Morpho-anatomical variations of leaves and seeds among three *Moringa* species

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Abstract: The main goal of this study is to elucidate the variation among three species of genus Moringa; namely, *M. oleifera*, *M. stenopetala* and *M. peregrine*. Morphological and anatomical characters and scanning electron microscopy of leaf and seed were investigated. In addition, numerical analysis of studied characters was carried out. Various obtained characters were used to construct a botanical key to differentiate between studied Moringa species. This work proved the importance of ultrastructure of leaves and seeds, in addition to leaf anatomical structure as complementary tools to identifying the *Moringa* species.
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Key words: macro and micromorphology, seed scan, leaf scan, *Moringa*

1. Introduction

Moringa, derived from the vernacular South Indian name; Tamil. The family is one of the fifteen families that produce mustard oil (glucosinolates), and related to other mustard oil plants like Brassicaceae and Caricaceaec, (Chase *et al.*, 1998). The family consists of 10 to12 species belong to the sole genus *Moringa*, (Mohan and Srivastava 1981; Somali *et al.*,1984 and John, 1998). The genus originated from sub-Himalayan tracts of Northern India, distributed worldwide in the tropics and sub-tropic, (Olson, 2002). Species of this gnus is a fast growing drought resistant trees or shrubs. All *Moringa* species are native to India from where they have been introduced into many warm countries in Africa, Arabia, Southeast Asia, South America and the Pacific and Caribbean Islands, (Willis, 1966; Sengupta and Gupta 1970; Verdcourt, 1985; Mabberley, 1990 and Iqbal and Bhanger 2006). Morton (1991) reported that the most common species are *Moringa peregrina* (Forssk) Fiori.; *M. arabica* (Lam.) Pens., *M. zeylanica* Sieb.; *M. stenopetala* Cufod.; *M.borziana* Mattel.; *M. longituba* Engl.; *M. concanensis* Nimmo; *M.ovalifolia* Dinter and Berger.

Its uses being a food source for humans and animals alike, coagulant for water purification, remedy for numerous ailments as well as a source for biofuel production, (Anwar *et al.*, 2007 and Rashid *et al.*, 2008). The leaves and twigs are used as fodder for cattle, sheep, goats, and camels. The flowers are a good source of pollen for honeybees. The immature seeds, which taste like peanuts after frying, are also consumed raw or cooked. The oil of *Moringa* seed is similar to the olive oil and is rich in palmetic, stearic, behmic, and oleic acids, and is used for human consumption, and in cosmetics and soaps. The oil is highly valued by perfumers for its power of absorbing and retaining odors.

Most research efforts are focused mainly on medicine uses (Ezeamuzie *et al.*, 1996), anatomical identification of plant (Jayeola, 2010), moringa anti-viral activity (Okoye *et al.*, 2010). Morphological and anatomical characters of plants have been used by many authors in plant identification (Noraini and Cutler, 2009; Soladoye *et al.*, 2010 and Sharma *et al.*, 2010). Taxonomic identification has been the basis on which plant breeding effort are founded such that diagnostic characters are assigned to specific or varietal parentage.

In the light of the above fact, the present study was conducted to analyze the morphological, anatomical and scanning electron microscope features of leaves and seeds of three species belong to genus *Moringa* aiming to identify the taxonomic relationship between these species.

2. Materials and methods

The Herbarium of Orman Botanical Garden, Ministry of Agriculture, Giza, Egypt during 2012–2013 was consulted to define the available species of genus *Moringa*. The following three species were found *M. oleifera* Lam., *M. stenopetala* (E.G. Baker) Cufod and *M. peregrine* (Forssk.) Fiori. Fresh samples of these species were generously secured and were subjected for the present investigation. Fifteen fresh specimens of the collected species and the same as herbarium specimens were examined and checked. Moreover mature plants were collected during the flowering stage and after seed maturation to define the morphological and anatomical traits and seed scan analyses. The detailed leaf and seed surfaces scan features were examined by using Scanning Electron Microscope (SEM) with different magnifications. Scanning was carried out by JEOL- JSM T 100 Model Scanning Electron Microscope, Central Laboratory, National Information and Documentation Center (NIDOC),

Dokki, Giza, Egypt**.** Descriptive terms for leaf and seed surfaces scan as cited by Murley (1951) and Claugher (1990) were followed. Investigation and identification criteria of the studied species were based on the authentic flora and taxonomic references, among of them; Hedge (1992) and Harley *et al*. (2004). The anatomical practices were done according to Nassar and El- Sahhar (1998).

Numerical analysis (Sneath and Sokal, 1973) was performed using Single Linkage Clustering Technique. The final results of this technique were constructed in a dendrogram representing the level of similarity in which the studied species have been shared.

3. Results and discussion

3.1 Macro and micro-morphological results of leaf and seed

To evaluate the taxonomic relationship between the studied species of genus *Moringa*; *M. oleifera, M. stenopetala* and *M. peregrina*. Morphological and scanning electron microscope (SEM) characters of leaves and seeds surfaces were studied. In addition, the anatomical structure of leaf was considered. The numerical analysis technique using these characters was also performed to facilitate the similarity or dissimilarity between these species.

M. oleifera

Leaves imparipinnate, average 9 leaflet, leaflet shape oboordate, 4.5 x 2.0 cm, emarginate apex, symmetric base, (Table 1). Leaflet upper surface hairy (non glandular, glandular), lower one smooth, stomata on upper epidermis not clear, actinocytic with raised level on lower one, colliculate sculpture of leaflet upper surface, tuberculate-reticulate on lower one (Figure 1). Seeds, brown, round with tan edges, 1.9 x 1.1 cm (Table 2), rough texture, reticulate epidermal cell walls, anticlinal walls raised (4-6 gonal)-straight, outer periclinal walls concave (Figure 2).

M. stenopetala

Leaves imparipinnate, average 7 leaflet, leaflet shape elliptic, 5.0 x 1.9 cm, obtuse apex, symmetric base (Table 1). Leaflet upper surface hairy, lower smooth, stomata on upper and lower epidermis anomocytic with depressed level, rugose sculpture of leaflet surface upper, reticulate-verrucate on lower one (Figure 3). Seeds reminiscent of almonds or pistachios, brown, 2.5 x 1.0 cm, (Table 2) rough texture, reticulate-foveate epidermal cell walls, anticlinal walls raised (5-6 gonal)-straight, outer periclinal walls concave (Figure 4).

M. peregrina

Leaves pinnate with around 3 pairs of leaflet, leaflet shape linear, 0.8 x 0.1 cm, acute apex, symmetric base (Table 1). Leaflet upper surface hairy (non glandular, glandular), lower smooth, stomata on upper epidermis anomocytic with superficial level, not clear on lower one, rugose-tuberculate sculpture of leaflet surface upper, rugose-tuberculate on lower one (Figure 5). Seeds angled, nut-like, white, 2.0 x 1.2 cm (Table 2), smooth texture, colliculate epidermal cell walls, anticlinal walls raised (4-5 gonal)–straight or wave with irregular channel, outer periclinal walls convex (Figure 6).

3.2 Leaf anatomical results

In this part of study the comparative numerical readings were used to describe the anatomical differences of features of the three *Moringa* species; *M.oleifera*, *M.stenopetala* and *M.peregrina*. The anatomical measurements and counts of leaves were shown in (Table 3) and the transverse section of the middle part of the leaves was studied (Figure 7). The data represented that the leaves of *M. peregrina* were thinner (331.1 µ) than the leaves of *M.oleifera* (501.6 µ) and *M. stenopetala* (493.3 µ). Well developed cuticle layer was formed on the surface of leaves of the last two species. The upper and lower epidermis consist of a single layer of rectangular or orbicular cells in *M. olifera* and *M. stenopetala*. While, *M. peregrina* showed barrel shaped swollen epidermal cells with different shape and size. There were many multicellular trichomes on both epidermis. Stomata occur on both epidermal surfaces, on the same level with neighboring cells. Also, stomata cavities were large in leaves of *M. oleifera* plants as compared with the other two species. Mesophyll consists of the palisade and spongy parenchyma (Figure 7). Thickness of leaf mesophyll of *M.stenopetala* (240.7 µ) was significantly thinner than the mesophyll of *M.oleifera* (261.2 µ) (Table 3). Leaf mesophyll of *M.oleifera* consists of 2 layers of elongated palisade cells while, the other two species showed single layer. Palisade cell had many chloroplasts and large intercellular cavities. The thickness of upper the epidermis (9.3 µ) and the lower (8.7 µ) of *M. peregrina* was thinner than those of *M.oleifera* and *M. stenopetala* plants, respectively (13.1, 12.7 and 11.5, 10.7 µ). Solitary vascular bundles surrounded by parenchymatous and orbicular cells. However, palisade parenchyma of *M.oleifera* leaves showed similar thickness, as well as palisade tissue of *M. stenopetala* (Table 3).

Vascular bundles are well developed in the leaves of all studied species and the comparisons could not be held due to different time of sampling and degree of secondary growth that may be occurred. The outcomes of this article confirm the fact that species belong to specific genus have a distinctive anatomical features. This was early reported by Metacalfe and Chalk (1950). They mentioned that, from time to time, anatomists though in general more interested in structure in relation to function than to classification, have made excursions into the realms of taxonomy, and have added some solid contributions to the knowledge

of systematic. In general, however, the work of anatomists has tended to be overlooked or mistrusted by their taxonomic colleagues. The chief reason for this is that anatomists have not always realized the limitations of their mode of investigation and have sometimes drawn conclusions that, to a taxonomist, are obviously highly improbable. Conversely many highly skilled taxonomists have sometimes been unable to assess the value of anatomical investigations. There have been signs in recent years, however, that as taxonomists have learned the value of co-operation with cytologists and geneticists, so they are coming to appreciate the contribution which anatomists can make to their investigations.

Numerical analysis results

Data obtained from the micro and macro morphological characters of leaves and seeds surfaces were analyzed by using a Single Linkage Clustering analysis technique (Sneath and Sokal, 1973). The final results of analysis were represented in a form of dendrogram (Figure 8). The dendrogram shows the level of similarity in which the studied species have been shared, in other words, determining the similarity or dissimilarity distance between these species.

From the illustrated dendrogram (Figure7), it could be stated that the studied species, according to the similarity or dissimilarity distance, split into two main clusters, the first includes *M. oleifera* and *M. stenopetala* which linked together at similarity level of 0.5. The second cluster, which started at similarity level 2.0, included *M. peregrina*. The studied species linked in the main cluster at 2.0 as they are all species belong to the same genus.

Key

Leaflet vary shape

- oboordate shape, emarginate apex, colliculate upper surface, tuberculate-reticulate lower surface, reticulate epidermal cell wall, palisade tissue 2 layers ……..*M. oleifera*
- elliptic shape, obtuse apex, rugose upper surface, reticulate-verrucate lower surface, reticulate-foveate epidermal cell wall, palisade tissue 1 layer ……..….. *M. stenopetala*

Leaflet linear shape

- Linear shape, acute apex, rugose-tuberculate upper and lower surface, colliculate, palisade tissue 1 layer …….. *M.peregrina*

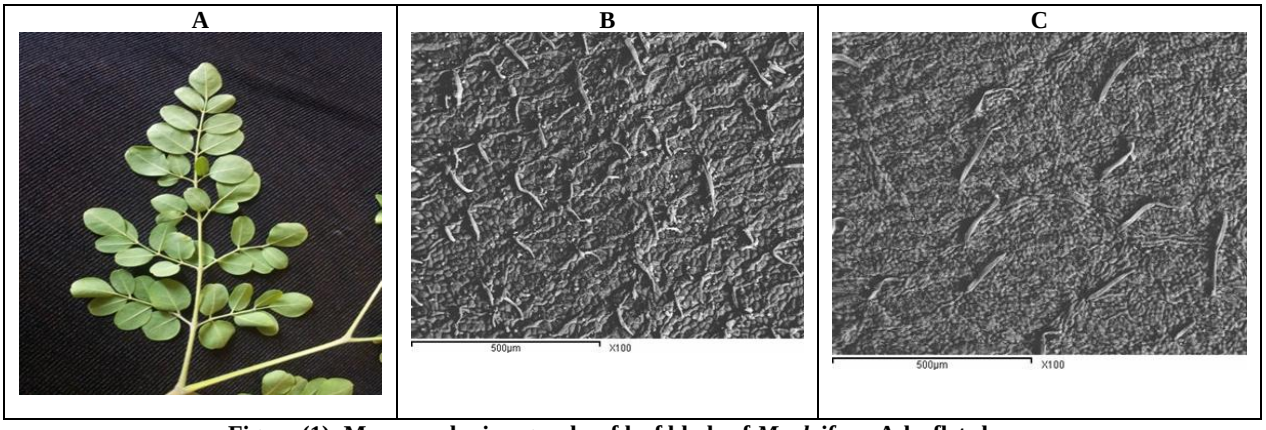


Figure (1): Macro and micrographs of leaf blade of *M. oleifera*. A:leaflet shape, B: upper epidermis surface, C: lower epidermis surface

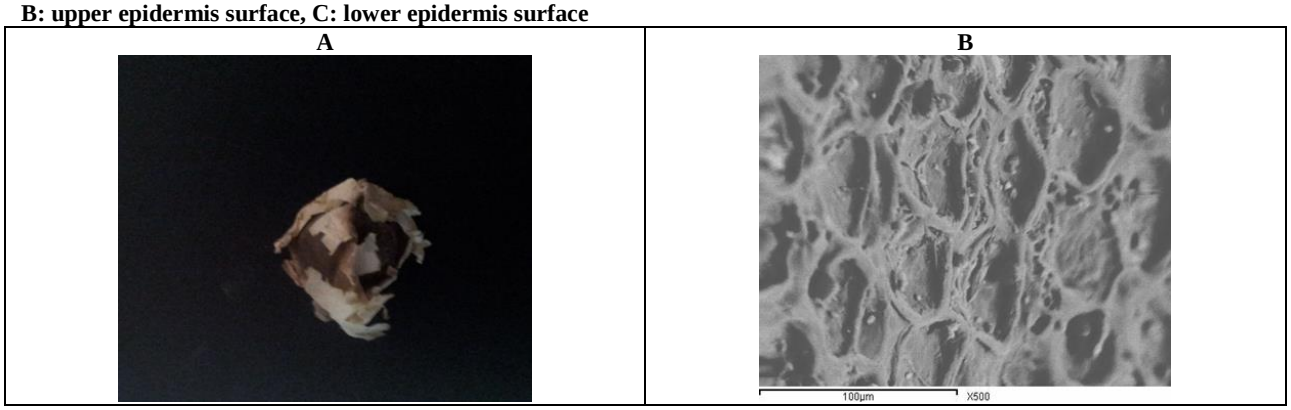


Figure (2): Macro and micrographs of seed of *M. oleifera*. A: Seed shape, B: Seed surface sculpture patterns.

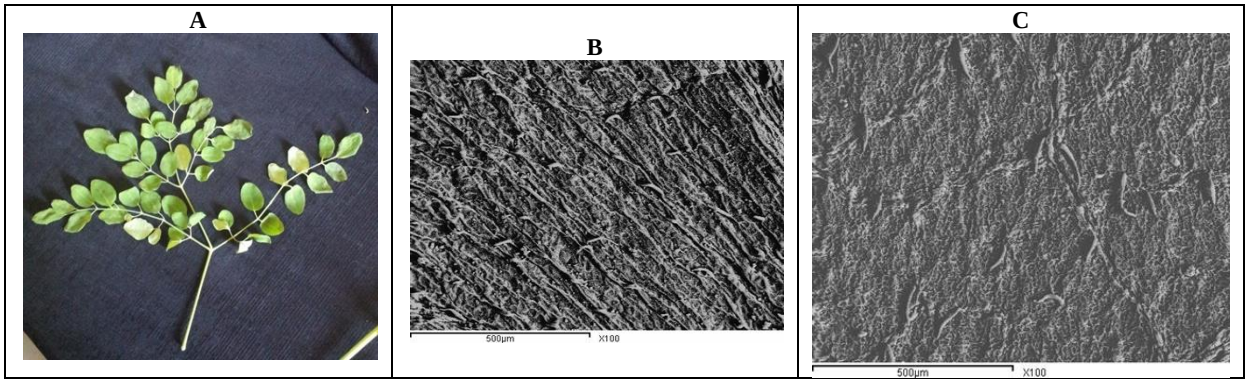


Figure (3): Macro and micrographs of leaf blade of *M. stenopetala*. A:leaflet shape, B: upper epidermis surface, C: lower epidermis surface

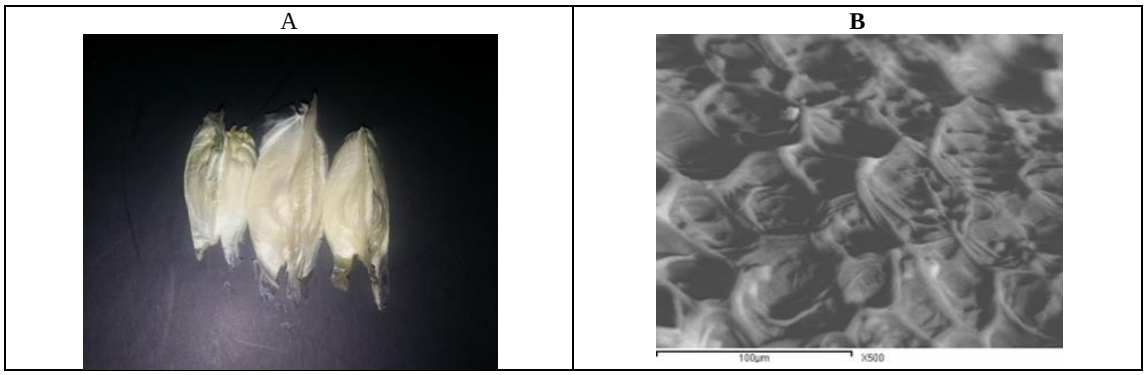


Figure (4): Macro and micrographs of seed of *M. stenopetala*. A: Seed shape, B: Seed surface sculpture patterns.

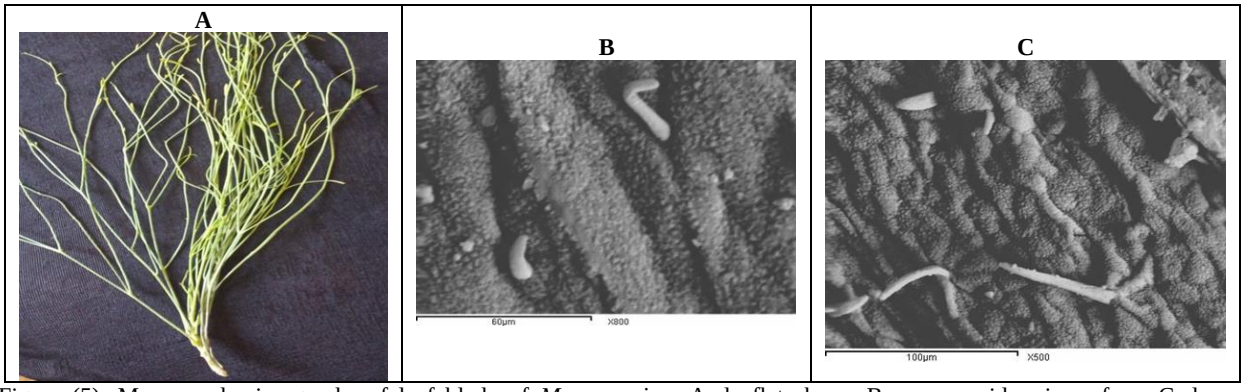


Figure (5): Macro and micrographs of leaf blade of *M. peregrina*. A: leaflet shape, B: upper epidermis surface, C: lower epidermis surface

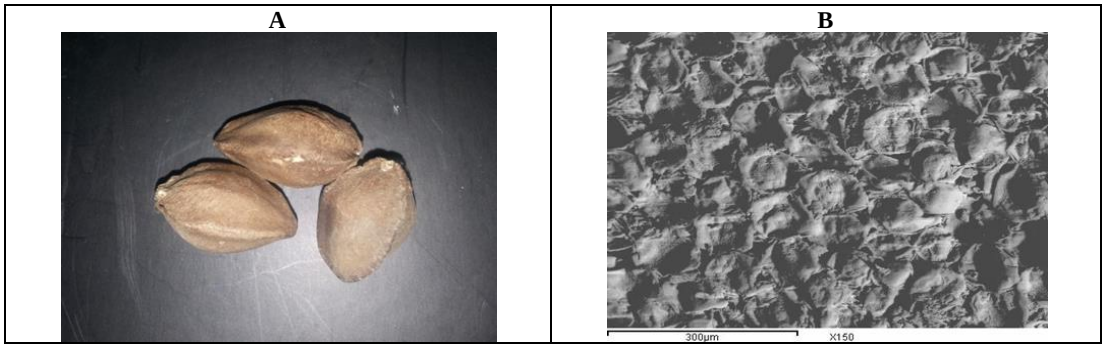


Figure (6): Macro and micrographs of seed of M. peregrina. A: Seed shape, B: Seed surfacesculpture patterns.

Table (1): Macro – micromorphological descriptions and measurements of leaf of the three *Morigna* species.

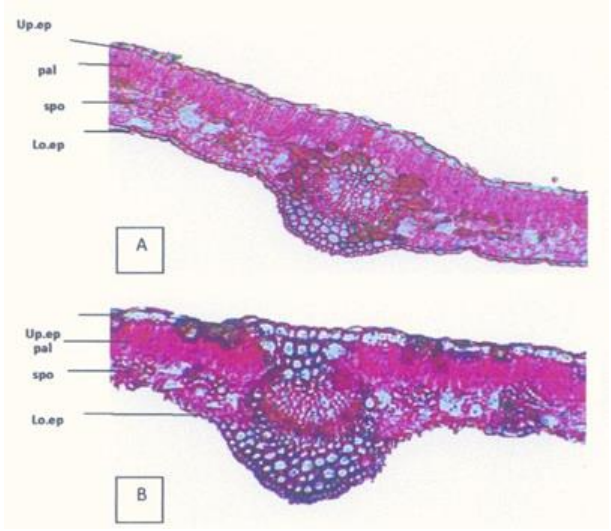
Species Characters	*M. oleifera*	*M. stenopetala*	*M. pregrina*
-Leaf type	imparipinnate	imparipinnate	pinnate
-Upper leaflet texture	Hairy (non glandular, glandular)	Hairy	Hairy (non glandular, glandular)
-Trichomes ornamentation	Smooth	Smooth	Tuberculate
-Lower leaflet texture	Smooth	Smooth	Smooth
-Leaflet shape	Oboordate	Elliptic	Linear
-leaflet apex shape	Emarginate	Obtuse	Acute
-leaflet base shape	Symmetric	Symmetric	Symmetric
-Leaflet length (cm)	4.5	5.0	0.8
-Leaflet width (cm)	2.0	1.9	0.1
-Leaflet number/leaf	9	7	6
-Upper leaf sculpture	Colliculate	Rugose	Rugose-tuberculate
-Lower leaf sculpture	Tuberculate-reticulate	Reticulate-verrucate	Rugose-tuberculate
-Stomata on upper epidermis	Not clear	Anomocytic with depressed level	Anomocytic with superficial level
-Stomata on lower epidermis	Actinocytic with raised level	Anomocytic with depressed level	Not clear

Table (2): Macro–micromorphological descriptions and measurements of seed of the three *Morigna* species

Species Characters	*M. oleifera*	*M. stenopetala*	*M. pregrina*
-Seed shape	Round with tan "frilled" edges	Reminiscent of almonds or pistachios	Angled, nut-like
-Seed color	Brown	Brown	White
-Seed length (cm)	1.9	2.5	2
-Seed width (cm)	1.1	1	1.2
-Seed grade	2.09	2.5	2.4
-Seed texture	Rough	Rough	Smooth
-Epidermal cell walls	Reticulate	Reticulate-foveate	Colliculate
-Anticlinal walls	Raised (4-6 gonal) – straight	Raised (5-6 gonal) - straight	Raised (4-5 gonal) – straight or wave with irregular channel
-Outer periclinal walls	Concave	Concave	Convex

Table (3): Anatomical measurements (µ) of different tissues of leaf lamina of the three studied species of genus *Moringa*

Species Characters	*M. oleifera*	*M. stenopetala*	*M. peregrina*
Av. Main vein thick.	501.6	493.3	331.1
Av. Mesophyll thick.	261.2	240.7	177.6
Av. Palisade thick.	127.4	124.2	78.1
Av. Spongy thick.	134.1	116.7	99.4
Av. Upper epidermis thick	13.1	11.5	9.3
Av. Lower epidermis thick.	12.7	10.7	8.7



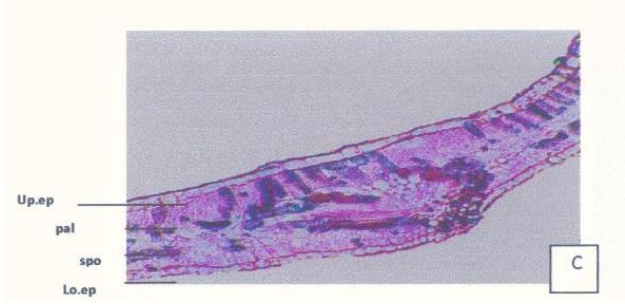


Figure (7): Transverse section on the middle part of the leaf of *Moringa* species
Key: A) *M. oleifera*, B) *M. stenopetala* and C) *M. peregrina*,
Details: up.ep (upper epidermis); pal (palisade tissue); spo (spongy tissue); lo.ep (lower epidermis) X 42

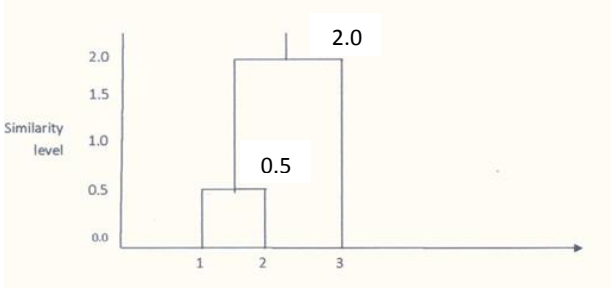


Figure (8): Dendrogram based on macro, micro-morphological and anatomical features of leaf and seed of *Morigna* plant using Single Linkage Clustering technique.
KEY: 1) *M. oleifera*, 2) *M. stenopetala* and 3) *M. peregrina*

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