

The Valuation of Genetic Diversity in *Tarchonanthus camphoratus* Plant Using ISSR Markers in Taif, KSA

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Abstract: Genetic variation among *T. camphoratus* individual plant samples collected from Taif region, Saudi Arabia was assessed using inter-simple sequence repeat (ISSR) markers. Eleven ISSR primers were used to evaluate *T. camphoratus* samples. The number of polymorphic bands per primer varied from 5 to 16, with 10.3 bands per primer on average. Nei's genetic distance between *T. camphoratus* samples ranged from 0.03 to 0.94. According to genetic similarity and intraspecific differentiation, the 15 *T. camphoratus* were grouped into two main different clusters with about 0.67 genetic similarity. It was not possible to classify the samples according to their geographic origin, showing that there is no structure in the gene bank. Cluster analysis is indicating good resolution of genetic diversity in *Tarchonanthus* germplasm using ISSR markers. Our results indicate that ISSR can be useful for genetic diversity studies, to provide practical information for parental selection, biodiversity and to assist breeding and conservation strategies.

Keywords: *Tarchonanthus camphoratus*, Genetic Variability, Molecular Markers, Genetic Resources

1. Introduction

The genus *Tarchonanthus* belongs to the family Asteraceae, the subfamily *Cichorioideae* and the tribe *Tarchonantheae*. *Tarchonanthus* is one of the rare Astareceae genera that consist of a tree [1-3]. Additionally, it is dioecious, with male and female flowers located on different plants. The exact name is generally selected to designate some outstanding features of the plant. The name *camphoratus* refers to the robust odor of camphor that given off whilst the leaves are crumpled. Many parts of *Tarchonanthus* species are used medicinally [4, 5]. Infusions and distillates of the leaves and branches are used for intestinal trouble, belly pain, headache, toothache, allergies, bronchitis and inflammation [4, 6]. Until currently the genus *Tarchonanthus* contained two species, viz. *T. camphoratus* and *T. trilobus*. During his revision and study on *T. camphoratus* complex, Herman on [3] *Tarchonanthus camphoratus* documented five species: *T. camphoratus*, *T. obovatus*, *T. littoralis*, *T. minor* and *T. parvicapitulatus*.

Molecular markers were used in several plant breeding and in studies associated with the preservation of genetic resources. Molecular markers have established to be precious methods for documentation, identification and assessment of the genetic assortment within and between cultivars. To meet the requirement for effective, precise, and fast documentation for genus *Tarchonanthus* and/or other plants, numerous molecular marker systems as RAPD [7, 8], ISSRs [9], and simple sequence repeats (SSRs) has been used effectively for molecular characterization of several plants [9, 10]. Among them, ISSR has been conveyed as a fast, reproducible, and reasonably-priced fingerprinting method based totally on the difference observed inside the regions among microsatellites [10-12]. Amongst the various DNA marker classifications, ISSR is acquisition acceptance for its advantage over other dominant DNA marker systems like RAPD for higher polymorphism and improved marker resolvability [13]. The marker technique has been effectively working in genetic assortment analysis and fingerprinting of some crop species [14-16]. The current research is considering the first molecular fingerprinting examination of the locally grown *T.*

camphoratus in Taif governorate using ISSR markers. The aims of the research can be concise as: 1) to assess the capacity of ISSR technique to discover the extent of assortment present in samples of Saudi Arabia *T. camphoratus* plant, and 2) to realize the genetic relationships amongst these genotypes for breeding studies of *T. camphoratus*.

2. Materials and Methods

2.1. Plant Material and DNA Isolation

A complete Fifteen individual plant samples of *Tarchonanthus camphoratus* gathered from Taif areas, Saudi Arabia, had been taken into consideration in the present research. From each genotype, the total genomic DNA was isolated from young leaves of grapevine plants using DNA Promega Kit DNeasy Blood & Tissue (Valencia, CA, USA) according to the instructions of the manufacturer.

2.2. ISSR-PCR Amplification

PCR amplification of ISSR were completed as previously stated [13]. Amplified DNA fragments have been examined via 1.5% agarose gel electrophoresis. The gels were stained and visualized with the useful resource of UV illumination and then photographed by means of the usage of a Bio-Rad Gel Doc 2000 tool. The C1000TM Thermo Cycler device, Bio-Rad Germany, was used for the amplification of the plant genomic DNA template. The program condition was beneath the conditions associated with denaturation at ninety-four Celsius for five min; forty cycles of denaturation at ninety-four Celsius for 30Sec, primer annealing at fifty Celsius for 60sec and primer extension at seventy-two Celsius for 2.5min. Finally, one more stem for addition extension step at seventy-two Celsius for 7min. The PCR products have been analyzed via 1.5% agarose gel electrophoresis. Next, the results were stained with ethidium bromide (5µg/ml) and envisaged by UV illumination, at that time, the results were photographed by a Bio-Rad Gel Doc 2000 device.

Table 1. ISSR Primers used in study of genetic variation among *T. camphoratus* samples.

Primers Name	Primers sequences 5'—3'
ISSR-2	GTG GTT GTG CTT GCC
ISSR-3	ACC ATG GCT ACC ACC GGC
ISSR-4	GCA SGT GTG CTC GCC
ISSR-9	CAG CAC ACA CAC ACA CA
ISSR-10	ACA ATG GCT ACC ACT ACC
ISSR-11	AAG CAA TGG CTA CCA CCA
ISSR-12	ACG ACA TGG CGA CCA ACG
ISSR-14	ACG ACA TGG CGA CCA CGC
ISSR-18	ACA CAC ACA CAC ACA CG
ISSR-19	AGA GAG AGA GAG AGA GTT
ISSR-28	GTG GTG GTG GTG GTG GTG

2.3. Data Analysis

The genetic relatives among plants were assessed by using Neighbour joined method of Jaccard's similarity coefficient.

Next, the dendrogram that showing the genetic assortment was built. The computations had been achieved via the program NTSYS-PC version 2.01 [17].

3. Results

3.1. Polymorphism of ISSR Markers

The results of the banding pattern of electrophoresis showed that the ISSR markers could demonstrate the medium level of diversity existing among the plant individuals. Consequently, the markers were functional for the 15 individual plant samples of *Tarchonanthus camphoratus* (Figures 1 and 2). PCR based molecular markers can play an important role in the analysis of genetic diversity in such species. Here, eleven ISSR primers were used and generated 113 bands ranging in length from 190 to 1950bp (Table 2 and Figures 1, 2). The total monomorphic pattern was 74.4%, while, the total polymorphic pattern was with low percentage of 25.6%. The maximum and minimum number of amplified bands were belonged to ISSR-11 and ISSR-4 that are produced 5 and 16 bands, respectively. The percentage of polymorphic bands was varied from 11.1% for ISSR-3 and ISSR-12 primers to 72.7% for ISSR-19 primer. The average band per primer was 10.3 and the average percentage of polymorphic bands was 25.6%. PCR-based molecular markers can perform a significant part in the investigation of genetic diversity in such plant species.

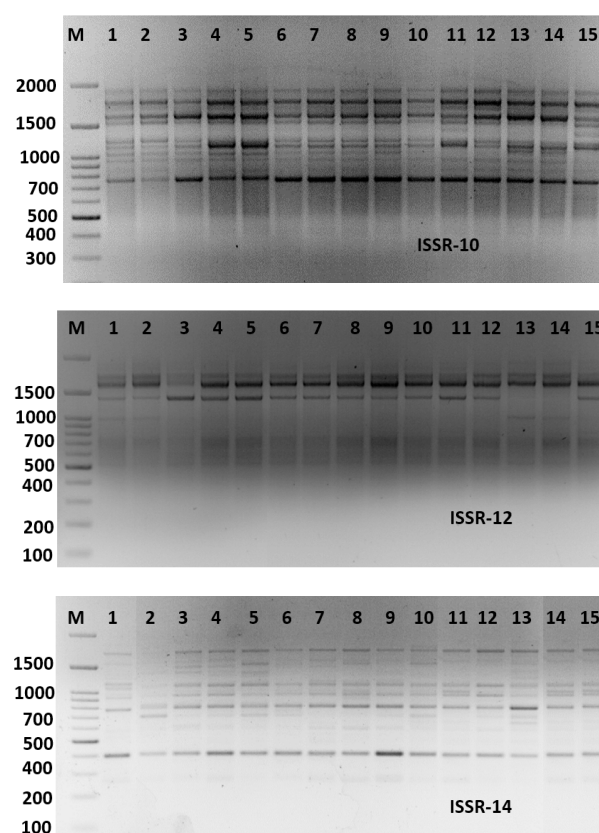


Figure 1. ISSR-PCR profile of 15 *T. camphoratus* samples generated with the respective ISSR primers: ISSR-10, ISSR-12, and ISSR-14. The first lane in each panel corresponds to 100-bp molecular weight markers.

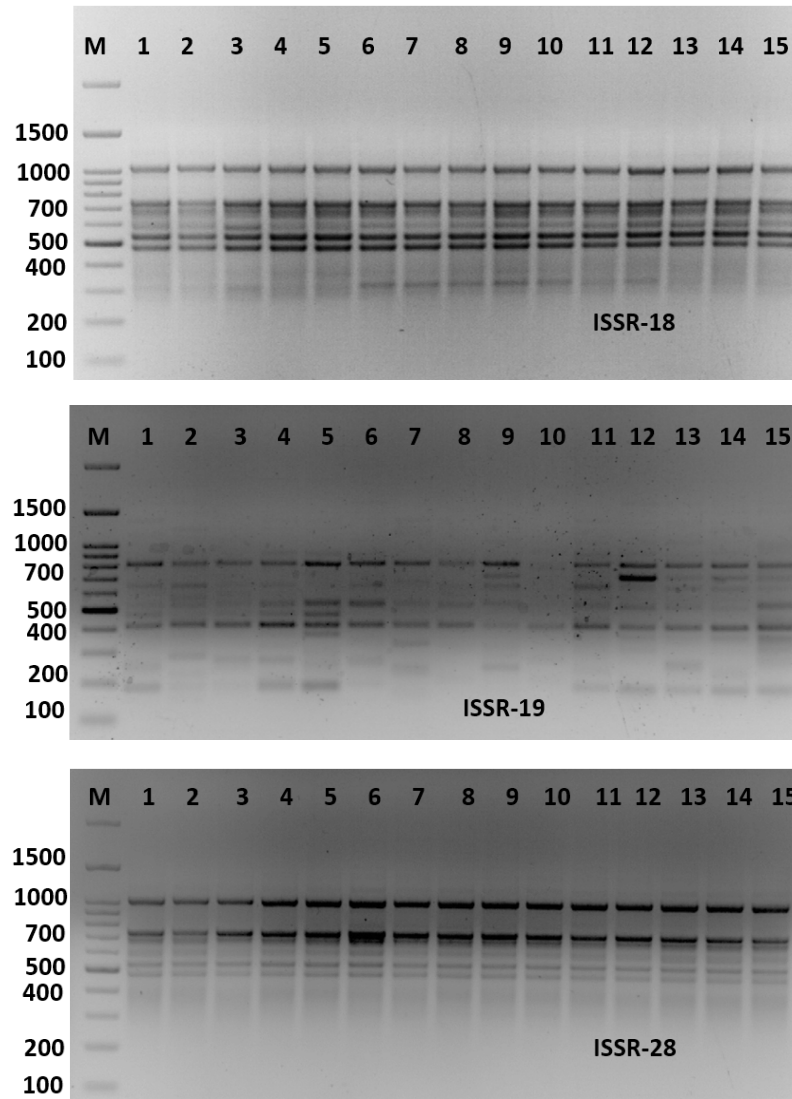


Figure 2. ISSR-PCR profile of 15 *T. camphoratus* samples generated with the respective ISSR primers: ISSR-18, ISSR-19, and ISSR-28. The first lane in each panel corresponds to 100-bp molecular weight markers.

Table 2. Eleven ISSR primers used to *T. camphoratus* plant samples, the total bands (TB), polymorphic bands (PB), monomorphic bands (MB), percentage of polymorphic bands (PPB) and percentage of monomorphic bands (PMB).

Primers Name	TB	PB	MB	PPB (%)	PMB (%)
ISSR-2	13	4	9	30.7	69.3
ISSR-3	9	1	8	11.1	88.9
ISSR-4	16	8	8	50.0	50.0
ISSR-9	11	3	8	27.3	72.7
ISSR-10	11	2	9	18.2	81.8
ISSR-11	5	0	5	0.0	100
ISSR-12	9	1	8	11.1	88.9
ISSR-14	12	2	10	16.7	83.3
ISSR-18	8	0	8	0.0	100
ISSR-19	11	8	3	72.7	27.3
ISSR-28	8	0	8	0.0	100
Total	113	29	84	25.6	74.4

3.2. Phylogeny Analysis of ISSR Marker

As the result of genetic similarity and intraspecific differentiation, the 15-plant individual of *T. camphoratus* were assembled into two major clusters with about 0.67

genetic similarity (Table 3 and Figure 3). Interestingly, the minor cluster has two plant only plant sample-1 and plant sample-2. While, the second major cluster grouped into two sub-cluster that contained the other 13 plants with 71% similarity (Figure 3).

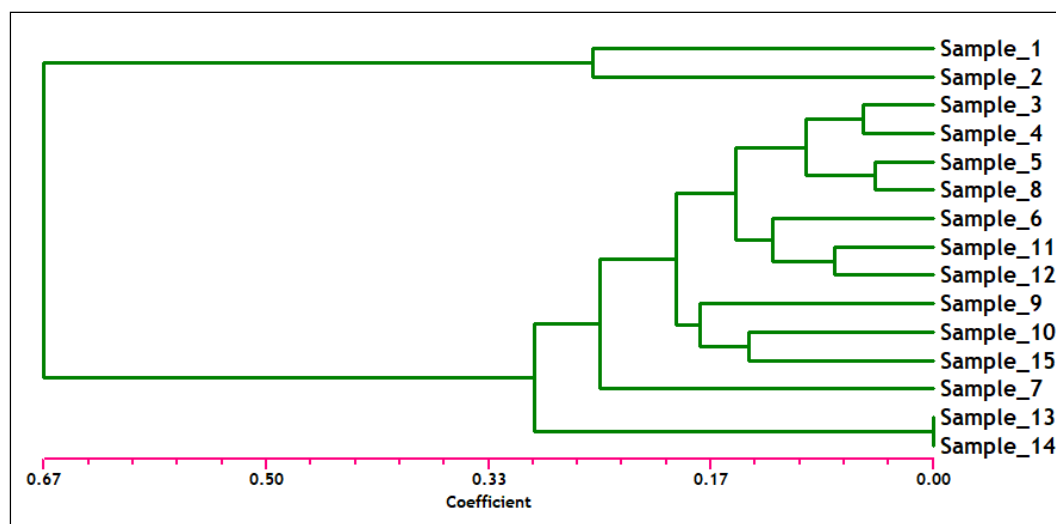


Figure 3. Dendrogram showing relationship of 15 individual plant samples of 15 *T. camphoratus* based on eleven ISSR marker using Jaccard's similarity coefficient.

Table 3. The genetic distance and similarity matrix between 15 individual samples of *T. camphoratus*.

S.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0														
2	0.255	0													
3	0.304	0.608	0												
4	0.356	0.66	0.052	0											
5	0.447	0.752	0.143	0.143	0										
6	0.558	0.558	0.708	0.218	0.153	0									
7	0.693	0.948	0.304	0.223	0.196	0.27	0								
8	0.404	0.708	0.1	0.047	0.043	0.095	0.153	0							
9	0.693	0.948	0.304	0.223	0.196	0.153	0.223	0.153	0						
10	0.404	0.708	0.218	0.153	0.138	0.2	0.27	0.095	0.153	0					
11	0.524	0.829	0.22	0.168	0.077	0.12	0.273	0.12	0.168	0.12	0				
12	0.524	0.829	0.22	0.168	0.077	0.12	0.273	0.12	0.273	0.215	0.074	0			
13	0.558	0.558	0.558	0.558	0.558	0.996	0.351	0.27	0.244	0.318	0.404	0.2	0		
14	0.558	0.996	0.351	0.27	0.244	0.318	0.404	0.2	0.404	0.318	0.2	0.404	0	0	
15	0.602	0.997	0.261	0.196	0.182	0.244	0.314	0.138	0.196	0.138	0.164	0.259	0.138	0.138	0

4. Discussion

The use of molecular markers was pointing to display fast and consistent discrimination of genetic relatives of *T. camphoratus* in Taif region, Saudi Arabia. In the present study, DNA fingerprinting data generated by using ISSR marker was found to be efficient to distinguish different *T. camphoratus* individual samples. In this case, a set of minimum number of primers are required to be selected for the varietal identification and to select a set with minimum number of primers, it is important to consider level of marker polymorphism [9, 11]. In case of ISSR markers, total genetic polymorphism of 25.6% was observed, showing that ISSR markers have low potential polymorphisms compared to SCoT marker in discriminating in some plant cultivars [14, 18]. On other hand, Schanzer and Vagina [19] reported that the total polymorphism amongst five *Rosa* varieties by using six ISSR primers were 90.6%. It was interesting to know the correlation among percent polymorphism and attributes of the markers for sequence motifs [9, 20].

Eventually, ISSR molecular markers method is a smooth to perform, high float-through technique, probably will characterize it rather approach for RAPD method and higher reproducibility due to the elevated annealing temperatures. The mainly appealing role of ISSR assessment is its flexibility in the view of experimental layout, wherein the variety of generated amplicons may be optimized by altering the number of the core repeat units and anchoring bases [13, 21, 22]. Therefore, for its excessive simplicity, ISSR investigation must be a stand by desire for genome mapping or gene tagging and marker assisted selection. The latter exploitation and further research studies might be substantial for the simple basic and applied research especially on *T. camphoratus* and expand the information of microsatellite conservation and evolution of *T. camphoratus* especially in Saudi Arabia.

5. Conclusion

Tarchonanthus camphoratus, is a strongly aromatic shrub found growing in the hillsides between 1200–2500m in KSA.

Traditionally, its aromatic leaves are important for medical uses such as wounds and infections. This makes it a potentially important species for medical production for many diseases. in Taif province *T. camphoratus* constitutes a rich source of biodiversity and conservation and utilization requires a good knowledge on its genetic variation. This may help for understanding the response of this species to climate changes as well as the mechanisms of its local adaptation. The study showed that ISSR is useful for genetic diversity in *T. camphoratus*, and indicated low potential of polymorphisms. There is therefore the need to further study, identify and preserve a broad genetic diversity in *T. camphoratus*. The findings of this study are therefore recommendable to all that working in decision maker for conservation of plant in Saudi Arabia.

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