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Research Article

Molecular Phylogenetics of Moringaceae Martinov with Emphasis on Ethnomedicinal Plant *Moringa oleifera* Lam. Grown in Egypt

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Abstract: The monogeneric family Moringaceae is remarkable for the great diversity of habit and floral morphology within its only genus; *Moringa*. The aim of the present study is to infere the phylogenetic relationships of seven species of *Moringa* using *rbc*L sequence and to find out some of the macro- and micromorphological characters as diagnostic features by which the *Moringa oleifera* can be identified in entire or powdered form. The present study included the investigation of macro- and micromorphological characters of *M. oleifera*. The sequences of the *rbc*L gene for seven taxa of Moringaceae and *Brassica rapa* as outgroup were extracted from the National Center for Biotechnology Information home page. The evolutionary history was inferred by using the Maximum Likelihood method. The morpho-anatomical traits are considered as valuable diagnostic elements for authentication of *M. oleifera*. It is concluded that genus *Moringa* is a monophyletic group. *M. peregrine* and *M. oleifera* are closely related as treated previously in current taxonomic systems.
Keywords: *Moringa oleifera*, Moringaceae, Morphology, Anatomy, Phylogeny, rbcL.

INTRODUCTION

Moringa oleifera Lam. is a multi-purpose plant that has been utilized for a tremendous variety of potential uses scince the ancient Romans, Greeks and Egyptians [1, 2]. It is cultivated as the source of nutritious leaf and fruit vegetables, high-quality of seed oil, pharmacologically active compounds and water clarification agents [3, 4].

Morphological and anatomical characters of plants have been used by many authors in plant identification [5]. The principal characteristics of the leaf venation pattern of a species are genetically fixed providing the basis for using the leaf venation as a taxonomic tool [6, 7]. Leaf epidermal studies have revealed that stomata can provide valuable taxonomic and systematic evidence in both living and fossil plants and also play a significant role in framing hypotheses about early angiosperm evolution [8].

Chloroplast DNA (cpDNA) has been used extensively to infer plant phylogenies at different taxonomic levels [9]. Usage of PCR and sequencing is higher compared with the other selected plant characterization genes such as matK; maturase K [10], as well as provided a good indication of major supra-generic groupings among the angiospermic plant families [11].

Moringaceae as monogeneric family comprises 13 species of *Moringa* from Africa, Madagascar, western Asia and the Indian subcontinent [12]. These species have generally been divided into three groups (bottle-tree clade, tuberous-tree clade and slender-tree clade) or sections (Moringa, Donaldsonia and Dysmoringa), but have also recently been categorised by habit, wood anatomy and phylogenetic trees [1, 13]; Section Donaldsonia or the 'bottle tree' group (radial symmetry) consists of four species of trees *viz*. *M. drouhardii*, *M. hildebrandtii*, *M. ovalifolia* and *M. stenopetala*, with swollen trunks and radially symmetrical flowers [1, 13]. A second group known as the 'tuberous clade', comprising section Dysmoringa (bilateral symmetry with a long hypanthium) and part of section Moringa (bilateral symmetry with a short hypanthium), consists of six species of tuberous shrubs and sarcorhizal trees with thick and fleshy tuberous roots viz. *M. arborea, M. borziana, M. longituba, M. pygmaea, M. rivae* and *M. ruspoliana* [1]. The third group is known as the 'slender tree clade'. It consists of three species of slender-trunked trees *viz*. *M. concanensis, M. oleifera* and *M. peregrina*, with tough roots and bilaterally symmetrical flowers [1].

Despite the great morphological diversity in the family, the monophyly of *Moringa* is supported by numerous distinctive synapomorphies such as gum ducts in the pith and monothecal, bisporangiate anthers [1]. Differing species groups within *Moringa* have been

proposed based on leaf and floral morphology [12], palynology [14], and wood anatomy [13].

The present study was conducted to analyze morpho-anatomical characters of *M. oleifera* with a refrence to phylogenetic relationships of seven species of *Moringa* using *rbc*L sequence data.

MATERIALS AND METHODS

Fresh samples of stem and leaf *M. oleifera* were collected from Botanical Garden, Botany Departement, Facculty of Science, Ain Shams University. The collected speciemens were matched against dried specimens in the Herbaria of Ain Shams University (CAIA), Cairo University (CAI), Flora and Phytotaxonomy at the Agriculture Research Center (CAIM) and Orman Botanical Garden, Cairo.

Macromorphological characters of the whole plant were described from the investigated specimens. Anatomy of stem and lamina were prepared corresponding to [15], leaf peels were prepared according to [16] then, examined under an Olympus Light Microscope (Model HSC 447591). Stomatography was carried following the method described in [17]. Lamina preparations for vein architecture were carried out according to [18]. Photomicrographs were taken using a Reichert Microstar IV microscope at the Plant Taxonomy Research Laboratory, Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt. Descriptive terminology based on [6] and [19].

The sequences of the large subunit of the ribulose-bisphosphate carboxylase gene (rbcL) of seven species of Moringa in addition to *Brassica rapa* as outgroup were extracted from USA National Center for Biotechnology Information home page (Table 1). The phylogeny of the examined species was inferred by using the Maximum Likelihood method and the phylogenetic and molecular evolutionary analyses were conducted using MEGA software version 6 [21].

Table 1: Taxa and gene accession numbers (extracted from NCBI) included in the present study

Sl. No.	Taxa	Gene accession number
1.	*Moringa drouhardii* Jum. -- Ann. Mus. Col. Marseille 1930, Ser. IV. viii. 115. (IK)	JX091929
2.	*M. hildebrandtii* Engl. -- Ann. Ist. Bot. Roma ix. 250. (IK)	JX091930
3.	*M. longituba* Engl. -- Ann. Ist. Bot. Roma ix. 251. (IK)	AF405248
4.	*M. oleifera* Lam. -- Encycl. [J. Lamarck & al.] 1(2): 398. 1785 [1 Aug 1785] (IK)	JX091931
5.	*M. ovalifolia* Dinter & A.Berger -- in Dinter, Neue Pfl. Deutsch-Sudwest-Afr. 45 (1914). (IK)	JX091932
6.	*M. peregrina* C.Chr. -- Dansk Bot. Ark. iv. No. 3, 17 (1922). (IK)	JX091933
7.	*M. rivae* Chiov. -- Boll. Soc. Bot. Ital. 1917, 26. (IK)	JX091935
8.	*Brassica rapa* L. -- Sp. Pl. 2: 666. 1753 [1 May 1753] (IK)	AY167977

RESULTS

Moringa oleifera is a deciduous tree, 15 m in height. With a single main trunk (10–45 cm wide), ending by wide, open and typically umbrella-shaped crown, and covered in a pale-grey and tough bark. The younger stems are finely pubescent and the younger shoots are greenish in colour. Leaves are tri-pinnate, petiolate with tiny stalked glands at the base. Five to 11 main branches (pinnae) that is pulvinate. Each of these branches has 5–11 smaller branches (pinnules). The smaller branches have stalks (petiolules) bearing 3–13 leaflets. Individual leaflet is ovate, elliptic or oblong in shape. However, the terminal leaflet on each petiolule is usually obovate and slightly larger. These leaflets are sparsely tomentose to puberulous when young, glabrous at maturity. Rounded to cuneate bases, entire margins and rounded to emarginate apices.

The inflorescence is axillary panicle. Flowers are white to cream, fragrant and pedicellate. Each flower has five, coloured, elongated sepals and five, spoon-shaped petals with prominent veins and acute apices. Five fertile stamens have waxy yellow or orange anthers, alternate with a row of five staminodes. Filaments are hairy at base. Ovary is oblong, hairy, unilocular, containing numerous ovules with a single slightly hairy style and a minute stigma. The large elongated capsules have nine ribs. Fruits are green and somewhat tomentose when young, pale brown at maturity. They are dehiscent and split open via three valves when fully mature. Seeds are numerous, about 20 in each fruit, sub-globose or slightly three-angled with three papery wings, dark brown or blackish in colour and embedded in the pits of the valves of the fruit.

Concerning the lamina vein architecture (Fig. 1A), the primary vein category is pinnate with 3 basal veins. The secondary vein category festooned brochidodromous with decreasing secondary vein spacing and angles toward base. The intersecondaries are weak. Tertiary veins arise at acute angle to primary vein with inconsistent angle variability, ramified course and random reticulate category. Quaternary vein category is dichotomizing. Areolation is moderately developed. The freely ending ultimate veins one-branched while the marginal ultimate venation looped.

The stomatographic investigation revealed that the abaxial epidermal cells appeared to be irregular shape in surface view with sinuous anticlinal walls. Trichomes are wanting. Stomata are elliptical in shape and paracytic type. The stomatal index is 26.180%. Adaxial epidermal cells are on the same ground plan as in the abaxial epidermis except in; stomata wanting (Fig. 1, B).

Concerning stem and lamina anatomy as in Fig. 1C, stem is terete. Epidermal cells are tangentially or radially elongated with cuticle thick. Trichomes are eglandular and unicellular. Cortex with 2-3 layers of sub-epidermal periderm followed by 7-10 layers of polyhedral parenchyma containing druses, starch grains and lysogenous ducts. Vascular cylinder crowned with isolated patches of past fibers which are stratified into hard fiber patches. Vascular tissue shows a continuous cylinder with angular ringporous vessels. Tyloses are common. Pith is solid of thin walled parenchyma with druses.

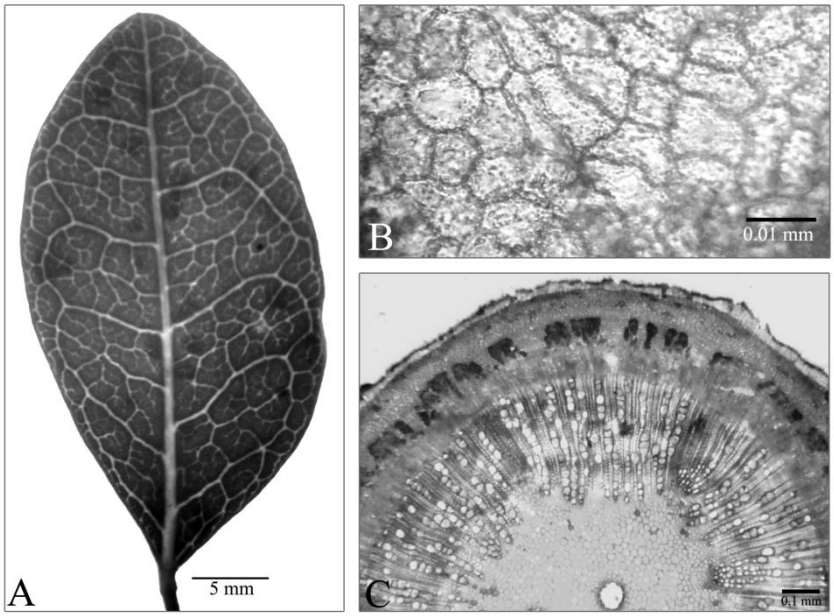

Fig. 1: Photographs of M. oleifera. A. Lamina architecture, B. Lamina adaxial epidermal strip, C. Stem anatomy

DISCUSSION

M. oleifera has main diagnostic elements that can be considered as reference for authentication of drugs extracted from it, including lamina vein architecture and epidermal cell wall pattern [21].

The obtained cladogram (Fig. 2) based on *rbc*L sequence data using Maximum Likelihood method showed that within the studied taxa of Moringaceae there is support for monophylly of *Moringa* this is in accord with [1]. *Moringa rivae* occupied the basal position to all studied species of *Moringa*, followed by *M. hildebrandtii*. *M. oleifera* and *M. peregrina* are closely related as the same as *M. ovalifolia* and *M. longituba*. [1] joined *M. oleifera* and *M. peregrina* in a distinct slender tree clade. In addition, there is a support for a sister-group relationship between *M. oleifera*, *M. peregrina* in one hand and *M. ovalifolia*, *M. longituba* in the other. The basal position of *M. drouhardii* is supported as is the monophyly of *M. peregrine*, *M. oleifera*, *M. ovalifolia* and *M. longituba*.

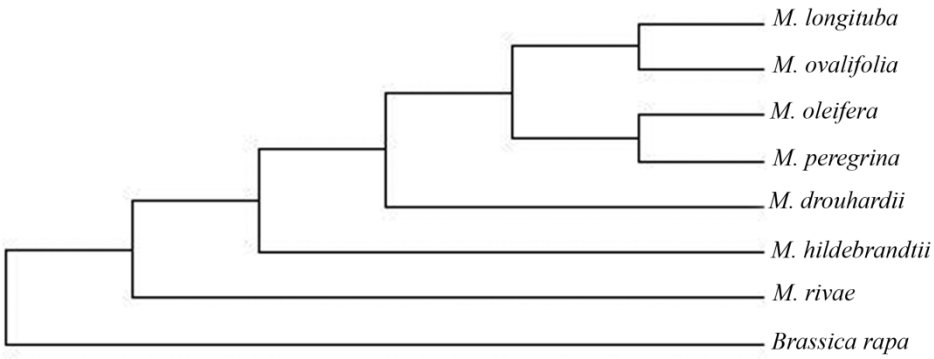

Fig. 2: Maximum likehood tree recovered in the analysis of rcbL sequence data of M. oleifera and related taxa using MEGA version 6 software

CONCLUSION

In conclusion, genus *Moringa* is a monophyletic group. *M. oleifera* and *M. peregrina* are closely related as treated previously in current taxonomic systems.

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