International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 20-05-2021, Accepted: 05-06-2021, Published: 21-06-2021
Volume 6, Issue 3, 2021, Page No. 751-752

Molecular analysis of the genus *Gentiana* growing in Azerbaijan

Aygun Zeynalova
Phd student, Department of Herbarium, Institut of Botany, Azerbaijan National Academy of Sciences, Baku, Azerbaijan

Abstract
Gentiana is the most common genus in the family Gentianaceae. The first description of the genus was given by K. Linney. The flower structure of this genus was first described by A. Grisebach. It is an annual or perennial plant of Mediterranean origin. The chloroplast DNA of 6 species from the genus was studied by PCR-RFLP analysis. The amplified DNA fragments were documented by using UV transilluminator-Biostep. Sequencing was performed on ABI 3730XL automated sequencer. The molecular- phylogenetic analysis has been conducted according to Tamura-Nei model.

Keywords: *Gentiana*, flower, molecular analysis, DNA, fragments

Introduction

Material and methods

Plant material: Plant materials were collected from 6 samples growing in Caucasus territories of (Nakhchivan, Guba) Azerbaijan.

Sample Preparation for DNA extraction

100-200 mg leaflet from each sample is placed into 2 ml tubes. After the closed tubes are stored in liquid nitrogen a few minutes, a plant material is crumpled thoroughly. 0.7 ml extraction buffer which was heated at 65 ^{0}C in advance is poured on the crushed sample (inside the tube). The tubes are put in the water bath with 65 ^{0}C within 60 minutes and mixed every 10 minutes carefully. The same volume (0.7 ml) of chloroform-isoamyl alcohol (24:1) is added and mixed carefully. The tubes are centrifuged at a velocity of 13000 rpm during 15 minutes. Then the solution accumulated on the upper part of the tube is transferred to another 2 ml tube cautiously and the same volume of cold isopropanol (-20 ^{0}C) is poured on it and it is put into - 20 ^{0}C refrigerator for a night. Afterwards, the tubes are centrifuged at a velocity of 13000 rpm again and the isopropanol is thrown by filtrating completely. 0.7 ml washing buffer is added on it and is centrifuged at a velocity of 13000 rpm during 5 minutes. This process is repeated twice. After the washing buffer is filtrated completely, the tubes are kept open for a night. 0.1 TE buffer is added on the dry DNA and it is kept in refrigerator with - 20 ^{0}C for 1 night.

Checking of the amount of DNA and degree of purity

The amount of the extracted DNA is fixed in 260 and 280 nm wave length in spectrophotometer (Nano Drop 2000c UV-Vis Spechtrophotometer-Thermo Scientific). The mixture used for determination of the amount consists of 20 µl extracted DNA and 1980 µl ddH2O or TE buffer. The density of DNA in solution is calculated as: the density of DNA (ng/µl) = (OS260x100 (factor of dilution) x 50 ng/ml)/ ratio between optical density in 1000.260 and 280 nm (OS260/OS280) shows the degree of purity of nucleic acids. Optimal degree of purity for Polymerase chain reaction is 1.8-2.0 (Table 1). Upon the determination of the amount of DNA, 50 ng/ µl thickness working solutions are made of every sample DNA for carrying out Polymerase chain reaction.

Table 1: Checking of the amount of DNA and degree of purity

Type name	Code	Date	Conc.	A260/280	Conc.	A260/280	Conc.	A260/280
Gentiana septemfida	A1G1	15.12.2017	110.1	1.94	111.1	1.93	112.4	1.94
Gentiana gelida	AG4	15.12.2017	125.8	1.79	126.4	1.80	124.5	1.82
Gentiana pyrenaica	AG2	15.12.2017	95.1	1.60	96.3	1.62	100	1.60
Gentiana nivalis	A6	15.12.2017	125.6	1.90	126.5	1.93	124.6	1.91
Gentiana blepharophora	A3	15.12.2017	410.2	1.99	416.8	1.98	409.4	1.99
Gentiana cruciata	A5	15.12.2017	155.3	1.83	155	1.88	155.2	1.85

The reaction volume per sample was 20 µl (2 µl sample DNA-18 µl reaction mixture). Two ITS primers were used for the analysis (Table 2). The whole reaction must be carried out in the ice and the received solutions must be vortexed firstly, then centrifuged. It is added to TAQ polymerase reaction mixture at the end by mixing and the general reaction mixture is vortexed well. The specified volume DNA is firstly put into the tubes or PCR plate and kept in the ice. Then 18 µl from the reaction mixture is poured on each sample and vortexed again. The tubes were placed into PCR apparatus (Gene Amp PCR System 2720, Applied Biosystems) and the following program was applied (table 1).

The PCR products were applied on a 1.5% ethidium bromide-stained agarose gel in 1× TBE (Tris–Borate–EDTA) buffer. (figure 1).

Table 2: Cycles and duration of Polymerase Chain Reaction.

PCR temperature	Duration	Cycle
94 ^{0}C	3 minutes	1
94 ^{0}C	15 minutes	
50 ^{0}C	15 minutes	35
72 ^{0}C	30 minutes	
72 ^{0}C	5 minutes	1

Table 2: Nucleotide sequence of ITS primers (5'-3').

DNA region	Primer	Nucleotide sequence 5'-3'	Reference
ITS	*ITS 1*	TCC GTA GGT GAA CCT GCG G	White and others. 1990
	ITS 4	TCC TCC GCT TAT TGA TAT GC	White and others. 1990

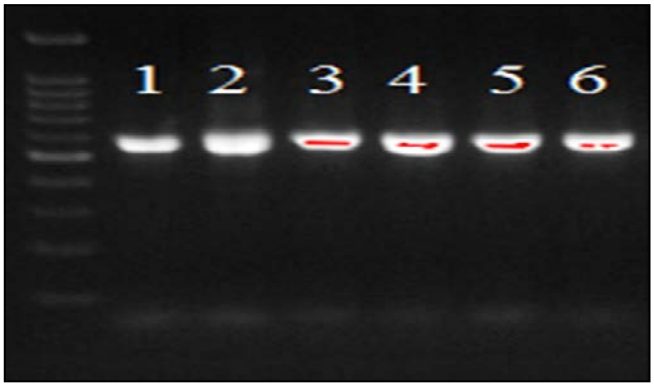


Fig 1: Checking of the quality of PCR products in agarose gel.

Results and Discussions

Sequencing

Obtained PCR products have been refined by using of QIAquick Gel Extraction Kit (Qiagen, GermanY) set before sequencing. The nucleotide sequences obtained have been sorted by means of ClustalW program (Thomson and others 1994). The results of some species have been taken from American GenBank information centre. By including the sorted nucleotide sequences in Molecular Evolutionary Genetics Analysis (MEGA 6.0) program, the molecular-phylogenetic analysis has been conducted. According to Tamura-Nei model, a phylogenetic tree has been built using the method of Maximum Likelihood (ML) by repeating 500 (Felsenstein 1985) [7].

By comparing the obtained nucleotide sequences with those in USA GenBank information centre, the following results have been gained:

S1 sample with GenBank data conforms with *Gentiana septemfida* type.

S2 sample with GenBank data conforms with *Gentiana pyrenaica* type.

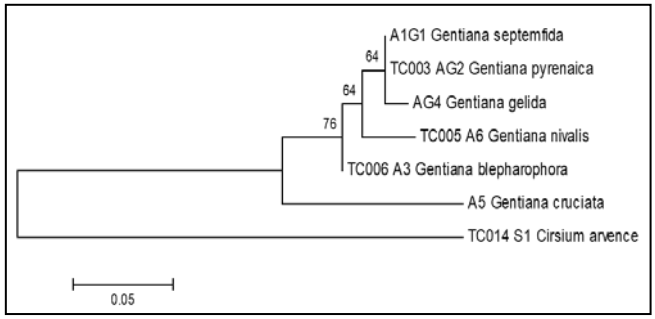
S3 sample with GenBank data conforms with *Gentiana gelida* type.

S4 sample with GenBank data conforms with *Gentiana nivalis* type.

S5 sample with GenBank data conforms with *Gentiana blepharophora* type.

S6 sample with GenBank data conforms with *Gentiana cruciata* type.

Circium arvence has been received for comparison (dendrogram 1.).



Dendrogram 1: Phylogenetic tree of *ITS* gene region in nuclear genome of some Gentiana species according to Maximum Likelihood (ML) method

Referenses

1. Flora of Azerbaijan. Baku, tt. VII. Izd. AN Azerbaijan. SSR, 1957, 87-99
2. Grossheim AA. Analysis of flora of the Caucasus. AzFAN USSR, 1939, 230.
3. Talibov TH, Ibrahimov A. Sh. Taxonomic flora of the Nakhchivan Autonomous Republic spectrum (Higher spore, bare-seeded and cover-seeded plants) Nakhchivan: Ajami, 2008, 364.
4. Xiao-Lan Zhang, Yu-Jin Wang, Xue-Jun Ge, Yong-Ming Yuan, Hui-Ling Yang, Jian-Quan Liu.
5. Molecular phylogeny and biogeography of Gentiana sect. Cruciata (Gentianaceae) based on four chloroplast DNA datasets
6. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue, Phytochem. Bull,1987:19:11-15.
7. Eva Häffner, Frank H. Hellwig. Phylogeny of the tribe Cardueae (Compositae) with emphasis on the subtribe Carduinae: an analysis based on ITS sequence data. Willdenowia.1999:29(1-2):27-39.
8. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution,1985:39:783-791.
9. Dong WP, Liu J, Yu J, Wang L, Zhou SL. Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding, 2012, PLoS One 7, e 35071.
10. Gadjiyev VD, Aliyev DA, Kuliyev VSh, Vagabov ZV. Alpine vegetation of the Minor Caucasus. Baku, 1990, 212.
11. Dequan Zhang, Bei Jiang, Lizhen Duan, Nong Zhou. Internal Transribed Spacer (ITS), an Ideal dna Barkode For Spesies Discrimination in Crawfurdia Wall. (GENTIANACEAE).