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Morphological Characterization of a New Biotype *Moringa* of Saudi Arabia

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ABSTRAK

Dari total 13 spesies *Moringa*, di Saudi Arabia hanya terdapat dua spesies, yaitu *M. peregrina* dan *M. oleifera*. Kedua spesies ini memiliki kandungan nutrisi dan bahan obat yang tinggi serta mampu bertahan pada iklim kering. Biotipe *Moringa* baru yang berbeda dengan kedua spesies ini telah teramati di daerah Al Bahah, Saudi Arabia. Pada penelitian ini, karakterisasi dari biotipe baru ini telah dilakukan dengan mengamati 22 karakter kualitatif dan kuantitatif dari daun, biji dan bunga. Semua karakter ini kemudian dibandingkan dengan *M. peregrina* dan *M. oleifera* untuk mengetahui hubungan biotipe baru dengan kedua spesies. Hasil pengamatan pada karakter kualitatif menunjukan bahwa biotipe baru ini memiliki 8 karakter (tepi anak daun, warna bunga, bentuk kelopak dan mahkota bunga, perlekatan kelopak bunga, warna dan orientasi kepala sari, dan bentuk dasar bunga) yang sama dengan kedua spesies, satu karakter (distribusi warna merah pada perhiasan bunga) dengan *M. peregrina* saja, tiga karakter (tipe susunan anak daun, kulit biji dan sayap biji) dengan *M. oleifera* saja. Untuk karakter-karakter kuantitatif, hasil pengukuran menunjukan semua karakter pada biotipe baru berada pada posisi antara *M. peregrina* and *M. oleifera*. Berdasarkan hasil ini, kami menyimpulkan bahwa biotipe baru dari *Moringa* ini adalah hibrid hasil persilangan *M. peregrina* dan *M. oleifera*.

Kata kunci: biotipe, bunga, *Moringa peregrina*; *Moringa oleifera*, daun, biji.

INTRODUCTION

Moringa is the only genus of the Moringaceae family. With just 13 species, the genus is one of the most morphologically varied groups of angiosperms. It ranges from very small tuberous shrubs to vast bottle trees and from radial to bilateral floral symmetry (Olson, 2002). All the species are originated from India and Africa (Amaglo, 2010), but then introduced into many tropical and subtropical countries as food, medicinal or ornamental plants. In Saudi Arabia, Migahid (1978) reported that only two species of *Moringa* are present i.e. *M. oleifera* and *M. peregrina*. Due to its edible leaves and high quality seed oil, *M. oleifera* is widely cultivated in the country. For *M. peregrina,* the species is not as famous as the former and is naturally distributed in South and North Hijaz.

Due to their conventional trunk and fibrous roots, *M. oleifera* and *M. peregrina* are classified within slender trees class of Moringaceae (Olson, 2002). Both species are tree with irregular flower, hairy ovary and smaller pollen grains. According to Olson (2002), the main morphological differences between *M. oleifera* and *M. peregrina* are that the former has winged seeds and deciduous

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leaf axes whereas the latter has unwinged seeds and their leaf axes are persistent.

In this study we aim to characterize a new biotype *Moringa* observed in Al Bahah Region, Saudi Arabia. The new biotype has both *M. oleifera* and *M. peregrina* characteristic in its leaflets and seeds. Its leaflets shapes are round, elliptic or oblanceolate, which are combination between leaflets shapes of *M. oleifera* (round) and *M. peregrina* (linear, elliptic or oblanceolate). In addition, while having similar size to those of *M. peregrina*, its seeds bear wings like those of *M. oleifera*.

As both *M. oleifera* and *M. peregrina* are known as the source of nutritious leaf and fruit vegetables, high-quality seed oil and pharmacologically active compounds (Tsaknis, 1998; Saleem and Meinwald, 2000; Jahn, 2001; Padayachee and Baijnath, 2012), detection of genetic diversity is of great value for the improvement of nutritional and medicinal value of these plants. Morphological variation is one indication of genetic variation and may serve as a basis for more detailed molecular studies. Thus in the present study, we morphologically characterized the new biotype *Moringa* by measuring its leaf, flower and seed traits and comparing them with of *M. oleifera* and *M. peregrina* to determine its relation with both species.

MATERIALS AND METHODS

Sample preparation and traits measurement

All leafs, seeds and flowers of the new biotype, *M. oleifera* and *M. peregrina* were collected from Al Bahah Region, Saudi Arabia, where the new biotype was observed (19°45'14.0"N 41°27'28.2"E).

For data presentation simplification, all measured traits were divided into qualitative and quantitative traits (Table 1). These traits are key morphological characteristics of *Moringa* which can be used to differentiate between species (Olson, 2002; Mgendi et al., 2011). For leaf traits measurement, a total of 12 individuals of the new biotype, 11 individuals of *M. peregrina* and 8 individuals of *M. oleifera* were examined in this study. From each individual, one leaf which fully exposed to sun light was selected and photographed. From these photographs, leaflet length and width, leaf area and petiole length were measured using ImageJ software (Schneider et al., 2012). Specific leaf area (SLA) of leaves was measured by dividing leaf area by its dried weight

Table 1. List of leaf, seed and flower traits examined to characterize the new biotype.

Traits	Leaf	Seed	Flower
Qualitative	Pinnate type	Seed cover	Color of perianth
	Leaflets shape	Wing	Petals shape
	leaflets margin		Sepals shape
			Sepals fusion
			Anther color
			Anther orientation
			Receptacle shape
			Distribution of red pigmentation
Quantitative	Leaflet length	Weight	
	Leaflet width	Weight to surface area ratio	
	Leaf area		
	Specific Leaf Area (SLA)		
	Petiole length		

(mm^2/mg). To get constant dried weight of the leaves, the samples were put in the oven at 70° C for 24 hours. For seed traits measurement, a total of 38, 45 and 37 seeds were randomly selected from the new biotype, *M. peregrina*, and *M. oleifera*, respectively. Similar to the leaves, seed surface area was measured using ImageJ software. As not all examined individuals produced flower during the study, minimum of 2 flowers were examined for flower traits measurement.

Data analysis

ANOVA one way was used to compare all quantitative traits between the biotype, *M. peregrina* and *M. oleifera*. The analyses were performed using PASW Statistic 18, release version 18.0.0 (SPSS Inc., 2009, www.spss.com).

RESULTS AND DISCUSSION

Most of the qualitative traits of the new biotype were similar to *M. peregrina* and *M. oleifera*. These similarities were observed for leaflets margin, flower color, petals and sepals shape, sepal fusion, anther color and orientation, and receptacle shape (Table 2 and Figure 1). All three studied samples had entire leaflet margin, white flower, unequal sepals and petals, fussed sepals, orange and centrally-pointed anther, and long tubular receptacle. Three traits of the new biotype namely pinnate type, seed cover and seed wing resembled *M. oleifera* (2 or 3-pinnate leaf, membranous seed cover, and winged seed), while only one trait i.e. distribution of red pigmentation on perianth resembled *M. peregrina*. Furthermore, leaflet shapes of the new biotype (round, elliptic or oblanceolate) were the only trait observed to have mixed characteristic of both *M. peregrina* and *M. oleifera* (Table 2 and Figure 1).

All quantitative traits of the new biotype were found in between *M. peregrina* and *M. oleifera*. The analysis of ANOVA one way revealed that both leaflet length and width of *M. peregrina,* the new biotype and *M. oleifera* was significantly different ($F(2,247) = 39.56$, $p < 0.0001$ and $F(2,247)$

Table 2. Comparison of leaf, seed and flower characteristics between *M. peregrina,* the new biotype and *M. oleifera.*

No	Traits	*M. peregrina*	The new biotype	*M. oleifera*
	Leaf			
1.	Pinnate	2-pinnate	2 or 3-pinnate	2 or 3-pinnate
2.	Leaflets shape	linear, elliptic or oblanceolate	round, elliptic or oblanceolate	Round
3.	Margin	Entire	Entire	Entire
	Seed			
4.	Seed cover	Hard	Membranous	Membranous
5.	Wing	Unwinged	Winged	Winged
	Flower			
6.	Color	White	White	White
7.	Distribution of red pigmentation	along midrib of petals	base up to middle of petals midrib	Absent
8.	Petals	Unequal	Unequal	Unequal
9.	Sepals	Unequal	Unequal	Unequal
10.	Sepals fussion	Fussed	Fussed	Fussed
11.	Anther color	Orange	Orange	Orange
12.	Anther orientation	centrally-pointed	centrally-pointed	centrally-pointed
13.	Receptacle shape	long tubular	long tubular	long tubular

Figure 1 Comparison of leaf, seed and flower of *M. peregrina*, *M. oleifera* and the new biotype. Black lines on photos are1 cm scale

= 175.99, $p < 0.0001$, respectively) (Figure 2). Leaflet length of *M. oleifera* (18.94 ± 4.36 mm) was significantly higher compared to of *M. peregrina* (10.93 ± 3.62 mm, $p < 0.0001$) but not significantly different with the new biotype (17.91 ± 6.37 mm, $p = 0.374$). For leaflet width, the highest value was observed in *M. oleifera* (12.54 ± 3.46 mm) which significantly differed compared to *M. peregrina* (2.72 ± 1.04 mm, $p < 0.0001$) and the new biotype (7.19 ± 3.17 mm, $p < 0.0001$).

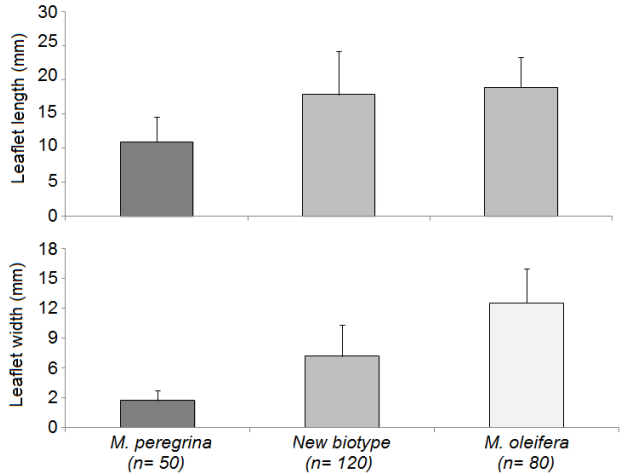

Figure 2. Comparison of leaflet length and width between *Moringa peregrina*, the new biotype and *Moringa oleifera*. Bars with line above them are mean values and their standard deviation and n is number of sample. Different colors of bars indicate statistically significant difference ($p < 0.0001$).

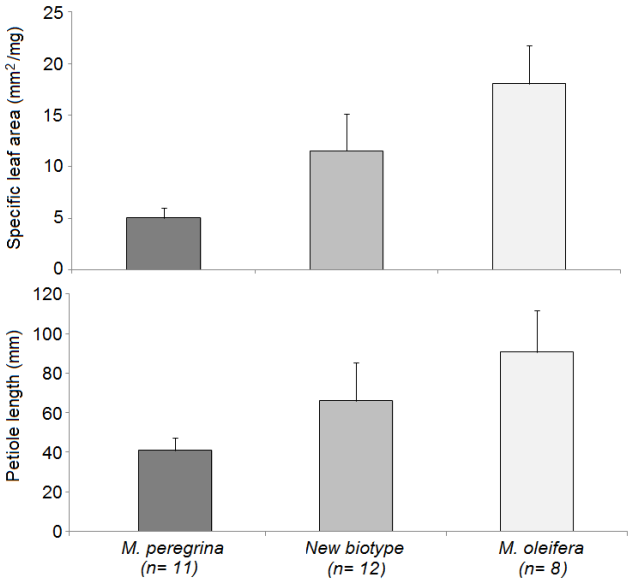

Figure 3. Comparison of leaf area, specific leaf area (SLA) and petiole length between *Moringa peregrina*, the new biotype and *Moringa oleifera*. Bars with line above them are mean values and their standard deviation and n is number of sample. Different colors of bars indicate statistically significant difference ($p < 0.0001$).

Leaf area and specific leaf area (SLA) of *Moringa peregrina*, the new biotype and *Moringa*

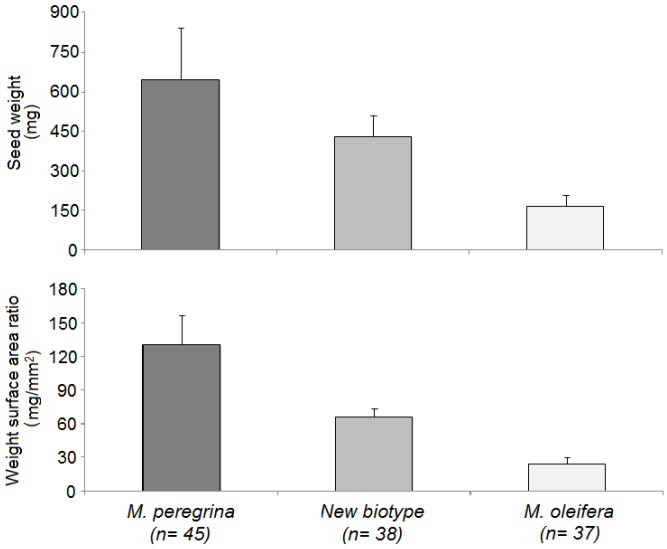

Figure 4. Comparison of seeds weight and weight to surface area ratio between *Moringa peregrina*, the new biotype and *Moringa oleifera*. Bars with line above them are mean values and their standard deviation and n is number of sample. Different colors of bars indicate statistically significant difference ($p < 0.0001$).

oleifera were significantly different (F (2,28) = 19.25, $p < 0.0001$ and F (2,28) = 45.01, $p < 0.0001$, respectively) (Figure 3). Leaf area of *M. oleifera* (19,829.83 ± 9,228.39 mm²) was significantly higher compared to of *M. peregrina* (3,545.47 ± 1,019.62 mm², $p < 0.0001$) and the new biotype (11,440.13 ± 5,156.03 mm², $p < 0.0001$). Similarly, SLA of *M. oleifera* (18.04 ± 3.67 mm) was significantly higher compared to of *M. peregrina* (5.03 ± 0.97 mm, $p < 0.0001$) and the new biotype (11.54 ± 3.59 mm, $p < 0.0001$).

Petiole length of *M. peregrina,* the new biotype and *M. oleifera* was significantly different (F (2,28) = 21.17, $p < 0.0001$) (Figure 3). The longest petiole was observed in *M. oleifera* (90.54 ± 21.25 mm) which significantly differed compared to *M. peregrina* (40.88 ± 6.31 mm, $p < 0.0001$) and the new biotype (65.9 ± 19.25 mm, $p < 0.0001$).

Similar pattern was also showed by seed characteristics. There was significant different of seeds weight and weight to surface area ratio between *M. peregrina,* the new biotype and *M. oleifera* (F (2,117) = 139.47, $p< 0.0001$ and F (2,117) = 384.54, $p< 0.0001$, respectively) (Figure 4). Seed weight of *M. peregrina* (646.22 ± 194.37 mg) was significantly higher compared to of *M. oleifera* (166.22 ± 41.19 mg, $p< 0.0001$) and the new biotype (428.95 ± 80.36 mg, $p< 0.0001$). For seed weight to surface area ratio, *M. peregrina* (130.22 ± 27.04 mg/mm²) had significantly higher value compared to of *M. oleifera* (24.41 ± 5.21 mg/mm², $p< 0.0001$) and the new biotype (65.97 ± 8.24 mg/mm², $p< 0.0001$).

The examination of qualitative morphological traits revealed that the new biotype shared eight characters (leaflets margin, flower color, petals and sepals shape, sepal fusion, anther color and orientation, and receptacle shape) with both *M. peregrina* and *M. oleifera,* one character (distribution of red pigmentation on perianth) with *M. peregrina* only, three characters (pinnate type, seed cover and seed wing) with *M. oleifera*, and none of unshared character. For quantitative traits, all measured characters of the new biotype showed intermediate position between *M. peregrina* and *M. oleifera*. Based on these results, we suggested that the new biotype is a hybrid between *M. peregrina* and *M. oleifera*. Previous studies on plant hybrid identification and characterization showed that most hybrids occupied intermediate position between their parental species, and were additive in their characters which reflected the contribution of both parental species characters (e.g. Takamiya *et al.*, 1999; Horandl & Greilhuber, 2002; Rieseberg *et al.*, 2003; Lin *et al.*, 2010; Hoyo & Tsuyuzaki, 2013; Hodac *et al.*, 2014). Further studies using co-dominant and highly polymorphic molecular markers are needed to test our conclusion. For this purpose, we suggest to use simple sequence repeat (SSR) and single nucleotide polymorphism (SNP). With simple cross-amplification, common loci can be selected and hybridization between involved *Moringa* species could be analyzed.

CONCLUSION

Using 22 qualitative and quantitative morphological markers, the present study was able to characterize the new biotype *Moringa* of

Saudi Arabia. Due to the high number of shared character with both *M. peregrina* and *M. oleifera*, we suggested that the new biotype is a hybrid between the two species. The results of the present study may serve as a basis for future studies on genetic diversity of *Moringa* in Saudi Arabia.

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