A novel Screening Methods using SSCP –PCR to Detect Variations Among three Varieties of Trigonella Foenum Graecum L. Collected from Different Regions in Iraq

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Abstract

Fenugreek is one of the most commonly used herbal medicinal plants. Another benefit of fenugreek seeds is that it is used to enhance the flavor, color and texture of fruit. Fenugrek comprises a number of chemical components such as trigonellines, choline, flavonoids and fixed oils. SSCP –PCR is useful to reach rare and endemic species' genetic diversity and also to resolve genetic links between populations. . PCR amplification of DNA. Genotyping of *ITS4* was performed using a polymerase chain reaction technique, followed by single-strand conformation polymorphism. Accordingly, these DNA polymorphisms were confirmed using DNA sequencing. The results appeared that the presence of three different haplotype patterns named according to the number of bands were 4-bands, 3-bands, 2 –bands using SSCP –PCR technique. The pattern of all resolved SSCP bands can however be difficult to establish using gel visualisation alone. This polymorphisms of DNA must then be verified by the use of DNA sequencing. The findings of the sequence shown that the haplotypes of the ITS region have been identified by several SNPs.

Conclusion: our findings indicated that SSCP-PCR technique is more informative for evaluation of genetic diversity and relationships among fenugreek populations.

Key words: *PCR, SSCP, sequencing, polymorphism, fenugreek*

Introduction

Fenugreek is an annual herb belonging to the Leguminoae family (Trigonella foenum graecum L). That is the well-known inhuman spices. Fenugreek's seeds and green leaves are both used in diet and medicine, the ancient tradition of human history [1].

The Fenugreek includes a number of chemical constituents, including trigonelline and choline, such as fibres, saponins, flavonoids, fixes, and alkaloids. Seeds of plants are economically attractive as they contain 1-2% steroidal saponins, particularly diosgenine [2]. Fenugreek is a versatile resource for diosgenin production, used as a synthesis medicine for more than 200 types of steroid, representing in fenugreek bioactive steroid saponin [3] and known in fenugreek as a fat-decreasing, antidiabetes, anti-cancer and antioxidants stressful substance [4]. [3]. The rDNA (ribosomal DNA) ITS region is made up of highly diverse areas which can be used for the study of recent diversification taxonomic classes, or indeed within populations. In contrast, ribosomal DNA, in the case of animals compared to ancient diversification, has strongly conserved areas [5]. However, studies so far have been limited by the nature of the markers utilized [6]. The single-stranded polymorphic conformation analysis is defined as an alternative method of genetic variants identification and genotyping in various species. The main advantage of the SSCP approach is that the genetic composition of multiple species can be sampled simultaneously and are considerably economical than other genetic analysis. In comparatively unrealized laboratory settings, the approach outlined here can be

used with very basic equipment and thus be useful for both clinical laboratories and research laboratories [9].

Due to its relative technical simplicity and high mutation detectability the PCR-coupled SSCP is a powerful tool to detect genetic polymorphism and to screen for genetic efficacy, the capacity of SSCP to detect point mutations in different Genes was clearly demonstrated and significant savings in cost, time and effort have been saved for the SSCP analysis of large samples [8].

Though these procedures have been shown to be useful for the ribosomal and mitochondrial DNA study, they have the ability to be used in any gene in an organism [9].

Rapid technological advancement has allowed biologists to generate large, high quality sequencing readings at reduced costs [10,11]. The key discrepancies between different DNA platforms in library generation, amplification technology and sequence identification recording methodology [12].

The objective of this work was to demonstrate the effectiveness of the use of SSCP-PCr technique for research into trigonella foenum graecum L'phylogenetic connections isolated from different areas of our country.

Methodology

Sampling

Fenugreek samples were collected from three different regions in Iraq, North (Sulaymaniyah) Middle (Hilla) and South (Basrah) between September 2020 to December 2020. Before being used, the samples were held in sterile plastic bags and transferred to the laboratory in the deep freeze.

Genotypic identification

DNA Extraction

DNA of the plant Trigonella foenum graecum was collected and purified using the "wizbio" extraction and clearing kit (south Korea).

Primers

For ITS region obtained from Bioneer, IDTDNA(USA). DNA of *Trigonella foenum graecum plant* were tested for specific primer for SSCP -PCR technique (table1).

Table (1): Primer Sequences used in this study

primer name	Sequence 5----------3
ITS 3	5- GCATCGATGAAGAACG CAGC-3
ITS4	5- TCCTCCGCTTATTGATATGC-3

PCR amplification

Final result of 25µl reaction volumes of 1 ul. forward and reverse primer, 12.5 ul. green Master, 3 ul. Genomic deoxyribonucleic acid, and even reaction rate, rounded off to 25ul. In a very thermo-cycler (Eppendorf), programmed for 5 minutes, amplification was given. At 94°C temperature, 35cycles 1 min at 94oC, 1 min at 55oC and 2 min at 72°C; and a final extension at 10 min at 72°C. A total amount is available to be achieved at 35cycles 1 min. At 94°C. In 2% agarose gels a product amplification was electrophored and then visualized by ethidium bromide. standard molecular markers were conjointly enclosed in every electrophoresis run. Ultraviolet trans-illuminated gels have been captures as photographs.

SSCP- Sequencing

It has been stated that SSCP goods are better to run on minigels to sum up time and cost [13]. The pattern of all resolved SSCP bands can however be difficult to establish using gel visualisation alone. This polymorphisms of DNA must then be verified by the use of DNA sequencing.

Results

Genotypes of *Trigonella foenum-graecum* L Using Universal Primer

For general genotyping the genomic DNA has been amplified with certain primers and carried out under the optimum conditions described above by the thermo-

cycling device The findings showed that a single band was present. (400 bp)

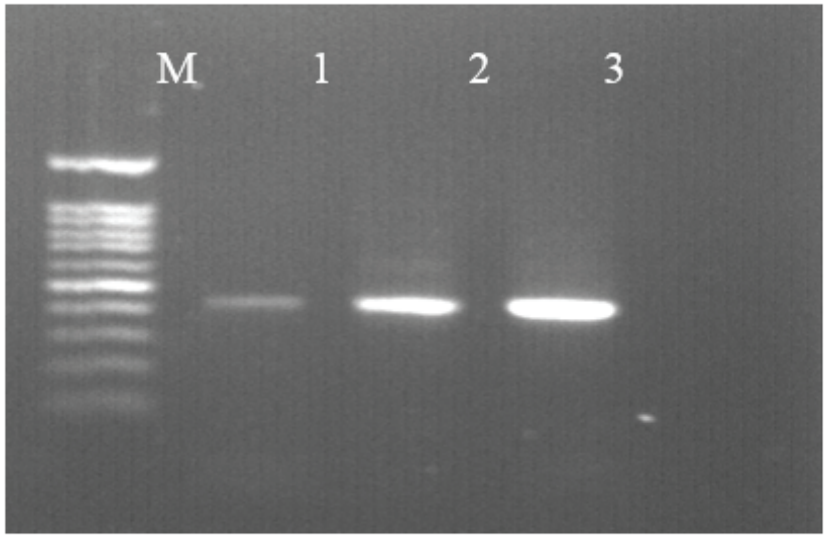

Fig. (1): Agarose gel electrophoresis of *ITS* 4 amplified product:1(North), 2 (South) and 3 (Middle) of Iraq.

4.3 PCR –SSCP

After the target area was amplified, the genotyping of the ITS4 region by PCR-SSCP approach was examined. The results indicated that, as shown in figure (2) of PCR-SSCP gel Electrophoresis (PCR-SSCP gel photo), there were three separate haplotype models named by the number of bands. The single stranded (ssDNA) DNA bands that occupy the top of the gel and the double stranded (dsDNA) bands that occupy the bottom portion of the gel have been detected. The ssDNA variations in SSCP gels are used to determine each amplified's genetic pattern.

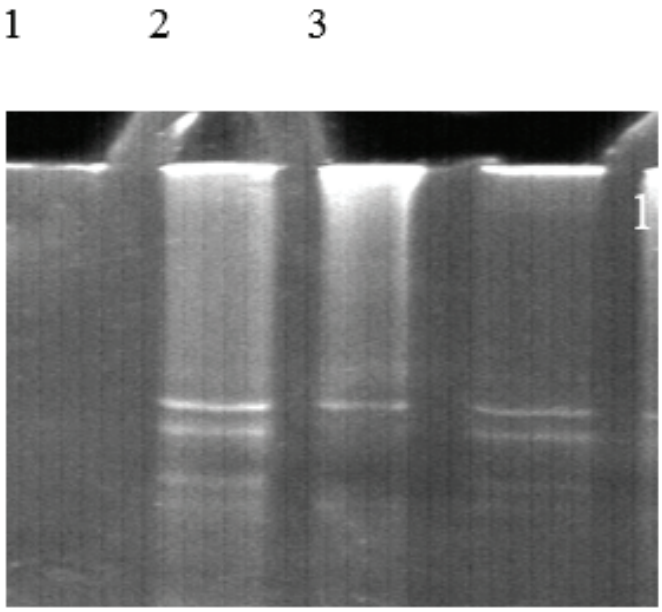

Fig. (2): *ITS* region polymorphisms according to the number of the bands using PCR-SSCP method

Electrophoresis conditions: 8% polyacrylamide gel concentration; 200V (7.5V/cm) – 100mA, run time 90 - 120 min. Staining method; ethidium bromide.

The pattern of all resolved SSCP bands can however be difficult to establish using gel visualisation alone. This polymorphisms of DNA must then be verified by the use of DNA sequencing. The sequence (Fig. 2) findings indicate that several SNPs have identified the ITS area haplotypes in line with the NCBI Blast

Discussion

The estimate of genetic diversity was a priority of plant curators, since it is the first step towards improving crops in developing global scenarios, including food safety, starvation, global warming and climate change. There has been a higher threshold of marginal plants and medicinal plants such as fenugreek because more services are being used for staple or commercial plants [14]. Different molecular and biochemical techniques are crucial to the detection of various seed genotypes [14,15,16].

After the target area was amplified, the genotyping of the ITS4 region by PCR-SSCP approach was examined. The results indicated that, as shown in figure (2) of PCR-SSCP gel Electrophoresis (PCR-SSCP gel proto), there were three separate haplotype models named by the number of stripes. The research findings accepted that the ITS regions are of large varying size and series with Chalmers et al[9]. Furthermore, the small size of the ITS region (>700 bp in angiosperms) and highly preserved sequences that fly in this region are easy to amplify from even herbary materials using universally constructed eukaryotic primers [5].

The SSCP theory is based upon the sensitivity of nucleic acid to both size and form of electrophoretic mobility of the non-denaturating gel. Denaturation processes have led dsDNAs with double stranded DNAs) to be converted into ssDNAs with one strand. In contrast to dsDNAs, ssDNAs are modular and follow a conformation that is peculiar to their sequence structure based on intramolecular interactions and basic folding. This conformation of ssDNAs can also influence the modification of one foundation which is identified by variations in substitution sequences such as insertions and deletions in separate electrophoretic movement [17]. Also, Genomic variations measured in total detectable fragments of the RFLP by the number of polymorphic fragments. The disparity may be due to the prevalence of genetic polymorphism in non-coding regions [18,5]. The methodology of SSCP, however, is good technique for polymorphic identification. And it demands quite a lot of high quality because the SSCP processes are dependent on nucleotid heterogeneity of haplotypes of homologous sequences between two genomes and it is not wonderful if we do not use the tool to detect a comparable estimate of genomic diversity.

A reproductive, fast and very easy approach to detect deletions/insertions/rearrangements of polymerase chain reaction amplified DNA [16, 20, 21] is a single strand conformation polymorphism [20, 21].

Homogeneity of DNA sequences is a requirement in the molecular phylogenetic analysis before they are implemented. In phylogenetics, internal ribosomal DNA transcribed spacer (ITS) area (nrDNA) is typical but it is also very difficult to achieve a homogenous sequence. For the identification of homogeneity for nine pooled amplified ITS products we use the single-stranded conformation polymorphism (SAPS) process. Our findings show that the SSCP mechanism was used before use in sequencing processes for detecting homogeneity of the ITS region [22].

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