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Molecular Marker Analysis Tools as A Cornerstone for the Phylogenetic Analysis of *Hibiscus* Species in Egypt

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Abstract

Various *Hibiscus* species such as *Hibiscus rosa sinensis linn*, *Hibiscus malvaviscus arboreus*, *Althaea rosea*, *Hibiscus rosasinensis* sp., *Hibiscus schizopetalus*, *Hibiscus china rosa*, *Hibiscus sabdariffa L.* and *Hibiscus cannabinus L.* (kenaf) were collected from Antoniades Gardens in Egypt. These species were very similar in the morphology of the flowers and leaves, thus morphological markers traits use to identify these different *Hibiscus* species is limited and less accurate because they are strongly influenced by environmental conditions. Therefore, using molecular markers of DNA for plants is considered to be more accurate than morphological markers. In this study we used eight primers from (RAPD), five (ISSR) primers, and four (SSR) primers for the analysis on these species of *Hibiscus*. The results showed for all primers were as follows; the total number of the polymorphic fragments of RAPD, ISSR and SSR were 89, 72, and 15; respectively, with an average polymorphism about 97.75%, 98.61%, and 100%, respectively. Moreover, the dendrogram for the phylogenetic tree analysis of RAPD was divided into two main clusters, the first cluster has C7 (*Hibiscus sabdariffa L*), and the second cluster comprised the other species. For ISSR phylogenetic analysis, the dendrogram was divided into two main major clusters, the first cluster had C3 (*Althea rosea*), C7 (*Hibiscus sabdariffa L.*), and C8 (*Hibiscus cannabinus*) and the second cluster comprised the other species. Whereas for SSR phylogenetic analysis, the dendrogram was divided into two main major clusters, the first cluster had C2 (*Hibiscus malvaviscus arboreus*), and the second group comprised the other species.

Keywords: *Hibiscus*, RAPD, ISSR, SSR.

Introduction

The *Hibiscus* genus belongs to the family Malvaceae (mallow family). This family of flowering plants contains over 200 genera with close up to 2300 species. It is commonly found throughout the tropics and subtropical areas. It is well known as an ornamental plant because of the beauty and variety of its flowers. The *Hibiscus* genus consists of 300 species with different growth characteristics ranging from small plants, shrubs, perennial crops, and annual crops (Prasad, 2014). The *Hibiscus* genus has a great variation of morphological characters such as flower colours and sizes, and the leaf shapes, its flower morphology can be used as a morphological marker to identify the genetic relation (Kadve et al., 2012). In Egypt, *Hibiscus* grows very well during the whole year. The flowers are large (generally red in the original varieties), firm, but generally lack any scent. Numerous varieties, cultivars and hybrids are available, with flower colours ranging from white through yellow and orange to scarlet and shades of pink, with both single and double sets of petals (Prasad, 2014). The most important species of the Hibiscus genus are *Hibiscus rosasinensis-linn*, *Hibiscus malvaviscus arboreus*, *Althea-rosea*, *Hibiscus rosasinensis sp*, *Hibiscus schizopetalus*, *Hibiscus china rosa*, *Hibiscus sabdariffa L.*, and *Hibiscus cannabinus L*. (kenaf). *Hibiscus rosa-sinensis* is a species that contains many flowering plants in the family Malvaceae. It is characterized by being an evergreen shrub or small tree growing 2.5–5 m (8–16 ft) tall and 1.5–3 m (5–10 ft) wide, with glossy leaves and solitary, brilliant colour flowers in summer and autumn. It is widely grown as an ornamental and medicinal plant throughout the tropics and subtropics. *Hibiscus rosa-sinensis* is one of the most important medicinal plants extensively used by traditional practitioners in India for its medicinal value. Flowers and leaves of *Hibiscus rosa-sinensis* are found to possess antioxidant, antifungal, anti-infectious, antimicrobial, anti-inflammatory, anti-diarrheic, and antipyretic activity (David & Leonard, 1998). *Hibiscus schizopetalus* Hook, f. also called “Fringed Hibiscus” with orange-red flowers has been popularly cultivated in Asian countries. The Rose of China, *Hibiscus rosa-sinensis* commonly called Chinese rose is a shrubby plant, probably native to tropical regions of Asia, and is widely used as ornamental species (Senapati et al., 2006).

Almost all the species of *Hibiscus* are known to produce fiber, but only two species, namely *Hibiscus cannabinus L*. ('Bimlipattam Jute', 'Mesta', 'Kenaf') and *Hibiscus sabdariffa L*. ('Roselle') are commercially exploited for fiber production. *Hibiscus cannabinus* is characterized by its short vegetative period (3-4 months) with a superior quality of fiber, while *Hibiscus sabdariffa* is a long duration crop (7-8 months) with comparatively inferior quality of fiber but is a heavy yielder. In Egypt, Karkadeh is considered a very popular beverage valuable medicinal plant due to its effect on lowering and/or adjusting the blood pressure without producing any side effects (Faraji and Haji Tarkhani, 1999). *Althaea rosea* (garden Hollyhock) is an ornamental and medicinal plant species. It is cultivated in a variety of colours including white, pink, and red. It is native to China. The plant grows best in medium-fertile, moist, but well-drained soil (Still, 1994), and Clay soil (Abraham, 1999).

Modern molecular techniques have been developed to meet the demands of the horticulture industry genetic variation, which range from morphological characterization to various DNA-based markers such as randomly amplified polymorphic DNA (RAPD), inter Simple Sequence Repeat (ISSR), and simple sequence repeats (SSR). Identification and characterization of germplasm are essential for the conservation and utilization of plant genetic resources. Characterization of plants with the use of molecular markers is an ideal way to conserve plant genetic resources. Molecular characterization helps to

determine the breeding behavior of species, individual reproductive success and the existence of gene flow, the movement of alleles within and between populations of the same or related species, and its consequences (Papa & Gepts, 2003).

The present study was designed to clarify the genetic relationships among many species of Hibiscus in different accessions from Egypt. This work is very important to document in gene banks for sustainable conservation of plant genetic resources.

Materials and methods

1- Plant Materials

The young fresh leaf material represents eight species; 'C1- *Hibiscus rosa sinensis linn*, C2- *Hibiscus malvaviscus arboreus*, C3- *Althaea rosea*, C4- *Hibiscus rosasinensis sp*, C5- *Hibiscus schizopetalus*, C6- *Hibiscus china rosa*, C7- *Hibiscus sabdariffa L*., and C8- *Hibiscus cannabinus L*. (kenaf) were used in the present study. The different species of *Hibiscus rosasinensis* have collected the leaves were wrapped between moist tissue paper from Antoniades botanical Gardens.

2- Morphological studies

Six morphological traits were studied for the identification and characterization of the different *Hibiscus* species and varieties. These were: height, growth habits, flower petal colour, flower shape, flower form, and leaf shape. All the measurements and observations were taken on eight different plants of each species.

3- Molecular Studies

All molecular work was carried out in the molecular biology laboratory, Genetics department, Faculty of Agriculture at Alexandria University.

3-1- DNA Extraction

Genomic DNA was isolated from young leaves of each one of the previously mentioned different eight *Hibiscus* species by using Biotech Kit (i-genomic plant DNA mini kit) South Korea.

3-2- Determination of DNA purity and concentration:

The purity and quantity of the isolated DNA were determined by Nanodrop spectrophotometer (ND1000) USA. The 260/280 ratio was found between 1.8 – 1.9 that indicating the DNA was pure enough for RAPD, ISSR, and SSR analysis.

3-3- Primers:

Eight random primers as well as five ISSR and four SSR primers were used in this study as shown in Tables 1, 2, and 3 respectively.

Table (1): Random primers name, sequences and melting temperature as well as guanine and cytosine content percentage.

Primer no.	Primer name	Sequence	GC%	Tm(c) Value
1	OPA-03	5' – TCG GCC ATA G -3'	60	32
2	OPA-05	5' – CCT TGA CGC A -3'	60	32
3	OPB-05	5' – GTG ACC CCT C -3'	70	34
4	OPB-07	5' – GGC GGT CTT T -3'	60	32
5	OPB-11	5' – TGT CTC GGT G -3'	60	32
6	OPD-02	5' – CGA CCC AAC C -3'	70	34
7	OPS-11	5' – AAA GTC GCG G -3'	60	32
8	OPA-06	5' – AGT CAG CCA C -3'	60	32

Tm = Temperature of melting. GC% = Guanine and Cytosine ratio (percentage).

Table (2): ISSR primers name, sequences and melting temperature as well as guanine and cytosine content percentage.

Primer no.	Primer name	Sequence	GC%	Tm(c) Value
1	HB-08	5' – GAG AGA GAG AGA GG -3'	57.14	44
2	HB-09	5' – GTG TGT GTG TGT GG -3'	57.14	44
3	HB-10	5' – GAG AGA GAG AGA CC -3'	57.14	44
4	HA-99	5' – CAC ACA CAC ACA AGT -3'	50.0	42.0
5	HB-13	5' – GAG GAG GAG GC -3'	72.73	38

GC% = Guanine and Cytosine ratio (percentage).

Tm = Temperature of melting

Table (3): SSR primers name, sequences and melting temperature as well as guanine and cytosine content percentage.

Primer	Primer name	Sequence of forward primers	Sequence of reverse primers
1	H- DAT3	5' – AAG CGA AAT CGA CTG AAG GA -3'	5'- TGT CGT AGA AAC TTC CAA TCC A - 3'
2	H- DAT2	5'- TGT CAA GCT GTC AAG GGT GA -3'	5'- CCG ATC CGT GTT TTT CAA GT - 3'
3	H- DAT1	5' - CCC TTC AAG TGC TCC TCT -3'	5' – TCA CAA GCT GTC AAG GGT GA – 3'
4	H-AMLFP1	5'- ACC GTT CTT TGT TTT AGA TT -3'	5'- CAG GGA AAA CCA CAA AGG AA - 3'

3-4-1- Random Amplified Polymorphism DNA (RAPD):
Each polymerase chain reaction was applied in 20µl final reaction and consisted of 10 µl master mix; 2 µl of primer and 50ng of genomic DNA. DNA amplification was performed in a Biometra T1 gradient thermal cycler for 45 cycles after initial denaturation for 2 min at 94°C. Each cycle consisted of denaturation at 95°C for 1 min; annealing at 37°C for 1.30 min; extension at 72°C for 2 min and final extension at 72°C for 2 min. (Patel et al., 2012).

3-4-2- Inter-Simple Sequence Repeat (ISSR): ISSR amplification was performed in final of 20 µl reaction volume. Each reaction contained 10 µl master mixes; 2 µl of primer and 50 ng of genomic DNA; the reaction profile was preceded at 94ºC for 2 min, and then 35 cycles were applied. Each cycle consisted of a denaturing step of 1 min at 95ºC; a primer annealing step of 1 min at 50ºC and a primer extension step of 2 min at 72ºC. At the end of the 45 cycles, final extension was applied at 72ºC for 2 min. (Patel et al., 2012).

3-4-3-Simple Sequence Repeats (SSR): SSR amplification was carried out in final volume of 20µl. Each reaction mixture contained 10 µl master mix; 1.5µl of 30 ng/µl of each forward and reverse primer and 50ng of genomic DNA. The amplification was done for initial denaturation step of 4 min at 95°C followed by 35 cycles. Each cycle was applied as

denaturation for 45s at 94°C; annealing for 45s at 50°C and extension for 45s at 72°C. Final extension was done for 8 min at 72°C. (Patel et al., 2012).

3-5- Agarose gel electrophoresis

Agarose solution was prepared by adding 0.75g (1.5%) agarose to 50ml of 1XTBE buffer (pH 8.0) in 50 ml flasks. The agarose gel was then dissolved by heating in a microwave oven for 2-3 min. a comb was inserted into the electrophoretic bed and the agarose was carefully poured on it. The gel solidified within 15 min; the electrophoresis apparatus was filled with TBE buffer (pH 8.0), and the comb was removed creating wells for sample application. Agarose gel 1.5% agarose was run for 40 min (100v) in 1xTAE buffer the gel was stained by ethidium bromide then visualized in UV light and photographed.

3-7- Scoring the data and Dendrogram Construction

The DNA fragment profiles were scored in the form of a matrix with 1 and 0, which indicate the presence and absence of bands of each distinct band to species samples respectively. The individual RAPD, ISSR, and SSR amplified profiles were analyzed for genetic similarity. Jaccard similarity coefficient was estimated from these binary data using PAST software version 3.14.

RESULTS

1- Morphological analysis

All the species showed high diversity of flower morphologies as shown in (**Fig. 1**). The variation of flower shapes was observed either with in these species.

Figure1. The eight species of *Hibiscus* species: *C1- Hibiscus rosa sinensis linn., C2- Hibiscus malvaviscus arboreus., C3- Althaea rosea., C4- Hibiscus rosasinensis sp., C5- Hibiscus schizopetalus., C6- Hibiscus china rosa., C7- Hibiscus sabdariffa L., and C8- Hibiscus cannabinus L. (kenaf).*

Table (4): Some morphological characteristics Between *Hibiscus* species.

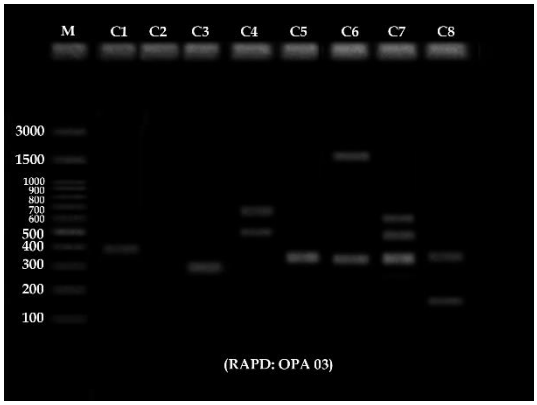
Species name	High	Growth habits	Flower petal colour	Flower shape	Flower form petal	Leaf shape
H.rosasinensis linn	2- 5 m	Shrub	Red	Funnel	Single	Alternate
H.malvaviscus arboreus	2 m	Shrub	Red	Half-closed	Single	Serrated oval
Althea- rosea	50cm /2m	herbs	Purple	Saucer	Single	Alternate heart
H. china rosa	2-5 m	Shrub	Red	Funnel	Single	Alternate
H.schizopetalus	2-5 m	Shrub	Whit	oval	Single	Oval
H. rosasinensis sp	2-5 m	Shrub	Pink	Funnel	double	Alternate
H. sabadariffera	1.5-2 m	herbaceous annual	Dark red	Trumpet -shape	Single	Palmate
H. cannabinus	1.5-2 m	herbaceous annual	Cream Pale yellow	Trumpet	Single Ivory petal	palmate

2- Molecular analysis

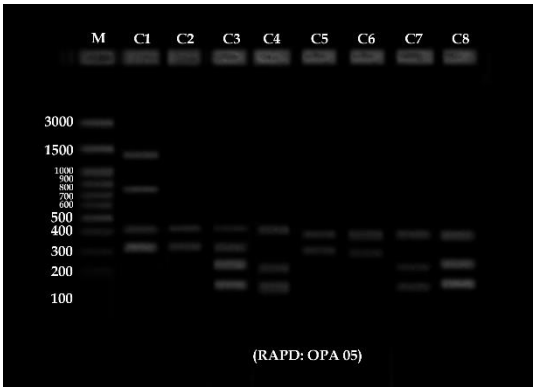
Screening of the eight *Hibiscus* species used in the study by RAPD. PCR analysis showed that every one of the eight primers generated different electrophoresis DNA patterns (clear bands on the RAPD amplification) for each of the studied the sample's studies (**Fig. 2**). In the present study, the numbers of the amplified bands varied from 8 to 19 for **OP-D02** and **OPS-11** primers, respectively, with a total of 89 bands and DNA lengths ranging from 100 to 3000bp. For all primers, the total number of the polymorphic fragments was 89 with an

2-1 RAPD analysis

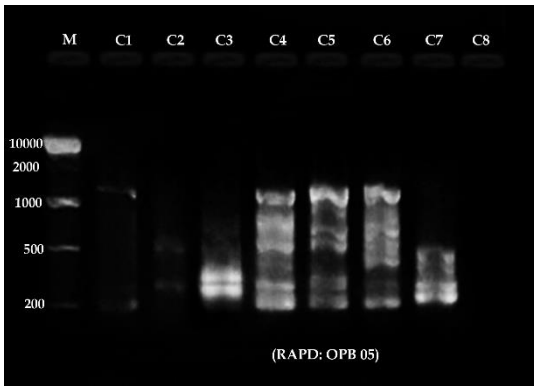
average of polymorphism about 97.75%. The maximum number of the polymorphic bands (eight) was recorded for primer **OP-DO2** with percentage of polymorphism 87.5%. On the contrary, primer **OPS-11** exhibited the minimum number of the polymorphic fragments and polymorphism (100%). These results also demonstrated that the presence of nine different amplified unique bands in the genome of the different eight *Hibiscus* species.



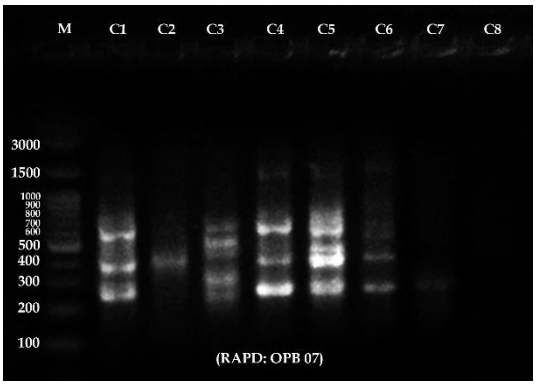
OPA03



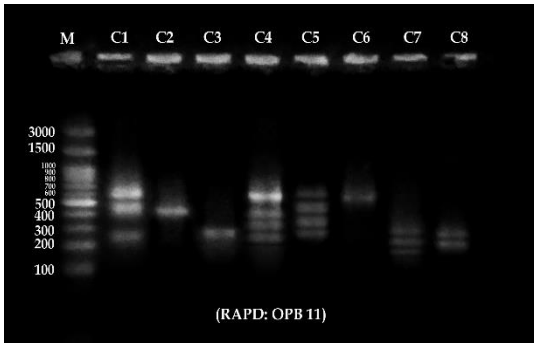
OPA05



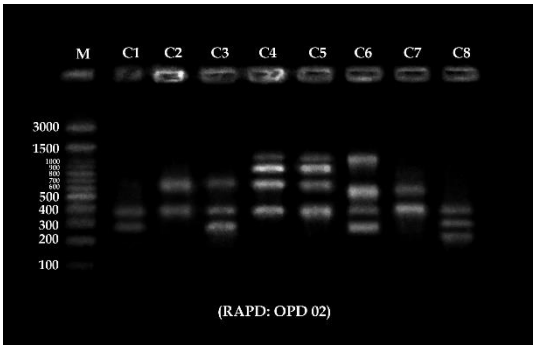
OPB05



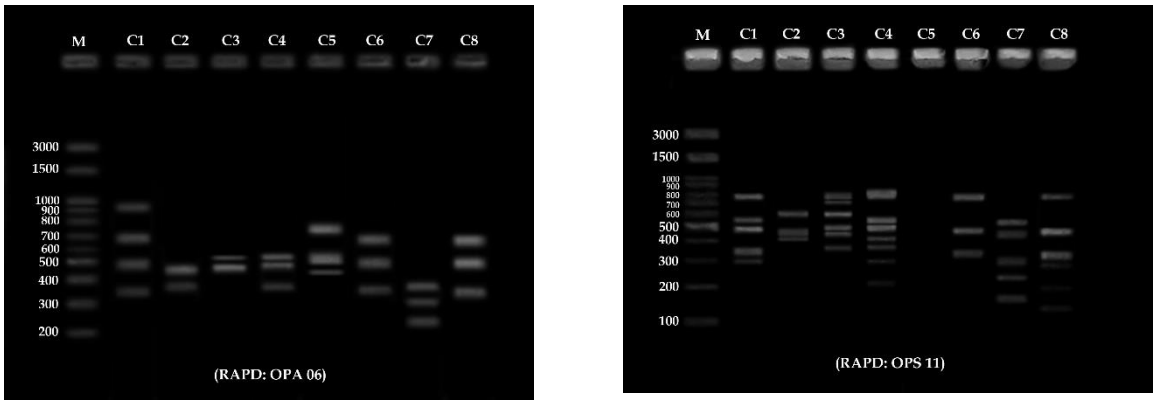
OPB07



OPB11



OPDO2



OPA06 OPS11

Figure 2: Banding of RAPD patterns of eight *hibiscus* species using eight selected random primers, 1-8: C1- *Hibiscus rosa sinensis linn..*, C2- *Hibiscus malvaviscus arboreus*, C3- *Althaea rosea*, C4- *Hibiscus rosasinensis sp.*, *C5- Hibiscus schizopetalus*, C6- *Hibiscus china rosa*, C7- *Hibiscus sabdariffa L., and* C8- *Hibiscus cannabinus L. (kenaf).*

Table (5): Total number of amplified fragments and number of polymorphic fragments generated by PCR using selected RAPD primers.

Primer	Total number of bands	Monomorphic bands	Polymorphic bands	Unique bands	Percent of polymorphism %
OP-AO3	10	0	10	0	100
OP-AO5	10	1	9	0	90
OP-BO5	10	0	10	0	100
OP-BO7	11	0	11	1	100
OP-B11	9	0	9	1	100
OP-D02	8	1	7	1	87.5
OP-AO6	12	0	12	2	100
OP-S11	19	0	19	4	100
Total	89	2	87	9	97.75

The obtained results from the phylogenic tree based on eight random primers as shown in **(Fig. 3)** revealed that the different eight *Hibiscus* species were divided into two (main) major clusters. Cluster (A) included only C7 species, whereas, cluster (B) consisted of two sub clusters. The sub cluster (B1) comprised C8 species and sub cluster includes two groups containing C1 and C6 species, while the other sub cluster (B2) contained to two sub cluster each sub cluster included two species the first group contained C4 and C5 species, while the second group included C2 and C3 species.

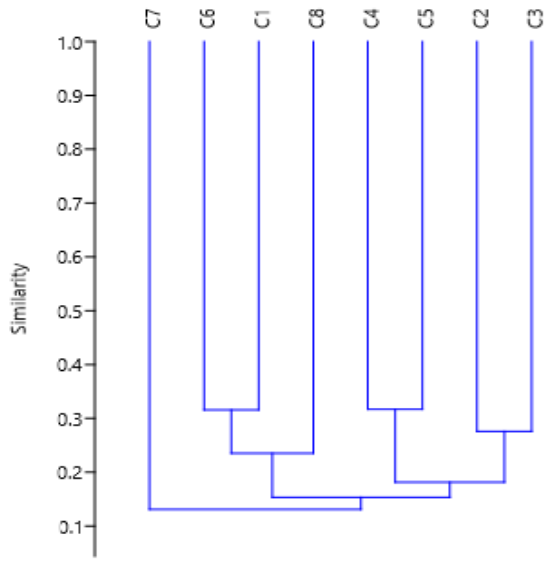


Figure 3: Dendrogram of relationships between *Hibiscus* species using jaccard index for RAPD primers.

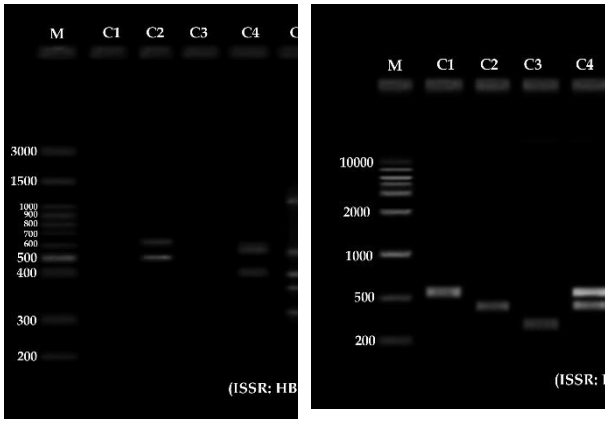
2-2 ISSR-ANALYSIS

Five ISSR primers were used in the present study to differentiate among eight species; presented in **Table (6)** and **Fig. (4)** polymorphism for each of the primers are The ISSR primers amplified produced numbers of the amplified bands varied from seven to 24 for **HA-09** and **HB -99** primers, respectively, with a total of 72 bands and DNA lengths ranged from 100 to 3000bp was reproducible fragments of which 98.61% were polymorphic. It clearly indicates how the primers produced the polymorphic bands mentioned earlier. (**Fig. 4**) These results also demonstrated that the presence of three different amplified unique bands in the genomic of the different eight *Hibiscus* species.

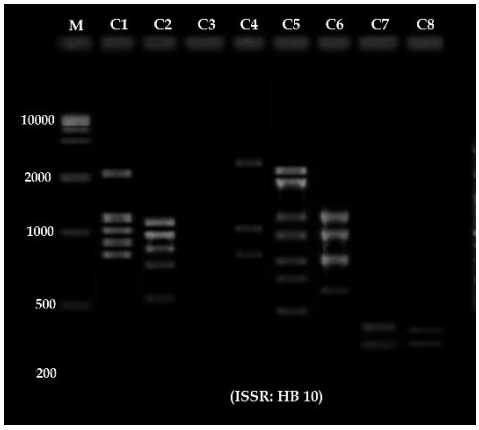
Table (6): Total number of amplified fragments and number of polymorphic fragments generated by PCR using selected ISSR primers

Primer	Total number of bands	Monomorphic bands	Polymorphic bands	Unique bands	Percent of polymorphism%
HA-08	10	0	10	1	100
HA-09	7	0	7	0	100
HA-10	20	0	20	2	100
HA-99	24	0	24	0	100
HB-13	11	1	10	0	90.90
Total	72	1	71	3	98.61

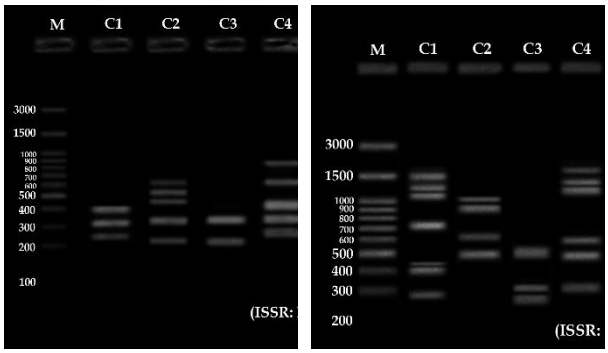
These results obtained that; in **(Fig. 5)** the phylogenic tree based on five ISSR primers showed that the different eight *hibiscus* species were divided into two major clusters. Cluster (A) comprised C3 species and sub cluster includes two groups containing C7 and C8 species, whereas, cluster (B) is divided into two sub clusters. The sub cluster (B1) comprised C2 species only while the other sub cluster divided into (B2) included C5 species only and sub cluster (B3) includes two groups containing the first group C1 cultivar and the second sub cluster contained C4 and C6 species.



ISSR: HB08 **ISSR: HB09**



ISSR: HB10



ISSR: HB13 **ISSR: HA99**

Figure 4: banding of ISSR patterns of eight *Hibiscus* species using five selected random primers, 1-8: the *Hibiscus* varieties C1- *Hibiscus rosa sinensis linn*, C2- *Hibiscus malvaviscus arboreus*, C3- *Althaea rosea*, C4- *Hibiscus rosasinensis sp.*, C5- *Hibiscus schizopetalus*, C6- *Hibiscus china rosa*, C7- *Hibiscus sabdariffa L.*, and C8- *Hibiscus cannabinus L.* (kenaf).

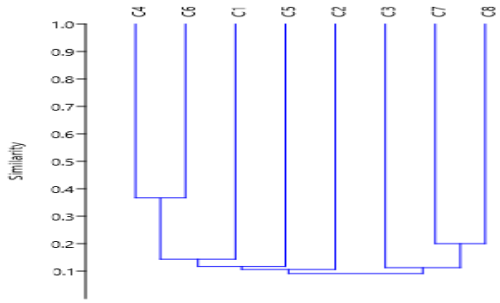


Figure 5: Dendrogram of relationships between *Hibiscus* species using jaccard index for ISSR primers

SSR ANALYSIS

Table (7) the use of SSR primers with the *hibiscus* species in this study produced a total of 15 amplified bands, which all of them were polymorphic bands with 100% percentage of polymorphism (**Fig. 6**) and **Table (7)**; These results also, demonstrated that the presence of two different amplified unique bands in the genomic of the different eight *Hibiscus* species which used as molecular markers to identify each one of these different eight *Hibiscus* species. As shown in Table the numbers of the amplified bands varied from three in **H-DAT3** and **H-DAT2** for five in **H-MFLP2** primers, with a total of 15 bands and DNA lengths ranging from 100 to 500 bp.

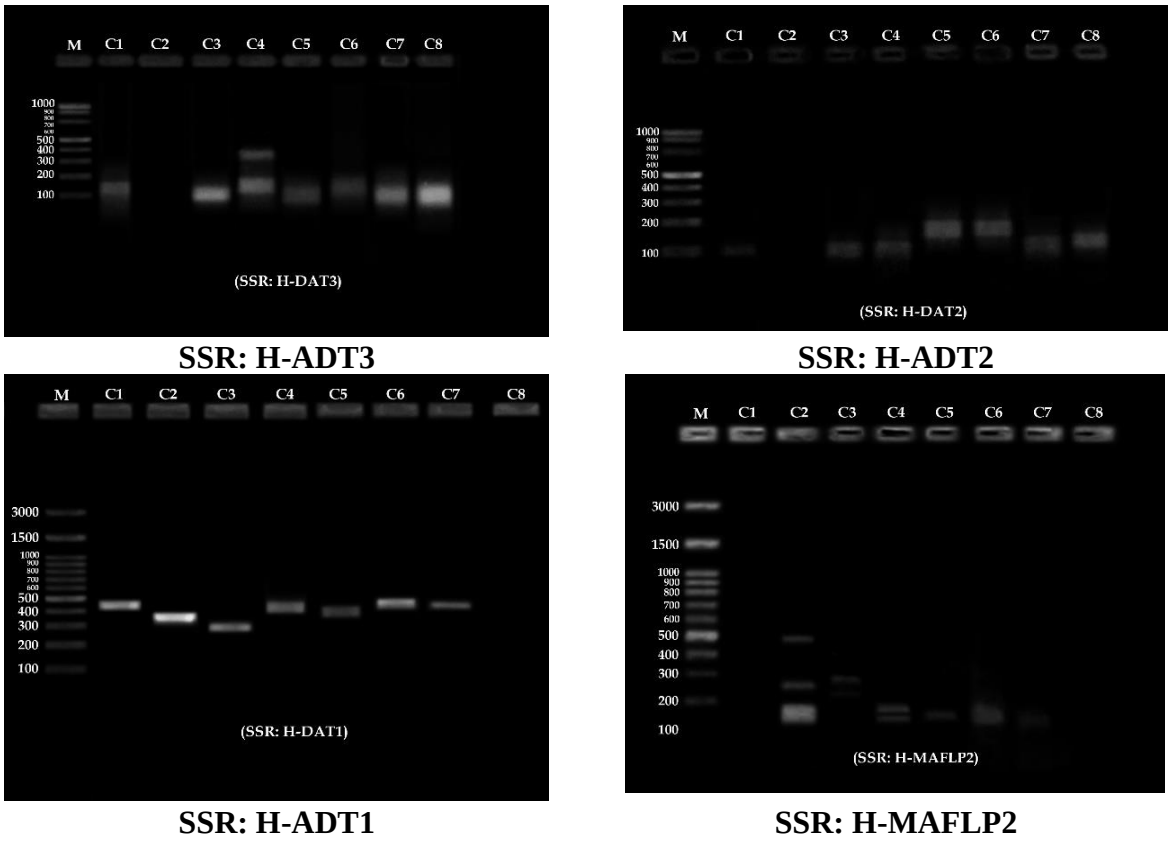


Figure 6: Banding of SSR patterns of eight *hibiscus* species using four selected random primers, 1-8: The *Hibiscus* species C1- *Hibiscus rosa sinensis lin*, C2- *Hibiscus malvaviscus arboreus*, C3- *Althaea rosea.*, C4- *Hibiscus rosasinensis sp.*, *C5- Hibiscus schizopetalus*, C6- *Hibiscus china rosa*, C7- *Hibiscus sabdariffa L.*, and C8- *Hibiscus cannabinus L.* (kenaf).

Table (7): Total number of amplified fragments and number of polymorphic fragments generated by PCR using selected SSR primers

Primer	Total number of bands	Monomorphic bands	Polymorphic bands	Unique bands	Percent of polymorphism %
H-DAT3	3	0	3	1	100
H-DAT2	3	0	3	0	100
H-DAT1	4	0	4	0	100
H-MFLP2	5	0	5	1	100
Total	15	0	15	2	100

The obtained results from the phylogenic tree based on four SSR primers as shown in **(Fig. 7)** revealed that the different eight *hibiscus* species were divided into two major clusters. Cluster (A) included C2 species only whereas cluster (B) divided into two sub clusters cluster (B1) included C8 and C5 species on the other hands' sub cluster (B2) divided to two sub clusters too, the first cluster included C4 and C6 species in a closed group, while the second cluster comprised C3 species and sub cluster

includes two groups contains the C1 and C7 species.

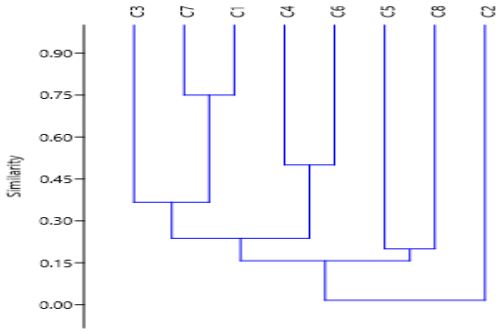


Figure 7: Dendrogram of relationships between *hibiscus* species using jaccard index for SSR primers

Discussion

1- Morphology

Greater variations were observed between these *Hibiscus* species, particularly in the treats of 'flower colour and shape'. (**Fig. 1**) The quantitative characters showed large different degrees of variation, which made them difficult to be used for characterization between *Hibiscus* species. This study showed that morphological characterization provided a rapid and satisfactory means to differentiate between *Hibiscus* species but Identification of some species, varieties and cultivars of *Hibiscus* can be problematic since flower colour, shape, and form are the only characters in which can be used to discriminate between the species and cultivars (Porebski et al., 1997).

2- Molecular analysis

The genetic variation through RAPD markers has been highlighted in several the ornamental plants including rose (Nei, 1978; Mohapatra & Rout, 2005), *Vanda sp.* (Hoon-Lim et al., 1999), Pelargonium (Renou et al., 1997), and Ixora cultivars (Rajaseger et al., 1997). In this study five *Hibiscus sp.* were used. This present finding showed that high degree of variations within the varieties of *Hibiscus rosasinensis* flower plants. Even though all the varieties of *Hibiscus rosasinensis* have the same DNA profile. However, they were different.

sinensis, the dendrogram revealed that cultivar 'ORANGE' and 'YELLOW' had equal similarities. Thus, these two cultivars could not be used in the hybridization

The obtained results from the phylogenic tree based on eight random primers as shown in (**Fig. 3**) revealed that the different eight *Hibiscus* species were divided into two (main) major clusters. Cluster (A) included only C7 *Hibiscus sabdariffa* cultivar, whereas, cluster B *Hibiscus cannabinus* consisted of two sub clusters and small results were similar to the obtained by (Prasad, 2014). In which results of the dendrogram categorized 15 genotypes into two main groups. The first group consisted of *Hibiscus cannabinus* (K1), *Hibiscus sabdariffa* (K2), and *Hibiscus mutabilis var rubra* (K3); the second group consisted of H. syriacus (K4), *Hibiscus schizopetalus* (K5), and *Hibiscus rosa-sinensis* (R1–R10).

Five ISSR primers were used in this study to differentiate among eight species; their polymorphism for each of the primers is used presented in **Table (3)** with a total of 72 bands. This result was similar to the results obtained by our research with (Khafaga, 2013) studied seven *Hibiscus* samples using eight primers of ISSR-PCR markers to assess the level of polymorphism the total number of amplified bands was 117 with 47.0 % polymorphism.

On the other hand, (Patel et al., 2012) used five ISSR primers on five cultivars with different flower colours (red, pink, white, orange, and yellow) of *Hibiscus rosa-*process. Also, this study could identify diverse genotypes like 'RED' and 'PINK' cultivars for their use in the hybridization program of *Hibiscus rosa-sinensis.* The

previous study obtained results were similar to the results obtained in this research. The first group C1 *Hibiscus rosa-sinensis* species and the second sub cluster contained C4 *Hibiscus china* and C6 *Hibiscus rosa-sinensis sp.* the also use in the hybridization program.

The percentage of polymorphism by using four SSR primers for eight species of *Hibiscus* was 100% polymorphism that indicated very high polymorphism and that clearly established the utility of these SSR markers in genetic analysis of *hibiscus* species (Satya et al., 2012).

The banding pattern of using three SSR primers with five species of *Hibiscus* showed very low percentage of polymorphism (34.87%) (Patel et al., 2012), which is not similar with the results obtained in this study showing high polymorphism.

Conclusion

RAPD, ISSR, and SSR analysis representing the molecular marker analysis tools is more reliable than those morphological analyses. The use of molecular marker demonstrated reliability for studying the phylogenetics relationships among the different *Hibiscus* species that can be reliable cornerstone for the *Hibiscus* breeding program. On the other hand, SSR and ISSR markers comparative evaluation in generating polymorphism and in *Hibiscus* as to assess genetic diversity, revealed higher polymorphism for SSR markers. This illustrates that the use of SSR markers is much useful in the discrimination, characterization, and differentiation of *Hibiscus* species than that of ISSR and RAPD markers.

Conflicts of interest: All authors that they have no conflicts of interest.

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