with nine longitudinal ridges, usually 20 to 50 cm long. The tree esquires an annual rainfall of between 250 and 3000 mm. It is drought-resistant, though in drought conditions it may lose its leaves (Akther *et al.,* 2014).

Although, there are no moringa commercial growers are reported in Sri Lanka, the crop has been cultivated in home gardens and used as a vegetable for a long period due to the unavailability of identified or developed moringa varieties/accessions for local farmers. Hence, this raises the crucial question of how to assess the local moringa germplasm, enhance it, and develop new breeding techniques and use it in addressing current food crisis. Hence, for the improvement of such crop, germplasm collection, analyzing of morphological, physical and molecular characteristics are needed. As the first step, investigating leaf-based characters of locally grown moringa is vital to unveil the latent potentials. In germplasm management, morphological characterizations are usually employed as markers to deter the variations either within or between species, varieties or accessions (Acquaah, 2007). These markers called descriptors of the crops (Esen and Hilu, 1989). Such information through basic plant features is vital and provides the breeder with the information on the genetic closeness of the accessions. Information acquired from these markers aid breeders in the selection of accessions with desirable traits for both farmers and consumers. Therefore, this study was conducted to characterize the local moringa germplasms based on leaf characteristics and to identify the best performing accessions for future moringa breeding programmers.

II. METHODOLOGY

Twenty *Moringa oleifera* accessions were collected from Batticaloa, Kurunegala, Kandy, and Jaffna and cultivated at Grain Legume and Oil Crops Research and Development Centre, Angunakolapelessa,SriLanka<https://www.latlong.net/place/kandy-sri-lanka-1085.html>The geographical coordinates are 6° 27' 0" North, 81° 1' 0" East. Each moringa accession was maintained as individual field plots with 3 replications in a Randomized Complete Block Design (RCBD). Stem cuttings with the length of 45 cm were planted in 30 cm x 30 cm x 30 cm size holes provided with 3 m x 3 m spacing. Then the crop management practices were done according to Department of Agriculture standards, regular irrigation, fertilizers (compost), weeding were practiced. At 90 days after field planting, moringa leaves qualitative and quantitative characters was observed in different 20 accessions in collected from the four districts of Sri Lanka.

Table 01. Details of Moringa olifera accessions collected from the four districts of Sri Lanka

Batticaloa	Jaffna	Kurunegala	Kandy
M02	M47	M72	M94
M13	M50	M74	M97
M14	M55	M78	M98
M25	M58	M84	M106
M05	M61	M86	M06

A. Leaf and leaflet measurements

Five leaves were taken from each moringa accessions from each field plots. Then the tripinnate leaf length and width, tripinnate leaflet length and width were measured using ruler and vernier caliper in mm (Zhigila *et al*, 2015).

B. Qualitative characters of moringa leaves

Leaf type, leaf and leaflet arrangements, leaflet shape, leaf color, growth habit, petiole pigmentation and stem bark color were recorded (Zhigila *et al*, 2015).

C. Moisture and dry matter content of moringa leaves

Moisture content of the sample was determined using the method described by (Prajapati.*et al*., 2003). One gram of sample in pre-weighed crucible was placed in an oven (105 °C) for 24 h, cooled, and reweighed. The moisture content was calculated as follows:

$$\text{Moisture (\%)} = (W_2 - W_3 / W_2 - W_1) \times 100$$

Where, W1 is the weight of the crucible, W2 is the weight of the crucible after drying at 105 °C and the sample, and W3 is the weight of the crucible and the sample after cooling in airtight desiccators.

Dry matter content of the sample was obtained using the following formula,

$$\text{Dry matter content} = 100 - [\text{moisture (\%)}].$$

D. Statistical analysis

Quantitative data was analyzed using the analysis of variance (ANOVA) using IBM SPSS software by using Tukey's post-hoc version 25. Dendrogram was generated by using IBM SPSS software to reflect their relatedness using cluster analysis.

III. RESULTS AND DISCUSSION

A. Morphological analysis

There were significant ($p<0.05$) differences among the accessions of *M. oleifera* for the leaf measurements determined in this study. Mean tripinnate leaf length ranged between 62.5 mm and 82.0 mm whilst the tripinnate leaf width ranged between 52.3 mm and 69.3 mm (Table 02). For both parameters, the maximum values were recorded in accession no. M14 collected from Batticaloa district while the minimum values of tripinnate leaf width were recorded in accessions no. M74 collected from Kurunegala district, as well as minimum tripinnate leaf length was recorded M25 originated from Batticaloa.

Tripinnate leaflet length and width ranged from 25.8m to 34.2 mm and 14.5 mm to 23.1 mm respectively. M14 Accessions collected from the Batticaloa district had the highest tripinnate leaflet length and width values of 34.2 cm and 23.1 mm respectively. M74 Accessions from Kurunegala district had the lowest tripinnate leaflet length (25.8 mm) and tripinnate leaflet width (15.3 mm).

Table 02: Variation in leaf and leaflet dimensions of Moringa olifera accessions

Acc. No	**Tripinnate leaf length (mm)**	**Tripinnate leaf width (mm)**	**Tripinnate leaflet length (mm)**	**Tripinnate leaflet width(mm)**
M 02	70.1 ± 3.79^{a}	60.5 ± 4.11^{ab}	30.4 ± 2.24^{a}	18.2 ± 1.59^{ab}
M 06	69.7 ± 2.95^{b}	62.3 ± 2.33^{a}	31.5 ± 1.18^{b}	19.7 ± 2.24^{b}
M05	70 ± 3.85^{a}	62.6 ± 2.34^{b}	30.9 ± 1.80^{c}	18.7 ± 0.98^{a}
M 106	67.0 ± 4.41^{ab}	61.2 ± 4.67^{a}	29.9 ± 2.28^{ac}	17.9 ± 1.54^{bc}
M 13	74.8 ± 4.99^{b}	59.1 ± 5.11^{b}	30.4 ± 2.34^{ad}	16.1 ± 1.36^{c}
M 14	$\underline{82.0 \pm 4.68^{c}}$	$\underline{69.3 \pm 4.90^{c}}$	$\underline{34.2 \pm 2.35^{a}}$	$\underline{23.1 \pm 0.99^{ac}}$
M 25	62.5 ± 2.72^{ac}	54.4 ± 3.40^{d}	27.6 ± 1.60^{b}	14.5 ± 0.69b
M 47	64.7 ± 0.75b^{c}	58.7 ± 1.36^{cd}	28.5 ± 1.21^{ab}	17.3 ± 0.59^{bd}
M 50	70.3 ± 1.79^{c}	58.6 ± 2.23^{d}	29.0 ± 1.01^{c}	15.9 ± 0.42^{a}
M 54	65.7 ± 2.14b^{c}	56.5 ± 2.51^{d}	27.7 ± 1.35^{bc}	16.1 ± 0.47^{ad}
M 55	66.1 ± 0.93b^{c}	61.8 ± 2.57^{c}	31.0 ± 0.99^{cd}	16.8 ± 0.28^{cd}
M 58	65.2 ± 3.85b^{c}	54.8 ± 4.77^{ad}	27.1 ± 2.33^{bd}	18.5 ± 1.53^{c}
M 61	64.5 ± 3.17^{c}	54.0 ± 2.69^{c}	27.9 ± 1.29^{cd}	15.9 ± 0.76^{cd}
M 72	76.4 ± 3.53^{d}	62.9 ± 3.47^{d}	31.2 ± 1.79^{ac}	18.3 ± 0.70^{d}
M 74	63.6 ± 3.47^{b}	52.3 ± 3.95^{e}	25.8 ± 1.80^{b}	15.3 ± 0.38^{c}
M 78	68.7 ± 5.58^{d}	57.7 ± 4.85^{c}	28.2 ± 2.30^{d}	17.6 ± 1.51^{d}
M 84	66.1 ± 3.90^{c}	53.9 ± 3.29^{ae}	32.2 ± 1.88^{cd}	15.7 ± 1.19^{ed}
M 86	72.2 ± 1.61^{d}	63.3 ± 2.25^{d}	26.8 ± 1.09^{b}	18.6 ± 0.98^{e}
M 94	62.2 ± 0.60^{c}	52.4 ± 1.19^{d}	34.0 ± 0.93^{c}	14.9 ± 0.47^{ce}
M 97	78.5 ± 2.19^{ed}	68.3 ± 1.92^{ae}	31.3 ± 1.11^{cd}	21.3 ± 0.44^{de}
M 98	72.4 ± 1.91^{ec}	63.0 ± 1.98^{e}	29.6 ± 0.43^{e}	19.3 ± 0.22^{c}
P	0.003	0.027	0.034	0.01

**Mean (±SD) followed by the same superscript letter in each columns are not significantly different (p>0.05)*

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B. Moisture content and Dry matter content of Moringa oleifera accessions.

There was significant (P<0.05) variation in the dry matter content of *Moringa oleifera* accessions. However there was significant variation in the moisture content. The moisture content ranged between 77.9% and 73.6%. The maximum value of 77.9% was recorded on the accessions of M54 and M58. Both accessions were collected from the Kandy district. The minimum value of moisture content was recorded on the accession of M94.The dry matter content ranged between 26.4% and 22.1%. The maximum value of 26.4% was recorded on the M94 which was collected from the Kurunegala district. Accessions of M54 and M58 had a minimum value of 22.1%. Those were collected from the Jaffna district (Figure 1).

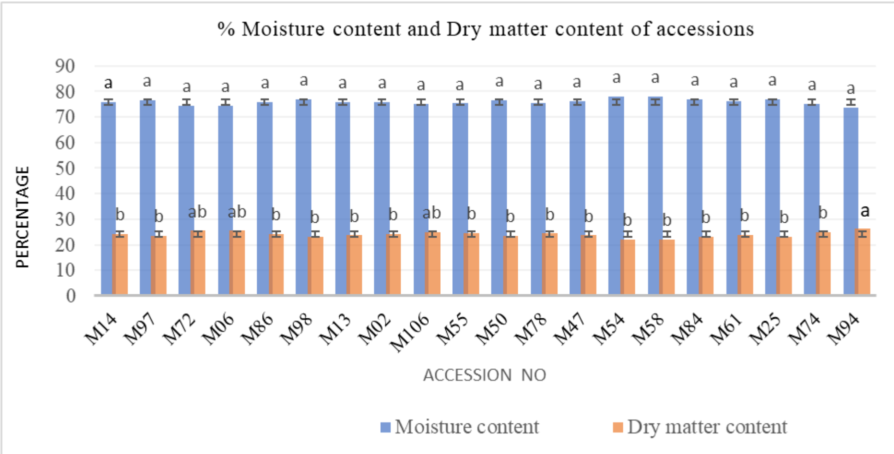

Figure 1: Moisture content and dry matter content of accessions.

C. Qualitative attributes of the Moringa oleifera accessions

The investigated *Moringa oleifera* accessions showed variance in the qualitative characteristics (supplementary Table 2). The compound leaves produced by all of the studied moringa accessions had alternate leaf configurations, and the leaflets were also placed alternately. As a result, the accessions' leaflet arrangement and shape seemed to be monomorphic for the characteristic. The hues of the leaves ranged from pale green to dark green and were all different shades of green. Since the leaf petiole's pigmentation ranged from dark green to light purple to purple, the pigmentation level of the various germplasms seemed to vary.

D. Cluster analysis for Quantitative of the Moringa oleifera accessions.

Cluster analysis resolved the quantitative data for moringa accessions into two major clusters A and B at a similarity level of 25 %. Major cluster-A further separated into two sub-clusters at a similarity level of 10 %. Sub-cluster I contained the accessions of M86, M98, M06, M72, M13, M106, M55, M50, M78, M02. Sub-cluster II contained; accession of M14 and M97.These two accessions had a high value for quantitative traits such as tripinnate leaf length, tripinnate leaf width, tripinnate leaflet length, tripinnate leaflet width. Then major cluster B separated into only one subcluster (cluster III) at a similarity level of 10 %. That contained the accessions of M74, M47, M54, M25, M74, M84, M61, M58 (Figure 2).

According to our findings, it was noteworthy to mention that there were variations in leaf traits among the germplasms collected from the four districts of Sri Lanka. Typically, the germplasms from drier regions (Batticaloa and Jaffna) and intermediate climatic zone (Kurunegala) tend to display improved leaf traits including dry matter accumulation and leaf dimensions. Conversely, the wet zone (Kandy) germplasm consisted of smaller leaf characteristics. The lowest percentage of dry matter among the accessions was observed in the accessions from the Kandy district, meaning fresh leaves derived from such germplasms may prone to deterioration since leafy vegetables with high moisture content tend to be more prone to perishability. Moreover, the accessions from the Kurunegala district were less prone to deterioration since they have the least amount of moisture content as such post-harvest handling and packaging, processing this data can be effectively utilized.

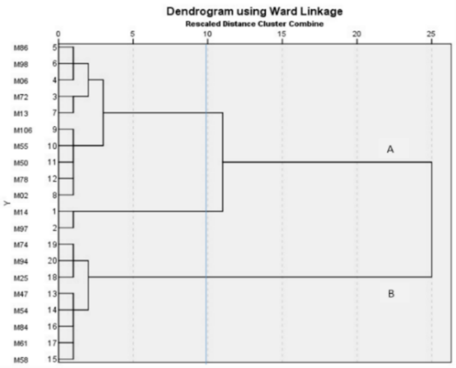

Figure 2: Dendrogram based on quantitative and qualitative of the Moringa oleifera accessions

IV. CONCLUSIONS

According to our research, the 20 evaluated moringa accessions varied in their tripinnate leaf and leaflet characteristics. According to the findings, *Moringa oleifera* accession from districts in the dry zone (Batticaloa and Jaffna) and intermediate zone (Kurunegala) displayed better leaf characteristics than those from wet zone (Kandy). Therefore, the M14, M94, and M97 are initially suggested for extensive field tests to back up the recent findings. For future research projects, suggest thorough characterization of leaf, flower, fruit, plant height and structure, sensory qualities, and nutrient analyses to have a clear grasp of the diversity that is accessible and their potential uses. It is also suggested to use molecular techniques, such as DNA marker assisted selection, to find variations in leaf characteristics among the moringa species.

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Supplementary table

Table 2 : Qualitative attribute of moringa accessions

ACC. No	Leaf arrangement	Leaflet arrangement	Leaflet shape	Leaf colour	Leaf type	Petiole Pigmentation
M 02	Alternate	Opposite	Compound	Green	Compound	Light Purple
M 06	Alternate	Opposite	Compound	Green	Compound	Dark Purple
M 106	Alternate	Opposite	Compound	Dark Green	Compound	Dark Green
M 13	Alternate	Opposite	Compound	Green	Compound	Dark Green
M 14	Alternate	Opposite	Compound	Light Green	Compound	Light Purple
M 25	Alternate	Opposite	Compound	Light Green	Compound	Dark Green
M 47	Alternate	Opposite	Compound	Light Green	Compound	Purple
M 50	Alternate	Opposite	Compound	Dark Green	Compound	Dark Purple
M 54	Alternate	Opposite	Compound	Light Green	Compound	Dark Purple
M 55	Alternate	Opposite	Compound	Green	Compound	Light Purple
M 58	Alternate	Opposite	Compound	Light Green	Compound	Dark Purple
M 61	Alternate	Opposite	Compound	Green	Compound	Dark purple
M 72	Alternate	Opposite	Compound	Green	Compound	Light Purple
M 74	Alternate	Opposite	Compound	Dark Green	Compound	Dark Purple
M 78	Alternate	Opposite	Compound	Green	Compound	Brown
M 84	Alternate	Opposite	Compound	Green	Compound	Brown
M 86	Alternate	Opposite	Compound	Dark Green	Compound	Dark Green
M 94	Alternate	Opposite	Compound	Green	Compound	Purple
M 97	Alternate	Opposite	Compound	Light Green	Compound	Green
M 98	Alternate	Opposite	Compound	Green	Compound	Purple

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