Buletin Kebun Raya Vol. 18 No. 2, Juli 2015 [99-110]
e-ISSN: 2460-1519 | p-ISSN: 0125-961X

CHARACTERIZATION OF A NEW BIOTYPE *Moringa* OF SAUDI ARABIA USING RAPD AND ISSR MARKERS

Karakterisasi Biotipe Baru *Moringa* dari Arab Saudi dengan Menggunakan Penanda RAPD dan ISSR

Iyan Robiansyah[1,2*], Ahmed Ramadan[1,3], Magdy A. Al-kordy[1,4], Ahmad Saeed Ghushash[5], and Abdulrahaman S. Hajar[1]

[1] Department of Biological Science, King Abdulaziz University
P.O. Box 80200 Jeddah 21589, Kingdom of Saudi Arabia
[2] Center for Plant Conservation Bogor Botanic Gardens, Indonesian Institute of Sciences (LIPI)
Jl. Ir .H. Juanda 13 Bogor 16003, Indonesia
[3] Agricultural Genetic Engineering Research Institute (AGERI), Agriculture Research Center (ARC)
Giza, Egypt
[4] Genetics and Cytology Department, Genetic Engineering and Biotechnology Division, National Research Center, Dokki, Egypt
[5] Faculty of Arts and Humanities, Albahah University Al Bahah, Kingdom of Saudi Arabia
Email: iyan.robiansyah@lipi.go.id

Abstrak

Moringa peregrina dan *M. oleifera* adalah dua spesies *Moringa* (Kelor) yang tersebar secara alami di Arab Saudi. Kedua spesies ini memiliki sifat tahan kekeringan serta memiliki kandungan nutrisi dan bahan obat yang sangat tinggi. Penelitian keanekaragaman genetik akan sangat berguna untuk meningkatkan kualitas nutrisi dan kandungan bahan obat kedua tanaman ini. Penelitian ini bertujuan mengkarakterisasi biotipe baru dari spesies *Moringa* Arab Saudi yang teramati di daerah Al Bahah. Sebanyak 11 primer RAPD dan 15 primer ISSR digunakan untuk mengkarakterisasi biotipe baru dan membandingkannya dengan *M. peregrina* dan *M. oleifera*. Level polimorfisme yang dihasilkan setiap penanda molekular dihitung dan koefisien Nei diperkirakan untuk mengetahui jarak genetik dari setiap spesies. Level polimorfisme dari RAPD dan ISSR secara berturut-turut adalah 59.7% dan 75%. Analisis dari RAPD dan ISSR menunjukkan bahwa biotipe baru memiliki 53 pita (43.44%) yang sama dengan *M. peregrina* dan *M. oleifera*, 29 pita (23.77%) dengan *M. peregrina* saja, 22 pita (18.03%) dengan *M. oleifera* saja, dan 18 pita (14.75%) unik. Berdasarkan data RAPD, jarak genetik antara *M. oleifera* dan *M. peregrina* adalah 0.59 sedangkan jarak genetik biotipe baru dengan *M. peregrina* dan *M. oleifera* secara berturut-turut adalah 0.41 dan 0.54. Berdasar data ISSR, jarak genetik antara *M. oleifera* dan *M. peregrina* adalah 0.98 sedangkan jarak genetik biotipe baru dengan *M. peregrina* dan *M. oleifera* secara berturut-turut adalah 0.59 dan 0.56.

Kata kunci: Biotipe, ISSR, *Moringa oleifera*, *Moringa peregrina*, RAPD

Abstract

Moringa peregrina and *M. oleifera* are the only *Moringa* (Kelor) species found in Saudi Arabia. Both species are drought resistant and have very high nutritional and medicinal properties. Detection of genetic diversity is of great value for the improvement of nutritional and medicinal value of these plants. The aim of the present

study was to characterize a new biotype *Moringa* observed in Al Bahah Region, Saudi Arabia. We used 11 RAPD and 15 ISSR primers to characterize and compare the new biotype with *M. peregrina* and *M. oleifera*. Level of polymorphism generated by each marker was calculated. We also calculate Nei's coefficient to measure the genetic distance between the studied species. Level of polymorphism generated by RAPD and ISSR was 59.7% and 75%, respectively. RAPD and ISSR primers revealed that the new biotype shared 53 amplicons (43.44%) with both *M. peregrina* and *M. oleifera*, 29 amplicons with *M. peregrina* (2377%), 22 amplicons (18.03%) with *M. oleifera*, and displayed 18 unshared amplicons (14.75%). Based on RAPD data, genetic distance between *M. oleifera* and *M. peregrina* was 0.59, whereas genetic distance between the new biotype and *M. oleifera* and *M. peregrina* was 0.41 and 0.54, respectively. For ISSR data, genetic distance between *M. oleifera* and *M. peregrina* was 0.98, whereas genetic distance between the new biotype and *M. oleifera* and *M. peregrina* was 0.59 and 0.56, respectively.

Keywords: Biotype, ISSR, *Moringa oleifera*, *Moringa peregrina*, RAPD

INTRODUCTION

Moringa (Kelor) is the only genus of the Moringaceae family which is comprised of 13 species. Suggested to have originated from India and Africa (Amaglo *et al*., 2010), all *Moringa* species have been widely introduced into many tropical and subtropical countries including Indonesia as food, medicinal or ornamental plants. In Saudi Arabia, Migahid (1978) reported that only two species of *Moringa* can be found i.e. *M. oleifera* and *M. peregrina*. The former species are widely cultivated in the country for its edible leaves and seed oil while the latter are naturally distributed in South and North Hijaz. Robiansyah *et al*. (2014) stated that seeds and leaves of *M. peregrina* are also edible and contain valuable nutrients for human diet. The species is recently grown as crop plant in Saudi Arabia but to a much lesser extent than *M. oleifera*.

M. oleifera and *M. peregrina* are classified within slender trees class of Moringaceae, which is characterized by a more conventional trunk and tough, fibrous roots (Olson, 2002). The main morphological differences between these two species are that *M. oleifera* has winged seeds and deciduous leaf axes whereas *M. peregrina* has unwinged seeds and their leaf axes are persistent (Olson, 2002). *M. oleifera* is a tree growing up to 15 m in height, usually with a large underground rootstock and a single main trunk. The trunk has pale–gray bark and is generally 10–45 cm wide. Leaves are 25–60 cm long and borne on petioles 4–15 cm long. Individual leaflets have round shape with 10–24 mm long and 5–18 mm wide. Flowers are white to cream, fragrant, 25 mm in diameter and borne on pedicels 12–21 mm long (ICRAF, 2001; Lu and Olson, 2001). For *M. peregrina*, it is an extremely fast growing tree with 5–15 m height, diameter of 20–40 cm, and grayish–green bark. It has 20–70 cm leaves with several tiny leaflets that drop when the leaf matures. The flowers (10–15 mm long) are generally yellowish white to pink, bisexual and harbor insect–pollination characteristics e.g. large, showy, slightly scented, and zygomorphic (Gomaa and Pico, 2011). In this study we aim to characterize a new biotype *Moringa* observed in a plantation in Al Bahah Region, Saudi Arabia. The new biotype has both *M. oleifera* and *M. peregrina* characteristic in its leaflets and seeds (Figure 1). 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).

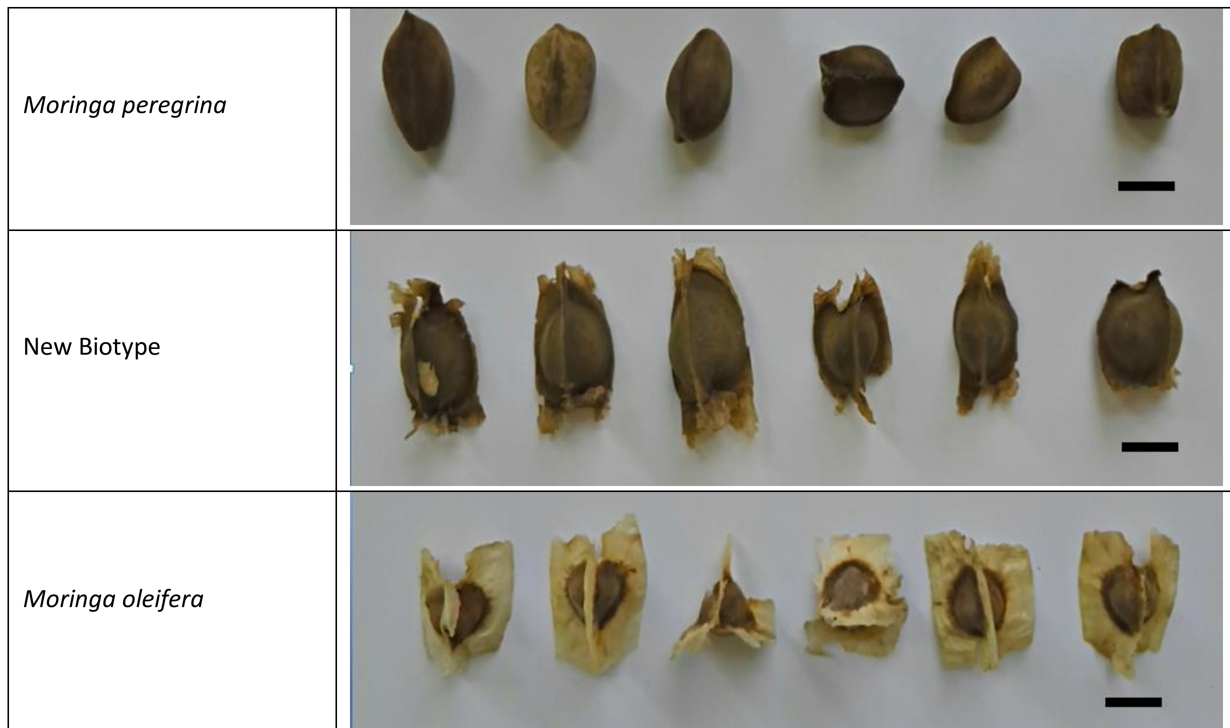

Figure 1. Seed comparison between *Moringa peregrina*, *Moringa oleifera* and the new biotype (Bar = 1 cm).

In addition, while having similar size to those of *M. peregrina*, its seeds bear wings like those of *M. oleifera*. Further information on morphological traits specific to both species and new biotype can be found in Robiansyah *et al*. (forthcoming 2015). The new biotype is suggested as a hybrid between *M. oleifera* and *M. peregrina* possibly as a result of spontaneous pollination and fertilization between the two *Moringa* in the plantation.

Compared to morphological and chemical markers, molecular markers have several advantages in determining genetic diversity. These advantages include free of environmental influence, unlimited in number and easier to analyze (Peleman and van der Voort 2003). Thus in the present study we use Random Amplified Polymorphic DNA (RAPD) and Inter–Simple Sequence Repeat (ISSR) markers to assess the molecular characteristic of the new biotype *Moringa* in comparison with *M. oleifera* and *M. peregrina*. The RAPD and ISSR are among the most commonly used molecular markers in taxonomic and genetic analysis. Both markers are efficient, cost effective and require no prior sequence information. Furthermore, they can produce reliable information on genetic diversity in genetically neutral regions (Karp, 2002). 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; Jhan, 2001; Padayachee and Baijnath, 2012), detection of genetic diversity is of great value for the improvement of nutritional and medicinal value of these plants.

MATERIALS AND METHODS

Plant material and DNA extraction

All plant materials of *M. oleifera*, *M. peregrina* and the new biotype were obtained from the plantation in Al Bahah Region, Saudi Arabia (19°45’14.0”N 41°27’28.2”E). From each species, leaves samples for DNA analysis was taken from one individual as only single individual tree of the first generation new biotype is observed in this locality. Further plant distribution study is needed to assess the presence of the new biotype in other areas. DNA extraction and purification was performed from leaves using GeneJET Plant Genomic DNA Purification

Mini Kit (Thermo Fisher Scientific Inc., 2011). DNA concentration of the samples was estimated by measuring optical density at 260 nm.

RAPD and ISSR PCR amplification

A total of 11 primers and 15 primers from Bioneer Company (South Korea) were used for RAPD and ISSR PCR amplifications, respectively (Table 1). Initial number of RAPD primers was 38 from which 11 primers gave reliable and reproducible results and subsequently were used in the further analysis. Both RAPD and ISSR reactions were performed in a volume of 10 µl. Amplifications were carried out in an automated thermal cycler (Multigene, Labnet International Inc.) programmed for a 5 minutes denaturation step before 40 cycles of 1 minute at 94 °C, 1 minute at 35 °C for RAPD and 40 °C for ISSR, 2 minutes at 72 °C as well as a final elongation cycle of 10 minutes at 72 °C. The reactions were replicated two times to ensure reproducibility of the results.

PCR products were detected using 1% agarose gel electrophoresis in 1X TAE buffer followed by staining with ethidium bromide (0.3 µg/mL). A 1 kb DNA ladder (Genedirex) was used as molecular size marker. Amplicons were visually observed under UV light and photographed using a CCD camera (UVP, UK).

Data analysis

Data were scored as (1) for the presence and (0) for the absence of a given fragment. Only clear and relatively intense fragments on the gel were scored. The number of amplicons generated in the new biotype which was shared with *M. peregrina* and *M. oleifera* was counted to assess their relationship. We also measured the number of monomorphic and polymorphic amplicons produced by each primer of each marker system. Level of polymorphism and Genetic similarity (GS) between the new biotype, *M. peregrina* and *M.oleifera* was measured using POPGENE software package (Yeh et al., 1999). The GS calculation used in the software was based on Nei's coefficien (Nei, 1978). The GS value was then converted to the genetic distance (GD) index using its complement (GD = 1–GS). To estimate the discriminating power of RAPD and ISSR, the polymorphic index content (PIC) was calculated by the formula: $PIC_i = 2f_i\,(1–f_i)$ (Roldán–Ruiz et al., 2000), where PIC_i is the PIC for locus *I* and f_i is the amplified band frequency. The PIC value from all bands of each primer was then averaged to get the PIC value for each primer. The PIC is useful to show the information content of a marker primer per assay.

RESULTS AND DISCUSSION

PCR products and level of polymorphism among different samples

RAPD analysis uses a single random 10–mer primer to randomly amplify target DNA sequences. Polymorphism is caused by sequence variation due to nucleotide deletion, insertion or substitution. We used 11 RAPD primers to screen the new biotype, *M. peregrina* and *M. oleifera* which produced reproducible polymorphic banding pattern. A total of 72 amplicons were scored with a mean number of amplicons per primer was 6.5. An example of amplicons variation detected in the present study is shown in Figure 2. The longest amplified fragment (2510 bp) was observed in primer RAPD–49, whereas the shortest (210 bp) was showed by primer RAPD–34. Primer RAPD–57 produced the highest number of amplicons (9), while primer RAPD–42 and RAPD–48 generated the lowest one (5) (Table 1). The total number of polymorphic amplicons was 43 (59.7% polymorphism) with the average of polymorphic fragments per primer was 3.9 (Table 1). The level of polymorphism for different RAPD primers ranged from 22.2% (form RAPD–57) to 100% (for RAPD–50). Previous studies on genetic diversity of *M. oleifera* have shown that level of polymorphism revealed by RAPD primers was varies across different populations. Some studies found higher polymorphism compared to the result of our study. Mgendi et al. (2010), for example, obtained 89.6% polymorphism when assessing cultivated and non–cultivated *M. oleifera* in Tanzania using 12 RAPD primers. In other studies by Abubakar et al. (2011)

and Ojuederie et al. (2013) on genetic diversity of *M. oleifera* in Nigeria, 74% and 81.5% polymorphism was generated using 24 and 10 RAPD primers, respectively. In addition, Cruz da Silva et al. (2012) found 62% polymorphism on *M. oleifera* of Brazil using 17 RAPD primers. The study by Rufai et al. (2013), however, found only 32.7% polymorphism for *M. oleifera* population in Malaysia using 24 RAPD primers. This level of polymorphism was lower compared to the result obtained by the present study. The relatively smaller result was also shown by study of Saini et al. (2013) when examining genetic diversity of *M. oleifera* in India. Using 17 RAPD primers, they obtained 48.68% polymorphism.

ISSR analysis is based on amplification of inter tandem repeat of short DNA sequences using anchored primers. Due to lack of genetic constraints, these sequences are known to be highly polymorphic even among closely related genotypes (Keightley and Gaffney, 2003). A total of 96 amplicons were generated by 15 ISSR primers used in the present study with a mean number of 6.4 amplicons per primer. An example of amplicons variation detected by ISSR primers is shown in Figure 2. The longest and shortest of ISSR amplified fragments was 2680 bp and 260 bp for primer ISSR–4 and ISSR–15, respectively. The highest number of amplicons (13) was revealed by primer ISSR–4, whereas the lowest number (3) was observed in primer ISSR–5 and ISSR–13 (Table 1). The total number of polymorphic amplicons was 72 (75% polymorphism) with the average of polymorphic fragments per primer was 4.8 (Table 1). The highest level of polimorphism (100%) was revealed by primer ISSR–6, whereas the lowest level (33%) was produced by primer ISSR–2, ISSR–11 and ISSR–15. Level of polymorphism generated by ISSR markers in this study (75%) was higher compared to the result obtained by Saini et al. (2013) on *M. oleifera* in India. Using 6 ISSR primers, they obtained 35 amplicons of which 17 amplicons were polymorphic (48.57%).

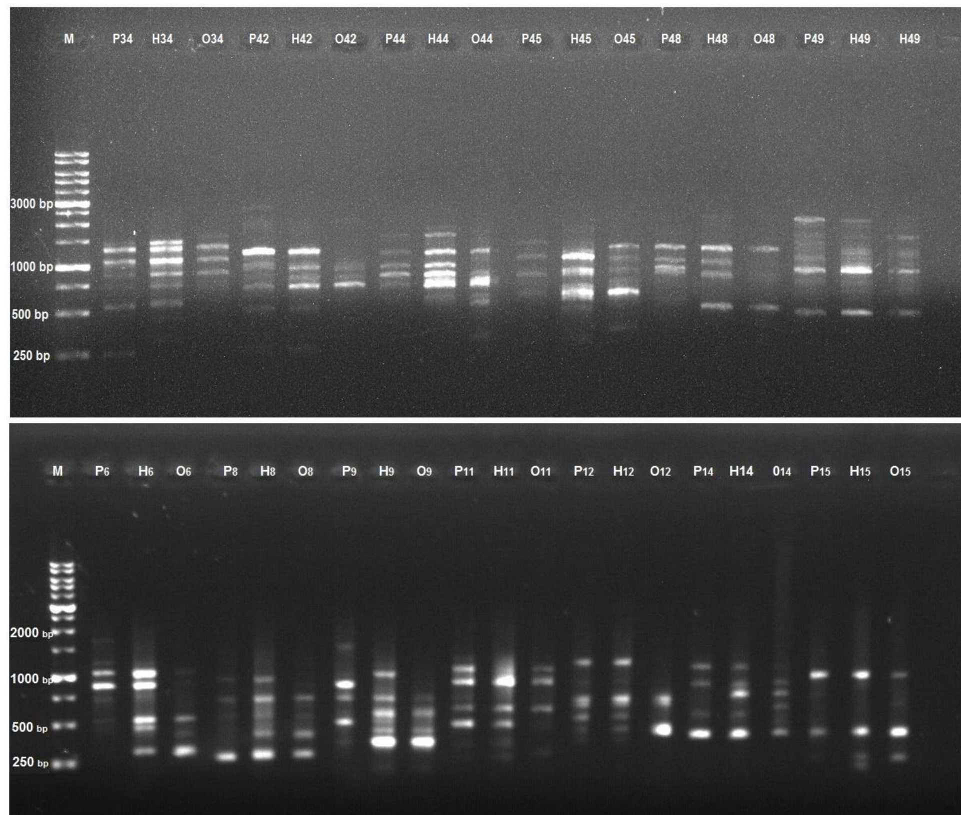

Figure 2. RAPD and ISSR profile of *Moringa peregrina* (P), the new biotype (H) and *Moringa oleifera* (O) for primer RAPD–34, RAPD–42, RAPD–44, RAPD–45, RAPD–48 and RAPD–49 (upper picture), and ISSR–6, ISSR–8, ISSR–9, ISSR–11, ISSR–12, ISSR–14 and ISSR–15 (bottom picture). M is a 1 kb DNA marker.

We used PIC values to identify the capacity of ISSR and RAPD primers in identifying polymorphic amplicons of the studied samples (Table 1). The highest PIC for RAPD primers (0.44) was showed by primer RAPD–50, while the lowest index (0.07) was obtained by primer RAPD–53. For ISSR primers, the highest value (0.44) was observed in primer ISSR–6, whereas the lowest value (0.22) was showed by primer ISSR–2, ISSR–11 and ISSR–15.

The new biotype characterization

In RAPD analysis, the new biotype shared 29 amplicons with both *M. peregrina* and *M. oleifera*, 14 amplicons with *M. peregrina*, 7 amplicons with *M. oleifera*, and displayed 10 unshared amplicons (Table 2). Relatively similar results were also shown by ISSR analysis. The new biotype shared 24 amplicons with both *M. peregrina* and *M. oleifera*, 15 amplicons with *M. peregrina*, 15 amplicons with *M. oleifera*, and had 8 unshared amplicons (Table 2).

We measured Nei's coefficient and its genetic distance complement to further examine the genetic relationship between the new biotype, *M. peregrina* and *M. oleifera*. Based on RAPD data, genetic distance between *M. oleifera* and *M. peregrina* was 0.59. The genetic distance between the new biotype and *M. oleifera* and *M. peregrina* was 0.41 and 0.54, respectively, and both genetic distances were less than the maximum genetic distance between the two species (Table 3). For ISSR data, genetic distance

Table 1. List of RAPD[a] and ISSR[b] primers, total number of amplicons, polymorphic amplicons, percentage of polymorphism, PIC value, and primer sequence used in this study.

Primer	No. amplicons	No. polymorphic amplicons	% polimorphism	PIC	Sequence
RAPD					
RAPD–34	7	5	71.4	0.32	TGCTCTGCCC
RAPD–42	5	3	60.0	0.27	CTCACCGTCC
RAPD–44	6	3	50.0	0.22	CCGCATCTAC
RAPD–45	6	3	50.0	0.22	GTGAGGTGTC
RAPD–48	5	4	80.0	0.36	TGTCTGGGTG
RAPD–49	6	4	66.7	0.30	GTTGCCAGCC
RAPD–50	8	8	100	0.44	ACACACGCTG
RAPD–52	8	6	75.0	0.33	GTCAGAGTCC
RAPD–53	6	2	33.3	0.07	CCAGGAGGAC
RAPD–54	6	3	50.0	0.22	AGGCTGTGCT
RAPD–57	9	2	22.2	0.10	CCGACAAACC
Total	72	43	59.7		
ISSR					
ISSR–1	8	7	87.5	0.39	(CA)6GG
ISSR–2	6	3	50.0	0.22	(CT)8TG
ISSR–3	8	6	75.0	0.33	(GTG)3GC
ISSR–4	13	10	76.9	0.34	(CAC)3GC
ISSR–5	3	2	66.7	0.30	(CTC)3GC
ISSR–6	6	6	100	0.44	(CT)8GC
ISSR–7	8	7	87.5	0.39	(GT)6CC
ISSR–8	5	3	60.0	0.27	(GT)6GG
ISSR–9	8	7	87.5	0.39	(GA)6CC
ISSR–10	10	8	80.0	0.36	(CA)6AG
ISSR–11	4	2	50.0	0.22	(CT)8AC
ISSR–12	4	3	75.0	0.33	(CA)6GG
ISSR–13	3	2	66.7	0.30	(GAG)3GC
ISSR–14	6	4	66.7	0.30	(CA)6AC
ISSR–15	4	2	50.0	0.22	(CA)6GT
Total	96	72	75.0		

[a] RAPD: Random amplified polymorphic DNA, [b] ISSR: Inter–simple sequence repeat

between *M. oleifera* and *M. peregrina* was 0.98. The genetic distance between the new biotype and *M. oleifera* and *M. peregrina* was 0.56 and 0.59, respectively, and both genetic distances were also less than the maximum genetic distance between the two species (Table 3).

Both RAPD and ISSR analysis showed that the new biotype shared most of the amplicons with *M. peregrina* and *M. oleifera* (85.25% in total), indicating the influence of both species to genotype of the new biotype. Furthermore, the genetic distance data showed that position the new biotype is in between *M. peregrina* and *M. oleifera.* Based on these shared amplicons and genetic distance analysis results, we suggest that the new biotype is a hybrid crossbreed between *M. peregrina* and *M. oleifera.* These results are also in line with the results of morphological characteristics study by Robiansyah et al. (forthcoming 2015) which showed that all quantitative traits of the new biotype were found in between *M. peregrina* and *M. oleifera*. Previous studies reported that most of hybrids occupied intermediate position between their parental species, and were additive in their characters reflecting the contribution of both parental species characters (e.g. Takamiya et al., 1999; Horandl and Greilhuber, 2002; Rieseberg et al., 2003; Hoyo and. Tsuyuzaki, 2013; Hodac et al. 2014). Further studies using codominant and highly polymorphic DNA markers (e.g. SSR and SNP) are needed to test our conclusion. With simple cross–amplification, common loci can be selected and hybridization between involved *Moringa* species could be analyzed.

Table 2. Number (and percentage) of shared and unshared amplicons for the new biotype.

Marker type	Amplicons shared with *M. peregrina*	Amplicons shared with *M. oleifera*	Amplicons shared with both species	Amplicons for new biotype only
RAPD[a]	14 (23.33%)	7 (11.67%)	29 (48.33%)	10 (16.67%)
ISSR[b]	15 (24.19%)	15 (24.19%)	24 (38.71%)	8 (12.90%)
Total	29 (23.77%)	22 (18.03%)	53 (43.44%)	18 (14.75%)

[a] RAPD: Random amplified polymorphic DNA, [b] ISSR: Inter–simple sequence repeat

Species-specific molecular markers

The total number of species-specific markers obtained across all studied samples was as high as 59, 21 from RAPDs and 38 from ISSRs (Table 4). Primer RAPD–50 scored the highest number of species-specific markers (5) for RAPD analysis, whereas no specific markers were detected for primer RAPD–42 and RAPD–44. For an individual sample, the highest number of species-specific markers from RAPDs was 10 for the new biotype, whereas *M. peregrina* and *M. oleifera* had specific markers of 6 and 5, respectively. For ISSR analysis, primer ISSR–4 scored the highest number of species-specific markers (9), while no specific markers were detected for ISSR–11, ISSR–12 and ISSR–13. The highest and lowest number of species-specific markers for an individual sample from ISSR analysis was 16 and 8 for *M. peregrina* and the new biotype, respectively. Due to their high percentage of polymorphism, high PIC value and high number of species-specific markers, the use of RAPD–50, ISSR–4 and ISSR–6 primer is highly recommended for further study on *Moringa* genetic diversity.

Comparison between marker systems

In the present study, ISSR marker systems were more efficient compared to RAPD in characterizing the new biotype *Moringa*. The ISSR method showed higher polymorphism, wider product size range as well as higher species-specific marker detection compared to the RAPD. Our results were in agreement with results obtained by Saini et al. (2013) who showed higher ISSR than RAPD diversity within eight Indian cultivars of *M. oleifera*. The higher effectiveness of ISSR compared to RAPD for plant identification has also been reported in numerous plant species, including peanut (Raina et al., 2001), rice (Qian et al., 2001), chickpea (Chowdhury et al., 2002), barley (Fernandez

et al., 2002), sugarcane (Da Costa et al., 2011), pepper (Thul et al., 2012) and *Miscanthus* (Cichorz et al, 2014). The distinction between RAPD and ISSR marker systems may be attributed to the nature of primer sequences and genome regions they amplified. While RAPD bands may be associated with specific function, the ISSR bands are not likely to be under functional constraints leading to rapid evolution and thus higher polymorphism (Esselman et al., 1999). In addition, ISSR primers amplify microsatellite regions, which are known to have high

Table 3. Genetic distance between *M. peregrina*, *M. oleifera* and the new biotype based on RAPD[a] (upper part) and ISSR[b] (down part) data.

	M. peregrina	*M. oleifera*	New biotype
M. peregrina	-	0.59	0.41
M. oleifera	0.98	-	0.54
New biotype	0.59	0.56	-

[a] RAPD: Random amplified polymorphic DNA, [b] ISSR: Inter–simple sequence repeat

Table 4. List of positive and negative species-specific markers of *M. peregrina,* the new biotype and *M. oleifera* based on RAPD[a] and ISSR[b] analysis.

Primer	Number (and MW[c] in bp) of species-specific markers			Total
	M. peregrina	New biotype	*M. oleifera*	
RAPD				
RAPD–34	2 (530, 240)	2 (970, 820)	-	4
RAPD–42	-	-	-	-
RAPD–44	-	-	-	-
RAPD–45	-	1 (530)	1 (870)	2
RAPD–48	1 (1120)	-		1
RAPD–49	1 (1280)	-	1 (1870)	2
RAPD–50	2 (920, 450)	2 (530, 250)	1 (1120)	5
RAPD–52	-	3 (1190, 990, 460)	1 (650)	4
RAPD–53	-	1 (580)	-	1
RAPD–54	-	1 (1300)	-	1
RAPD–57	-	-	1 (900)	1
Total	6	10	5	21
ISSR				
ISSR–1	2 (986, 526)	-	2 (490, 430)	4
ISSR–2	1 (1500)	-	1 (880)	2
ISSR–3	1 (1270)	1 (1010)	2 (840, 730)	4
ISSR–4	6 (2680, 2280, 1980, 1600, 680, 550)	2 (750, 360)	1 (620)	9
ISSR–5	1 (750)	-	-	1
ISSR–6	-	1 (460)	1 (410)	2
ISSR–7	1 (980)	-	3 (1390, 780, 400)	4
ISSR–8	-	1 (590)	-	1
ISSR–9	3 (1630, 930, 510)	1 (1110)	-	4
ISSR–10	1 (500)	1 (860)	3 (1760, 1330, 610)	5
ISSR–11	-	-	-	-
ISSR–12	-	-	-	-
ISSR–13	-	-	-	-
ISSR–14	-	-	1 (670)	1
ISSR–15	-	1 (260)		1
Total	16	8	14	38

[a] RAPD: Random amplified polymorphic DNA, [b] ISSR: Inter–simple sequence repeat, [c] MW: Molecular weight

level of polymorphism due to DNA polymerase slippage and un–equal crossing over during DNA replication (Da Costa *et al.,* 2011).

CONCLUSIONS

Using 11 RAPD and 15 ISSR primers, the present study was able to characterize the new biotype *Moringa* of Saudi Arabia. In general, ISSR marker systems were more resourceful compared to RAPD in characterizing the new biotype. The results revealed that the new biotype shared most of the amplicons with *M. peregrina* and *M. oleifera*, indicating the influence of both species to genotype of the new biotype. Furthermore, the genetic distance data showed that position the new biotype is in between *M. peregrina* and *M. oleifera.* The use of highly polymorphic DNA markers is needed to further test the relationship of the new biotype with both species. The results of the present study may serve as a basis for future studies on genetic diversity of *Moringa* in Saudi Arabia.

REFERENCES

Abubakar, B.Y., R. Wusirika, S. Muazu, A.U. Khan and A.K. Adamu. 2011. Detection of genetic variability using random amplified polymorphic DNA marker in some accession of *Moringa oleifera* Lam. from Northern Nigeria. *International Journal of Botany* 7 (3): 237–242.

Amaglo, N.K., R.N. Bennett, R.B. Lo Curto, E.A.S. Rosa and V. Lo Turco. 2010. Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L., grown in Ghana. *Food Chemistry* 122 (4): 1047–1054.

Chowdhury, M.A., B. Vandenberg and T. Warkentin. 2002. Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L.). *Euphytica* 127 (3): 317–325.

Cichorz, S., M. Goska and A. Litwiniec. 2014. *Miscanthus*: Genetic Diversity and Genotype Identification Using ISSR and RAPD Markers. *Molecular Biotechnology* 56 (10): 911–924.

Cruz Da Silva, A.V., A.R. Ferreira Dos Santos, A.S. Ledo, R.B. Feitosa, C.S. Alameida, G. Melo Da Silva and M.S.A. Rangel. 2012. *Moringa* genetic diversity from germplasm bank using RAPD markers. *Tropical and subtropical agroecosystems* 15 (1): 31–39.

Da Costa, M.L.M., L.L.B. Amorim, A.V.C. Onofre, L.J.O.T. De Melo, M.B. De Oliveira, R. De Carvalho and A.M. Benko–Iseppon. 2011. Assessment of genetic diversity in contrasting sugarcane varieties using inter–simple sequence repeat (ISSR) markers. *American Journal of Plant Sciences* 2 (3): 425–432.

Esselman, E.J., L. Jianqiang, D.J. Crawford, J.L. Windus and A.D. Wolfe. 1999. Clonal diversity in the rare *Calamagrostis porteri ssp. insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers. *Molecular Ecology* 8(3): 443–451.

Fernandez, M.E., A.M. Figuireas and C. Benito. 2002. The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. *Theoretical and Applied Genetics* 104 (5): 845–851.

Gomaa, N.H. and X.F. Pico. 2011. Seed germination, seedling traits, and seed bank of the tree *Moringa peregrina* (Moringaceae) in a hyper–arid environment. *American Journal of Botany* 98 (6): 1024–1030.

Hodac, L., A.P. Scheben, D. Hojsgaard, O. Paun and E. Horandl. 2014. ITS Polymorphisms Shed Light on Hybrid Evolution in Apomictic Plants: A Case Study on the *Ranunculus auricomus* Complex. *PLOS ONE* 9(7) e103003.

Horandl, E. and J. Greilhuber. 2002. Diploid and autotetraploid sexuals and their relationships to apomicts in the *Ranunculus cassubicus* group: insights from DNA content and isozyme variation. *Plant Systematics and Evolution* 234 (1): 85–100.

Hoyo, Y. and S. Tsuyuzaki. 2013. Characteristics of leaf shapes among two parental *Drosera* species and a hybrid examined by canonical discriminant analysis and a hierarchical Bayesian model. *American Journal of Botany* 100 (5): 817–823.

ICRAF. 2001. Agroforestree (AFT) Database. World Agroforestry Centre (ICRAF), Nairobi, Kenya. http://www.icraf.cgiar.org/Sites/TreeDBS/aft.asp (accessed 3 December 2014).

Jahn, S.A. 2001. Drinking water from Chinese rivers: challenges of clarification. *Journal of Water Supply: Research and Technology* 50 (1): 15–27.

Karp, A. 2002. The new genetic era: will it help us in managing genetic diversity?. In: Engels, J.M.M., V.R. Rao, A.H.D. Brown and M.T. Jackson (eds) *Managing plant genetic diversity* (pp 43–56). CAB Publishing, Wallingford.

Keightley, P.D. and D.J. Gaffney. 2003. Functional constraints and frequency of deleterious mutations in noncoding DNA of rodents. *Proceedings of The National Academy of Sciences of The United States of America* 100 (23): 13402–13406.

Lu, L. and M. Olson. 2001. Moringaceae. In: Wu, Z–Y. and P. Raven (eds.) *Flora of China, Volume 8: Brassicaceae through Saxifragaceae*. Missouri Botanical Garden Press, St. Louis, Missouri.

Mgendi, M.G., M.K. Manoko and A.M. Nyomora. 2010. Genetic diversity between cultivated and non–cultivated *Moringa oleifera* Lam. provenances assessed by RAPD markers. *Journal of Molecular Cell Biology* 8(2): 95–102.

Migahid, A.M. 1978. *Flora of Saudi Arabia Volume 1 Dicotyledon* (p.101). Riyadh University Publication, Riyadh.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89 (3):583–590.

Ojeuderie, O.B., D.O. Igwe, S.I. Okuofu and B. Faloye. 2013. Assesment of genetic diveristy in some *Moringa oleifera* Lam. Landraces from western Nigeria using RAPD markers. *African Journal of Plant Science and Biotechnology* 7(1): 15–20

Olson, M.E. 2002. Combining data from DNA sequences and morphology for a phylogeny of Moringaceae (Brassicales). *Systematic Botany* 27(1): 55–73.

Padayachee, B. and H. Baijnath. 2012. An overview of the medicinal importance of Moringaceae. *Journal of Medicinal Plants Research* 6(48): 5831–5839.

Peleman, J.D. and J.R. van der Voort. 2003. The challenges in Marker Assisted Breeding. In: van Hintum, T.J.L., A. Lebeda, D. Pink and J.W. Schut (eds) *Eucarpia leafy vegetables*. Center for Genetic Resources, The Netherlands.

Qian, W., S. Ge and D.Y. Hong. 2001. Genetic variation within and among populations of wild rice *Oryza granulate* from China detected by RAPD and ISSR markers. *Theoretical and Applied Genetics* 102 (2): 440–449.

Raina, S.N., V. Rani, T. Kojima, Y. Ogihara, K.P. Singh and R.M. Devarumath. 2001. RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species. *Genome* 44 (5): 763–772.

Rieseberg, L.H., O. Raymond, D.M. Rosenthal, Z. Lai, K. Livingstone, T. Nakazato, J.L. Durphy, A.E. Schwarzbach, L.A. Donovan and C. Lexer. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* 301 (5637): 1211–1216.

Robiansyah, I., Ghushash, A.S. and Hajar, A.S. (forthcoming 2015). Morphological characterization of a new biotype Moringa of Saudi Arabia. Jurnal Biologi Papua 7(2).

Robiansyah, I., Hajar, A.S., Al–kordy, M.A. and Ramadan, A., 2014. Current status of economically important plant *Moringa peregrina* (Forrsk.) Fiori in Saudi Arabia: a review. *International Journal of Theoretical & Applied Sciences* 6 (1): 79–86.

Roldán–Ruiz, I., Dendauw, J., Van Bockstaele, E., Depicker, A. and De Loose, M. 2000. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). *Molecular Breeding* 6 (2): 125–134.

Rufai, S., M.M. Hanafi, M.Y. Rafii, S. Ahmad, I.W. Arolu and J. Ferdous. 2013. Genetic Dissection of New Genotypes of Drumstick Tree (*Moringa oleifera* Lam.) Using Random Amplified Polymorphic DNA Marker. *BioMed Research International*. Doi: http://dx.doi.org/10.1155/2013/604598

Saini, R.K., K.R. Saad, G.A. Ravishankar, P. Giridhar and N.P. Shetty. 2013. Genetic diversity of commercially grown *Moringa oleifera* Lam. cultivars from India by RAPD, ISSR and cytochrome P450–based markers. *Plant Systematics and Evolution* 299 (7): 1205–1213.

Saleem, R. and J. Meinwald. 2000. Synthesis of novel hypotensive aromatic thiocarbamate glycosides. *Journal of the Chemical Society, Perkin Transactions* 1 (3): 391–394.

Takamiya, M., C. Takaoka and N. Ohta. 1999. Cytological and reproductive studies on Japanese *Diplazium* (Woodsiaceae; Pteridophyta): apomictic reproduction in *Diplazium* with evergreen bi- to tripinnate leaves. *Journal of Plant Research* 112 (4): 419–436.

Thul, S.T., M.P. Darokar, A.K. Shasany and S.P.S. Khanuja. 2012. Molecular profiling for genetic variability in *Capsicum* species based on ISSR and RAPD markers. *Molecular Biotechnology* 51 (2): 137–147.

Tsaknis, J. 1998. Characterization of *Moringa peregrina* Arabian seed oil. *Grasas Aceites* 49 (2): 170–176.

Yeh, F.C., Yang, R.C. and Boyle, T. 1999. *POPGENE version 1.32: Microsoft windows based freeware for population genetic analysis, Quick User Guide*. Center for International Forestry Research, University of Alberta, Canada.