The Pharmaceutical and Chemical Journal, 2020, 7(4):61-69

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Genetic variations among some *Jasminum* species and cultivars from Egypt using SCoT and ISSR markers

El-Hawary Seham[1], El-Hefnawy Hala[1], Osman Samir[2], EL-Raey Mohamed[3], Mokhtar Fatma Alzahraa[1]*

[1]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt
[2]Department of Pharmacognosy, Faculty of Pharmacy, Oct. 6 University, Giza, Egypt
[3]Phytochemistry and Plant Systematic Department, National Research Centre, Dokki, Cairo, Egypt
* Corresponding author: Mokhtar Fatma Alzahraa, e-mail: drfatmaalzahraa1950@gmail.com

Abstract Egypt is considered the top producer of Jasmine concrete; made from *Jasminum officinale* L,. The study of the genetic variabilities between different Jasminum species cultivated in Egypt in order to propagate the desired species. In this study, start codon targeted (SCoT) and inter-simple sequence repeats (ISSR) markers were used as genetic markers for the selected Jasminum species. SCoT primers produced out of 28 bands were visualized across the species, the results obtained 28 total bands with molecular weight from 230 to 1815 bp, 6 of total amplified bands were polymorphic (21.42 %) and the highest polymorphic percentage was 44.44 % produced with primer SCoT 1 and the lowest percentage was 16.66 % produced with primer SCoT 2 and SCoT4. ISSR primers obtained 31 total bands with molecular weight from 115 to 3400 bp, 18 of total amplified bands were polymorphic(58.05 %) and the highest polymorphic percentage was 71.42 % produced with primer HB-15 and the lowest was 50 % produced with primer 44B. Unweighted pair group method with arithmetic mean (UPGMA) was used for genetic relationships illustration.All the ISSR and SCoT primers used in the present study allowed for enough distinction among Jasminum species and cultivars.

Keywords DNA; inter-simple sequence repeats (ISSR); start codon targeted (SCoT); Jasminum; Unweighted; pair group method with arithmetic mean (UPGMA)

1. Introduction

Genus *Jasminum* is one of the most important genus in the Oleaceae family and includes over 2000 pants distributed all over the world, the flowers mostly white in color except few are yellow, they are native to Eurasia especially Italy, Iran, India and mediterranian region [1]. *Jasminum* flowers are characterized by very pleasant aroma, used for thousands of years in the perfume industry [2].

Among *Jasminum* species; *Jasminum azoricum* L (*J. azoricum*), *Jasminum humile* L (*J. humile*), *Jasminum multiflorum* Burm.f. (*J. multiflorum*), *Jasminum officinale* L (*J. officinale*), *Jasminum sambac* Ait. "Arabian nights" (*J. sambac* (A)) and *Jasminums ambac* Ait. "Grand Duke of Tuscany" (*J. sambac* (G)) are cultivated in Egypt.

J. officinale, *J. sambac*(A) and *J. sambac*(G) are the major sources of raw material for the perfume industry as Jasmine oil [3].

Egypt is considered the top producer of *J. officinale* concrete worldwide, the concrete is exported to France, England, and the USA [4] for the production of high grade perfumes. The Egyptian concrete is synthesized mainly from the flowers of *Jasminum officinale* L by solvent extraction technique [5] and have a remarked economical value. Most

Jasminum species have pharmacological activities as antitumor [6-7], antimicrobial activity [8], anthelmintic activity [9], antioxidant [10], effects on CNS [11].

Augmentation of *J. officinale* flower production is necessary to increase its contribution in the national income by increase the exact species on a wide scale as the Egyptian *J. officinale* concrete is the highest quality concrete worldwide.

Recently, plant molecular biology techniques and molecular genetic techniques have been developed for the evaluation of genetic diversity and phylogenetic relationships among different species and varieties.

Inter-simple sequence repeats (ISSR) analysis [12] offers many advantages, such as the requirement of only low quantities of template DNA, no need for sequence data for primer construction, random distribution throughout the genome, the generation of many informative bands per reaction [13]. This method uses a single primers of 15–20 nucleotides with a 30 or 50 anchor sequence [14], Inter-simple sequence repeat (ISSR) has been widely employed to detect intraspecific polymorphisms in plants such as in Tuber species [15], peanut (*Arachishypogaea* L.) [16].

SCoT markers were reported to be more informative and the efficiency for fingerprinting of varieties more than other markers based on the average percentage polymorphism, PIC and overall Shannon index [17-18], it is one of the most modern, effective, simple, gene targeted marker system. Scot technique was successfully used to assess the genetic diversity of many varieties and species; wheat [19].

This study was performed to assess the genotypic differences and relationships of *Jasminum* species using gene targeted markers and to characterize the genetic variability in *Jasminum* species cultivated in Egypt.

2. Materials and Methods

Jasminum species and cultivars

The Egyptian *Jasminum* species and cultivars *Jasminum azoricum* L (*J. azoricum*), *Jasminum humile* L (*J. humile*), *Jasminum multiflorum* Burm. f. (*J. multiflorum*), *Jasminum officinale* L (*J. officinale*), *Jasminum sambac* Ait. "Arabian nights" (*J. sambac* (A)) and *Jasminum sambac* Ait. "Grand Duke of Tuscany" (*J. sambac* (G)) were collected from the exact same position (alkeram farms), Albehaira government, Egypt, they were collected from the same position to eliminate location variabilites and they were identified and authenticated by specialist scientists in the ministry of agriculture, Egypt.

DNA Isolation

The genomic DNA was isolated from the *Jasminum* species using the DNeasy plant Mini Kit (QIAGEN) following kit instructions.

PCR reactions

A-Scot markers

PCR reactions were performed as described by Collard and Mackill [20], using five SCoT primers (Table 1), the DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94º C for 4 min followed by 45 cycles of 1 min at 94º C, 1 min at 57º C, and 2 min at 72º C. the reaction was finally stored at 72º C for 10 min [20]. The amplification reaction products were detected by 1.5% denatuing agarose gel, stained with ethidium bromide and photographed under UV.

B- ISSR markers

PCR reaction analysis was performed according to the method described by Godwin et al (1997), using five ISSR primers (Table 1), the DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94º C for 4 min followed by 45 cycles of 1 min at 94º C, 1 min at 58º C, and 2 min at 72º and the final extension at 72º for 8 min [14, 21]. The amplification reaction products were detected by 1.5% denatuing agarose gel, stained with ethidium bromide and photographed under UV.

Data analysis

Bands were scored as 1 for presence and 0 for absence. The similarity matrices were done using Gel works ID advanced software UVP-England Program. The relationships among genotypes as revealed by UPGMA dendrograms were done using SPSS windows (Version 10) program. DICE computer package was used to calculate the pairwise difference matrix and plot the phenogram among cultivars [22].

3. Results and Discussion

SCoT analysis

Five SCoT primers were successful out of ten SCoT primers to determine SCoT polymorphism of the six selected *Jasminum* species (Figure 1). The resulted in amplified bands and their densitometric analyses are illustrated in, Banding patterns were scored as present (+) or absent (-)

Five SCoT primers were used to study the genetic variation among six *Jasminum* species cultivated in Egypt (*J. azoricum, J. humile, J. multiflorum, J. officinale, J. sambac*(A), *J. sambac* (G)). PCR analysis produced a total number of 28 bands were visualized across the six *Jasminum* species and cultivars, the results obtained 28 total bands with molecular weight from 230 to 1815 bp. 6 of total amplified bands were polymorphic (21.42 %) and the highest polymorphic percentage was 44.44 % produced with primer SCoT 1 and the lowest polymorphic percentage was 16.66 % produced with primer SCoT 2 and SCoT 4. On the other hand, a total of 22 monomorphic bands have appeared and one specific marker band in all five primers. Finally, Primer ScoT 1 was the highest amplified bands (9 bands) whereas, primer SCoT 6 was the lowest amplified bands (3 bands). (Table 2)

Genetic relationship between selected *Jasminum* species based on SCoT analysis

The similarity between the six *Jasminum* species and cultivars using SCoT primers ranged from 0.07 to 1.00 and the highest similarity was between each of *J. sambac* (G) cultivar and each of *J. azoricum*, *J. multiflorum* and *J. officinale* and also, between *J. sambac* (A) and each of *J. multiflorum* and *J. officinale* at 1.00 and the lowest similarity was between species *J. multiflorum* and *J. officinale* at (0.00) (Figure 2).

ISSR analysis

Five ISSR primers were successful out of fifteen ISSR primers to determine ISSR polymorphism of the six *Jasminum* species and cultivars under study. The resulted amplified bands and their densitometric analysis are illustrated (figure 3).

A total number of 31 bands were visualized across the six *Jasminum* species and cultivars investigated, the results obtained 31 total bands with molecular weight from 115 to 3400 bp. 18 of total amplified bands were polymorphic (58.05 %) and the highest polymorphic percentage was 71.42 % produced with primer HB-15 and the lowest polymorphic percentage was 50 % produced with primer 44B. On the other hand, total of 13 monomorphic bands have appeared and nine specific marker bands in five primers. Finally, primer HB-15 was the highest amplified bands (7 bands) whereas, primer 14A were lowest amplified bands (5 bands) (Table 3).

Genetic relationship between selected *Jasminum* species based on ISSR analysis

The similarity between the six *Jasminum* species and cultivars ranged from 0.07 to 1.00 and the highest similarity was between each of *J. azoricum* and *J. humile* at 1.00 and the lowest similarity was between species *J. officinale* and *J. sambac* (A) at (0.07) (Figure 4).

The investigated *Jasminum* species across five primers of SCoT and five primers of ISSR, the results obtained 59 total bands with molecular weight from 115 to 3400 bp, 24 of total amplified bands were polymorphic (40.67 %) and the highest polymorphic percentage was 58.05 %% produced with ISSR primers and the lowest polymorphic percentage was 21.42 %% produced with SCoT primers. On the other hand, a total of 35 monomorphic bands appeared and ten specific marker bands in ten primers (Table 4).

Genetic relationship between the selected *Jasminum* species based on combining SCoT and ISSR analysis

By combining SCoT and ISSR, The similarity between the six *Jasminum* species and cultivars (Figure 5) ranged from 0.08 to 1.00 and the highest similarity was between each of *J. sambac* (A) and *J. sambac* (G) at 1.00 and the lowest similarity was between species *Jasminum azoricum* and *J. humile* species at (0.08).

Discussion

All the ISSR and SCoT primers used in the present study allowed for enough distinction among the six *Jasminum* species and cultivars. Overall comparison the six *Jasminum* species and cultivars across the used primers revealed the power of studied molecular genetic markers in distinguishing genetic identification and phyllogenetic relationship between *Jasminum* species under study and these results were in line with UPGMA cluster and analysis based on genetic similarity values for SCoT markers showed two main groups [23]. The first main group was included two species *J. sambac* (A) and *J. sambac* (G) the second main group was divided into two sub main groups: the first sub main group included *J. azoricum* alone and the second sub main group was divided into two sub sub group: the first sub sub group was included *J. humile* alone and the second sub sub group was included each of *J. multiflorum* and *J. officinale* species. SCoT markers are reported to success in genetic determination of variation and association analysis for pod yield and other agronomic and quality characters in an Indian Himalayan collection of broad bean *Viciafaba* [24].

UPGMA cluster and analysis based on genetic similarity values for ISSR markers was used to construct classify the selected *Jasminum* species showed two main groups: the first main group was included two species *J. azoricum* and *J. humile* and the second main group was divided into two sub main groups: the first sub main group included specie *J. sambac* (A) alone and the second sub main group was divided into two sub sub group: the first sub sub group was included species *J. multiflorum* alone and the second sub sub group was included each of *J. officinale* and *J. sambac* (G) These results agreed with previously published data focused on Iranian jasmine [23] with slight differences.

UPGMA cluster dendogram and analysis based on genetic similarity values for SCoT and ISSR markers was used to construct classify six *Jasminum* species into two main groups: the first main group was included two species *J. sambac* (A) and *J. sambac* (G) the second main group was divided into two sub main groups: the first sub main group included species *J. azoricum* alone and the second sub main group was divided into two sub sub group: the first sub sub group was included specie *J. humile* alone and the second sub sub group was included each of *J. multiflorum* and *J. officinale* species. Combination of ISSR and SCoT markers was proved to be an excellent procedure to establish a good genetic relationship among different species of *Jasminum* cultivated in Egypt and these results are consistent with the results performed for genetic variation and differentiation among annual Cicer species [25], assessment of genetic diversity among mango cultivars [26], and results that validate the different tissue cultures genetic diversity from *Alhagi maurorum* [27] and was an efficient tool for the Assessment of genetic diversity, population structure and sex identification in dioecious crop, *Trichosanthes dioica* [28].

Table 1: Lists of ISSR and SCoT primers (Sigma, Egypt) used in intergenetic evaluation of *Jasminum* species and cultivars

ScoT primer	Sequence	ISSR primer	Sequence
ScoT 1	ACG ACA TGG CGA CCA CGC	14A	5 CTC TCT CTC TCT CTC TTG 3`
SCoT 2	ACC ATG GCT ACC ACC GGC	44B	5`CTC TCT CTC TCT CTC TGC 3`
SCoT 3	ACG ACA TGG CGA CCC ACA	HB-12	5`CAC CAC CAC GC 3`
SCoT 4	ACC ATG GCT ACC ACC GCA	HB14	5´ CTC CTCCTC GC 3`
SCoT 6	CAA TGG CTA CCA CTA CAG	HB-15	5` GTG GTGGTG GC 3`

Table 2: List of SCoT primers of the six *Jasminum* species and cultivars. Percentage of polymorphism and Specific Marker bands

Primer Name	Total Band	Monomorphic Band	Polymorphic Band	Unique Band	Polymorphic %
SCoT 1	9	5	4	1	44.44%
SCoT 2	6	5	1	-	16.66%
SCoT 3	4	4	-	-	-
SCoT 4	6	5	1	-	16.66%
SCoT 6	3	3	-	-	-
Total	28	22	6	1	21.42%

Table 3: List of ISSR primers of the selected *Jasminum* species. Percentage of polymorphism and Specific Marker bands (SM)

Primer Name	Total Band	Monomorphic Band	Polymorphic Band	Unique Band	Polymorphic %
14A	5	2	3	1	60%
44B	6	3	3	2	50%
HB-12	7	3	4	1	57.14%
HB-14	6	3	3	3	50%
HB-15	7	2	5	2	71.42%
Total	31	13	18	9	58.06%

Table 4: List of SCoT and ISSR primers of the selected *Jasminum* species and cultivars. Percentage of polymorphism and Specific Marker bands

Primer Name	Total Band	Monomorphic Band	Polymorphic Band	Unique Band	Polymorphic %
SCoT	28	22	6	1	21.42%
ISSR	31	13	18	9	58.06%
Total	59	35	24	10	40.67%

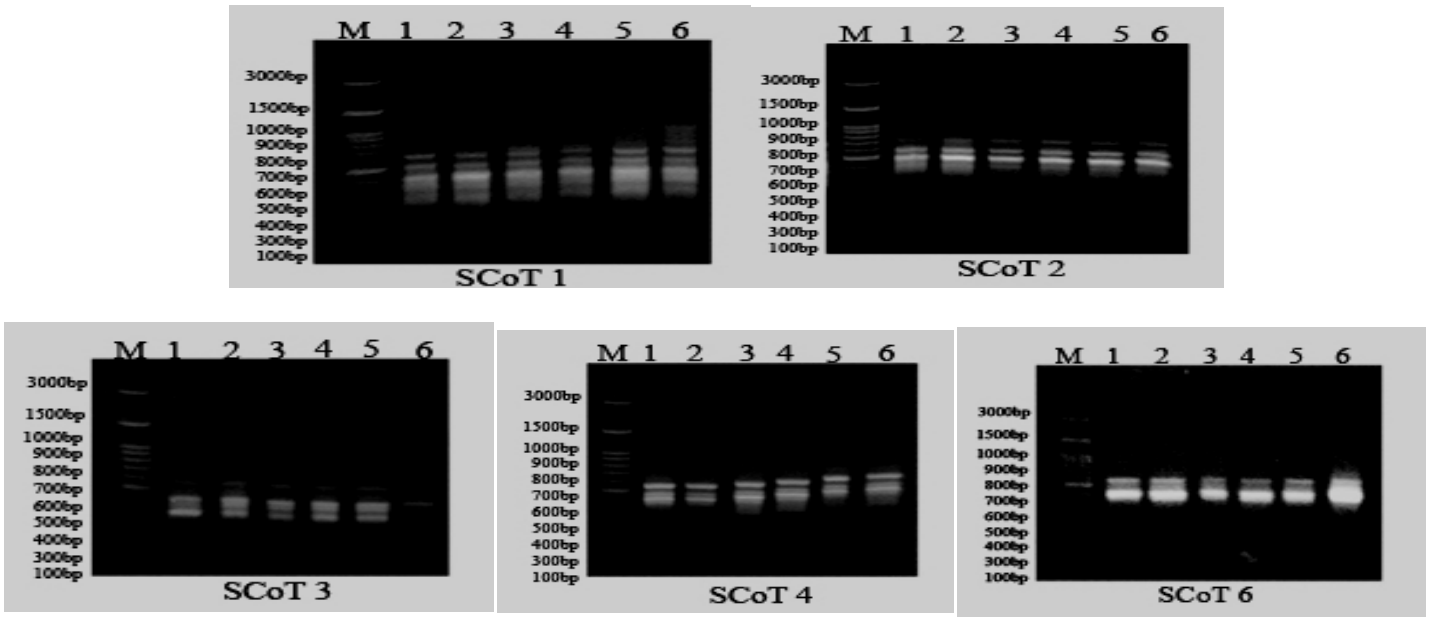

Figure 1: SCoT fingerprinting of Jasminum species: M; DNA marker, lanes 1-6 (J. azoricum, J. humile, J. multiflorum, J. officinale, J. sambac(A), J. sambac (G)) respectively.

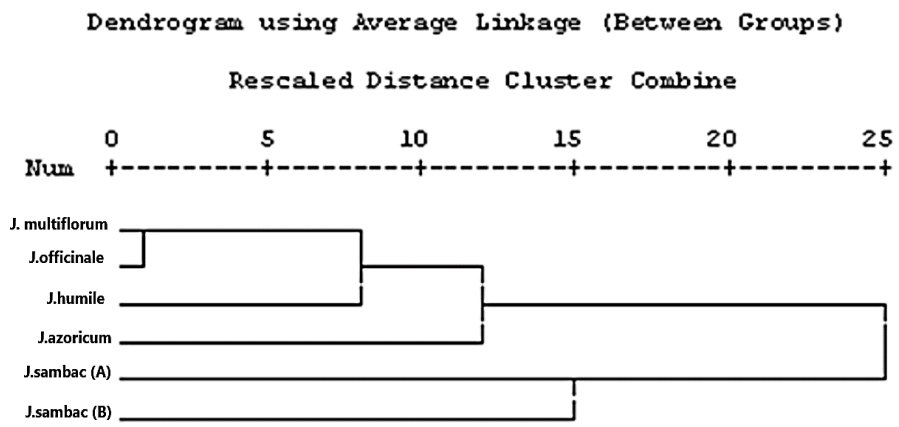

Figure 2: Unweighted pair group method with arithmetic mean (UPGMA) dendrogram illustrating the genetic relationships between six Jasminum species and cultivars based on SCoT analysis

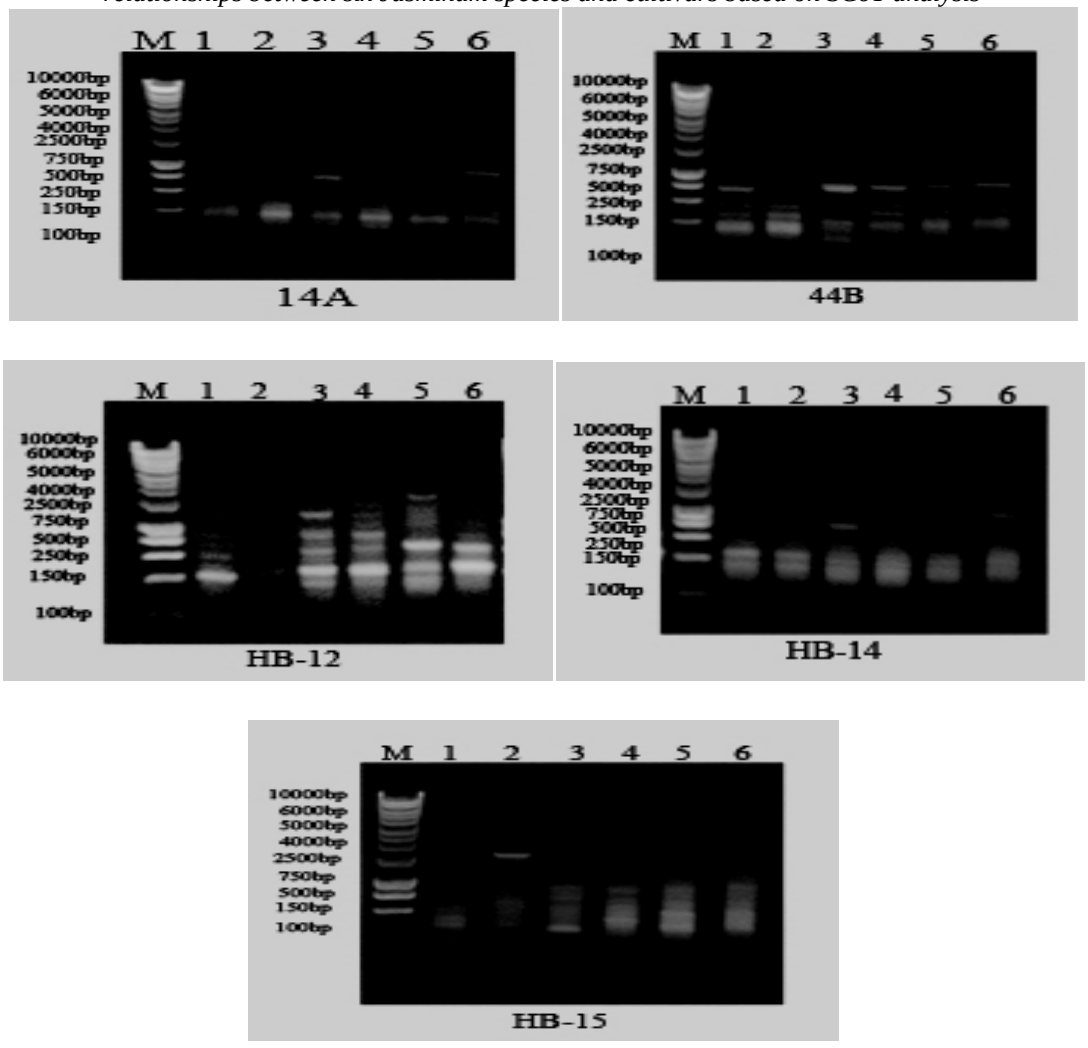

Figure 3: ISSR finger printing of Jasminum species and cultivars: M; DNA marker, lanes 1-6 (J. azoricum, J. humile, J. multiflorum, J. officinale, J. sambac(A), J. sambac (G)) respectively

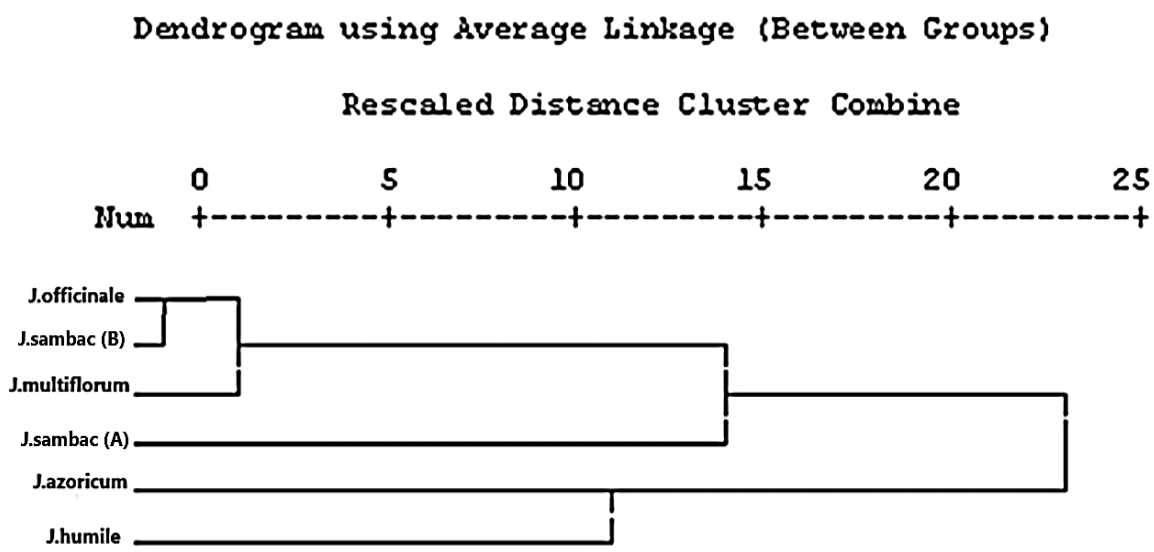

Figure 4: Unweighted pair group method with arithmetic mean (UPGMA) dendrogram illustrating the genetic relationships between the selected Jasminum species and cultivars based on ISSR analysis

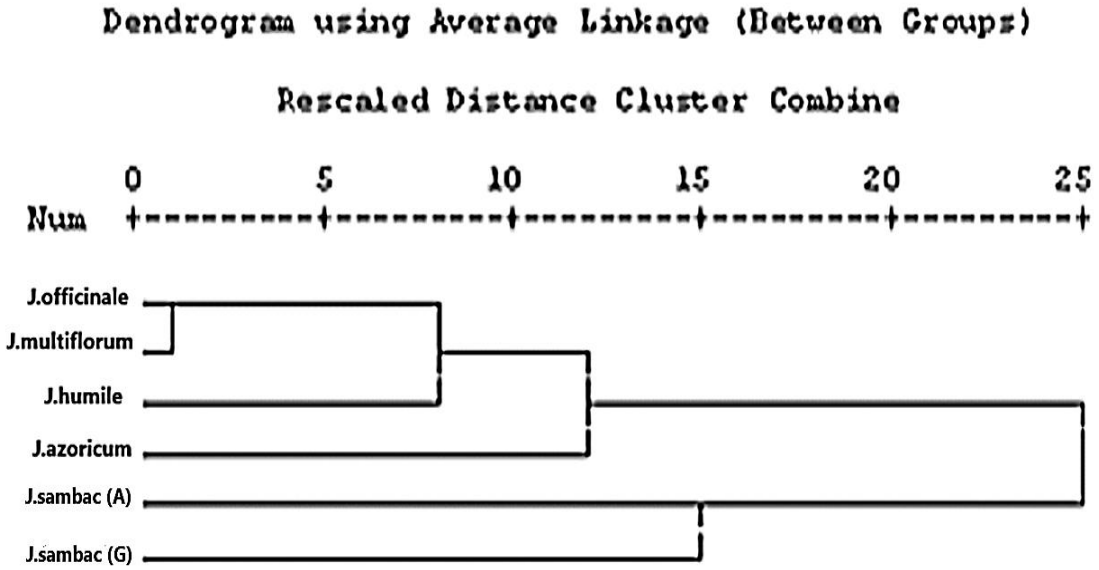

Figure 5: Unweighted pair group method with arithmetic mean (UPGMA) dendrogram illustrating the genetic relationships between six Jasminum species and cultivars based on combining SCoT and ISSR analysis

4. Conclusion

Overall comparison the six *Jasminum* species and cultivars across the used primers revealed the power of studied molecular genetic markers in distinguishing genetic identification and phyllogenetic relationship between *Jasminum* species under study

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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