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RESEARCH ARTICLE

Inter-simple sequence repeat (ISSR) analysis to molecular characteristic determination of *Heliotropium digynum* in Saudi Arabia

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Abstract

Heliotropium digynum, is a member of Boraginaceae family. The growth of the plant, as well as its size, length of inflorescence and speed of development depends on the amount of rain in their habitat. In this study, we aim to the applicability of inter-simple sequence repeat (ISSR) with polymorphism in *Heliotropium digynum* in different region of Saudi Arabia. Fifteen primers were used for ISSR-PCR optimization through ISSR analysis while, only five primers (UBC810, UBC811, UBC818, UBC834 and UBC849) gave the best amplification results which produced a total of 43 polymorphic bands. The number of polymorphic loci was 20 and the percentage of polymorphism was 90.47%. The similarity result indicates the presence of high level genetic diversity between populations and a dendrogram constructed by UPGMA method.

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Introduction

Polymorphism between species can result from different events and is an important aspect of plant breeding (Zietkiewicz *et al.* 1994). Microsatellites (SSR) represent the most abundant source of polymorphism from repetitive sequences. SSR are often used as molecular markers even if this technology is time-consuming and expensive. Inter simple sequence repeat (ISSR) is an alternative technique to study polymorphism based on the presence of microsatellites throughout genomes (Zietkiewicz *et al.*, 1994).

ISSR markers are DNA sequences delimited by two inverted SSR composed of the same units which are amplified by a single PCR primer, composed of few SSR units with or without anchored end. ISSR-PCR gives multilocus patterns which are very reproducible, abundant and polymorphic in plant genomes (Bornet and Branchard 2001 and Bornet *et al.* 2002a). Comparison of ISSR and other PCR-based markers have shown their efficiency in plant breeding (Adams *et al.* 2003; Archak *et al.* 2003; Galvan *et al.* 2003).

Heliotropium digynum, a member of Boraginaceae family, is an erect perennial shrub, reaching a height from 30 to 50 cm above the ground. The growth of the plant, as well as its size, length of inflorescence, and speed of development, depends on the amount of rain in its habitat. The study by Forssk (1877) is regarded as the first work mentioning the presence of the genus *Heliotropium*, in the Arabian region. It normally grows in areas with deep sand, and is found in north and north east Africa, the Arabian Peninsula, and some other areas of the Middle East (Chaudhary, 2001). In Saudi Arabia, it is found in the form of scattered families spread in the Hijaz region, and in the south region. It is also found in some areas of central Saudi Arabia such as Kharj, Muzahimiyah, Dir'iyah, Zufi, Unaizah and Rimah.

Hence, the present study, we measured the genetic diversity within and among natural populations of *Heliotropium digynum* sampled from different region in Saudi Arabia (Rumah, Kharj, Diriya and Derab) using ISSR markers.

Material and methods:

1. DNA extraction

DNA was isolated from *Heliotropium digynum* leaves as described in the protocol of *Levi and Thomas, 1999*. The DNA concentration of samples was measured with visible spectrophotometer (ULTRA SPEC 2100) at A260.

2. ISSR analysis

A set of 15 ISSR primers were used (UBC810, UBC811, UBC818, UBC834, ISSR 41, ISSR 40, ISSR 34, ISSR 35, ISSR 36, ISSR 37 HBIO836, HBIO835, HBIO864, HBIO842 and ISSR 40) were used in this study to amplify the genomic DNA. Five of them gave as a result nucleotide sequencing according to the (Table 1). Different concentrations of template DNA and Taq polymerase were tested for optimal amplification products. The optimal amplification mixture of 25 µl contained 100 ng DNA, 1 µM ISSR primer (Sangong Inc.) 0.5 mM dNTPs, 1 mM MgCl₂, 1x PCR buffer and 1 U Taq DNA polymerase (Sangong Inc.). PCR amplifications were performed in a Peltier Thermal Cycler PTC-200 (MJ Research) with an initial step at 95°C for 1 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing temperature of 50°C for 30 s and elongation at 72°C for 2 min. Finally, an additional extension for 10 min at 72°C was used.

3. Agarose gel electrophoresis:

After completion of the amplification process of DNA, separation process was conducted on agarose gel concentration of 1.3 % with (1.3 g Agarose + 50 ml DDW + 900 µl TAE 1X) put into a glass beaker, packaged with aluminium foil flask and placing in the microwave until boiling, then outed and leaved to cool slightly. Ethidium bromide (2-5 µl) were added before freezing and Shacked manually then asting in a mold (Gel cast) after comb is put in place right and left even hardened gel. Samples processing to take (8 µl) of each sample and added to (2 µl) of the dye solution (bromophenol blue) and injected them into wells formed by comb and filling up electric device thank with (1X TAE) solution. Separation process was conducted using an electric current strongly (80 - 100 V), samples began move from the cathode to the anode until reached the end of the template gel After the passage of time from 2 to 3 hours after, separated the electrical from the horizontal slab and removing the gel, after gel were transferred to the imaging device.

4. Data analysis: Statistical analysis was conducted with MVSP 3.1 (Multi Variate Statistical Package) programme for protein and DNA (<http://www.kovcomp.com/mvsp>). Bands were scored visually and recorded as presence (1) or absence (0) of bands. Similarity coefficients were used to construct the dendrogram using the unweighted pair group method with arithmetic means (UPGMA)

Results:

Table (1): The list of ISSR primers sequences

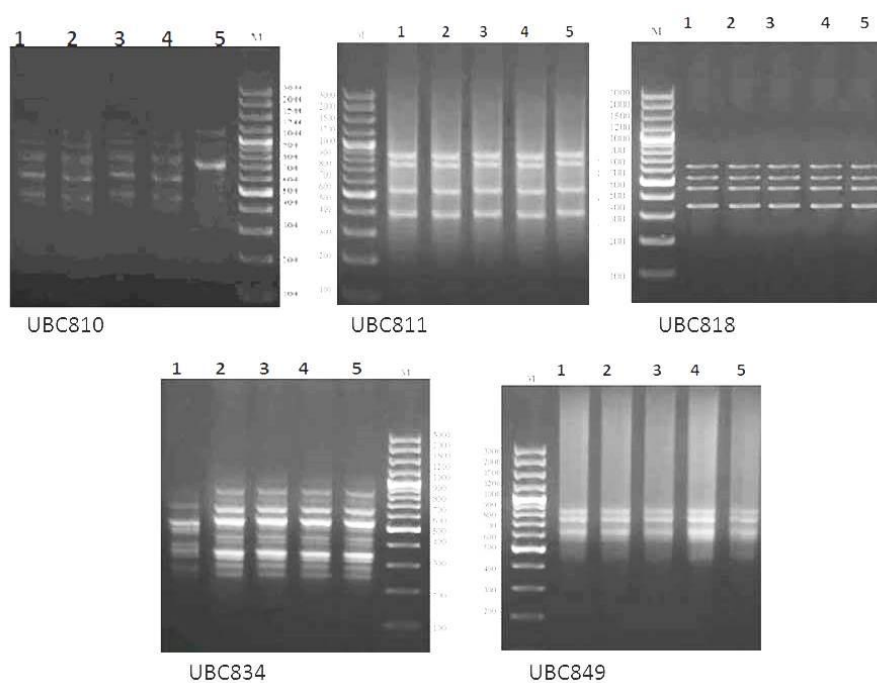
No.	ID	Sequence(5'→3')	Annealing Temp.
1	UBC810	GTGTGTGTGTGTGTGTCA	33.125
2	UBC811	GAG AGA GAG AGA GAG AC	51.78
3	UBC818	CAC ACA CAC ACA CAC AG	51.78
4	UBC834	GAGAGAGAGAGAGAGAGAT	54.877
5	UBC849	GAGAGAGAGAGAGAGAT	47.406

A: Adenine, T: Thymine, G: Guanine, C: Cytosine.

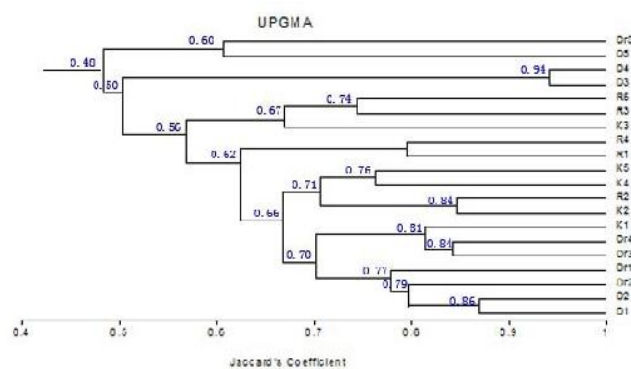
A total of 15 primers were used for ISSR-PCR optimization trials, 5 primers, which gave the best amplification results with the entire sample DNA, were selected for the final ISSR-PCR analysis. Five primers yielded 43 clearly identifiable bands ranging in size from 200 bp to 1000 bp. The number of polymorphic loci was 20 and the percentage of polymorphism was 90.47% table (2).

Figures (1) showed gel sliced for ISSR primers that gave clear segments, ranging from 200 to 1000 bp in size. Which gave primer UBC810 (8) bands, (11) bands in UBC811 whereas in UBC818 (7) bands, for UBC834 (12) bands, while (4) bands in UBC849, The total number of bands resulting from 5 primers 42 bands, matched 20 genetic bands .The percentage of polymorphic bands is 90.47% .

The total dendrogram obtained using Jaccard's Coefficient has one main cluster. UPGMA dendrogram (Figure 2) shows the two clusters. Cluster one consists of one sub cluster with similarity 60.6%. The cluster two consists of one sub cluster dividing to two branches with similarity 91.4% .

Figure (1). ISSR banding pattern of *Heliotropium digynum***Table (2): The list of the primers and number of ISSR markers obtained**

Number of Primer	Primer	No. of bands	Polymorphic Bands	Monomorphic Bands	% Polymorphism
1	UBC810	8	6	2	75
2	UBC811	11	9	2	81.8
3	UBC818	7	7	0	100
4	UBC834	12	12	0	100
5	UBC849	4	4	0	100
Total		42	38	4	90.47%

Figure (2): UPGMA dendrogram of *Heliotropium digynum* for ISSR analysis

Discussion

Genetic fingerprinting results obtained using the ISSR technique, with five different primers (UBC810, UBC811, UBC818, UBC834 and UBC849) were able to distinguish between genotypes well as the percentage of genetic variation between untreated varieties were (90.47%)Table (2) and Figure (1). In this study, the highest genetic variability between varieties may be due to the historical, eco-geographical and genetic isolation of populations this different environmental conditions consistent with the finding of *Kumar and De Britto ., 2011* . Also the reason for higher genetic diversity revealed by microsatellite markers may be their codominant or multiallelic nature, hypervariability, high information content and amenability to automation (*Powell et al ., 1996*).

The fast evolutionary rate and the hypervariability of ISSR may suggest that ISSR bands represent neutral markers (*Esselman et al., 1999*) .

This result also confirms that ISSR marker is efficient in detecting polymorphism within and among populations and/or varieties of sunflower. In the present study, it suggested the existence of higher diversity among wild sunflower varieties. Thus, ISSR marker systems will provide a useful tool in the future design of collection strategies for conservation and use of wild sunflower varieties . Moreover, this marker observed to be very useful in detecting genetic diversity and population structure. These results were in agreement with many investigators who studied the genetic diversity of sunflower and other species using molecular markers with morphological traits (Mahmoud and Abdel-Fatah, 2012).

They suggested that the main factor responsible for the high level of differentiation among populations is probably the historical, geographical and genetic isolation of populations in a harsh mountainous environment (Kumar and De Britto ., 2011).

Conclusion:

The ISSR technique used here was found to be quite effective in determining the genetic variation among *Heliotropium digynum* genotypes. By knowing about the diversity of this species, used for conserving programme and exploit the highly quantitative active principle compound for medicinal purposes.

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