Proceedings of Pakistan Society for Horticultural Science
2nd International Conference on Horticultural Sciences, February 18-20, 2016
Theme: Production Challenges and Food Security
Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Punjab 38040, Pakistan

Analysis of Genetic Diversity as a Key to Conserve *Berberis baluchistanica* ahrendt. An Endemic Species to Balochistan

Shazia Saeed*[1], Muhammad Younas Khan Barozai[1], Alia Ahmed[1], Rasool Baksh Tareen[1], Sania Begum[2], Ghulam Muhammad Ali[2]
[1]Department of Botany, University of Balochistan Quetta
[2]National Institutes of Genomics and Advanced Biotechnology (NIGAB), NARC, Islamabad, Pakistan
Email: shazia_botany@yahoo.com (S.S)

Abstract

Berberis is an important genus of Berberidaceae. This genus is of great economic and medicinal value. *Berberis baluchistanica* (endemic to Balochistan) selected for this study is used traditionally by local communities to cure different ailments such as internal injuries and infection of human and livestock, thus over collection of this significant taxa leads to its population depletion. Genetic diversity of *B. baluchistanica* has been studied for the first time from different sites of Zarghoon and Sra Ghurgai (Takatu-mountain range) of Balochistan from 2012-2014 by using Simple Sequence Repeats (SSR). Out of total sampled genotypes six were selected for this study. The morphological and molecular variation was estimated by Numerical Taxonomy and Multivariate Analysis System (NTSYS pc). DNA Amplification of all genotypes was studied by using SSR markers. Out of seven primer pairs of SSR markers, four amplified twenty-seven allelic variants with twenty-two polymorphic bands (81%) with an average of 5.5 amplified per primer. UPGMA method was used for cluster analysis indicating some genetic variability in investigated population. The average genetic similarity coefficient among the genotypes indicated that Zarghoon area population is genetically more diverse when compared with Sra Ghurgai (Takatu mountain range) may indicate that area is more disturbed anthropogenically resulting in fragmented population pattern. Data analyzed providing a base line study for this important species to be conserved by implementing different conservation strategies.

INTRODUCTION

Berberis baluchistanica Ahrendt, Vern. Zrlag of family Berberidaceae is one of the most valuable plant of Balochistan. This province is well known for its wealth of large number of medicinal plants and unique plants being used by human and animals. These plants are endemic to Balochistan, Pakistan. *B. baluchistanica* is a wild plant used as fodder for grazing animals this plant is also used as medicine by local communities.

Stem color vary from reddish to brown with smooth to sulcate surface. Leaves are simple, color differ from olive to pale green in cluster beneath which spines are present. Flowers yellow mostly raceme. Berries are ovoid to sub-orbiculate colour ranged from reddish to brown. Ethyl acetate soluble fraction of this plant is rich in valuable antioxidative phyto constituents and can play its role to protect human body cells from damaging due to oxidative stress. It might also be utilized to preserve food products (Abassi *et al.*, 2013). Methanolic extract of roots of *B. baluchistanica* exhibit antibacterial activity (Khan *et al.*, 2012). The antifungal activity of the methanolic extract and different fractions from roots of *B. baluchistanica* retain good antifungal activity against *Microsporum canis, Candida albicans*, *Aspergillus flavus*, and *Candida glaberata* (Khan *et al.*, 2012)

Genetic diversity is the level of biodiversity that refers to the total numbers of genetic characterizations in the genetic makeup of a species and plays an important role in the survival and adaptability of species (Volis *et al.*, 2001). Genetic diversity among individuals or populations can be assessed using different markers. Although earlier studies have reported some results of research on chemical, morphological, anatomical and ecological characters of other species, and few reports on genetic diversity elucidated the identification and characterization of Microsatellite markers in *Berberis microphylla*, these simple sequence repeats markers are very polymorphic and potentially useful in genetic studies in any species of the genus Berberis Varas *et al.*, 2013.

It should be emphasized that genetic diversity of natural populations of this significant wild medicinal plant *Berberis baluchistanica* was not studied previously. This is the first report on morphological and genetic variation of *B. baluchistanica* endemic to Balochistan by using microsetallite markers developed by Varas *et al.,* 2013 from *Berberis microphylla.*

MATERIALS AND METHODS

Site Selection

Two sites were selected for the study (Table 1) From each site three samples were selected.

Table 1: Ecological characteristics of study sites.

Sr. no.	Sampling site	Site code	Latitude (N)	Longitude (E)	Elevation (m)	Annual rain fall	Temp.
1	Sra gurgai	A	30.17	67.01	1660-2133	104-200 mm	-15 to 30ºC
2	Zarghoon	B	30.21	67.12	2200-3200	200-400 mm	-16 to 34ºC

Plant Material

Plants were sampled from different locations grouped in two geographic Zones at different altitude. Fresh and young leaves were collected and stored at ultra-low freezer at NIGAB, NARC Islamabad. Samples were submitted into Herbarium of Botany Department, University of Balochistan for future references.

DNA Extraction and Purification

Extraction of total DNA was performed using CTAB protocol according to Badr *et al.*, 2012. Also modified with phenolic extraction to remove the phenolic content by

using phenol: chloroform: isoamyl Alcohol (25:24:1). Samples were extracted without using liquid nitrogen. DNA concentration was determined using visual method. The DNA samples were electrophoresed in 1% agarose gel against 10 µl of a DNA marker (100bp Ladder). The estimation of DNA concentration in each sample was further checked on Biospec-Nano at 260/280 nm.

PCR Reaction:

Seven SSR primers was used to detect polymorphism among the six genotypes, 4 of them were able to detect the polymorphism (Table 2). The amplification reaction was carried out in 20 µl reaction volume containing 5X Dream Taq buffer, 2 mM dNTPs mix, 20 pmol primers from Gene Link USA, 10 ng DNA, and 0.2 µl Dream Taq DNA polymerase (5 U/µl).

Table 2: Primers information gives DNA amplification.

Locus primer	sequences (5 ′ –3 ′)	Size range (bp)
BmLP-05	F: AACACCTGGTTCAACTTGCG R: TGCTGCTACTGACTCTTCCG	110–130
BmLP-07	F: CGAAAATCTCGGGAATGGGC R: TGCCTGAAAAGTGTTGGCAC	110–140
BmLP-09	F: CATCCATCTCTTGGGAATTCAAC R: CGAAAATCTCGGGAATGGGC	110–130
BmLP-11	F: GGAAGGAGAGCGAAAATCGAC R: TGAGATGAAGGCATACATGAGC	90–120

Table 3: Morphological Characteristics Status of *Berberis baluchistanica* from Sra Ghurgai and Zarghoon.

Sr. no.	Character	Status	Code
SG	Stem color	Redish brown	1
ZR		Brown	0
SG	Stem surface	Sulcate	1
ZR		Smooth	0
SG	Internode	2.5cm	1
ZR		2.5-3cm	1
SG	Spines	2cm	1
ZR		1.2cm	0
SG	Leaf shape	Obvate-oblong	1
ZR		Sub-orbicular	0
SG	Leaf color	Green	1
ZR		Yellowish green	0
SG	Berries	Ovoid	1
ZR		Sub-orbiculate	0
SG	Berry color	Brown	1
ZR		Red	0

PCR amplification was performed through 35 cycles after an initial denaturation cycle for 3 minutes at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 45 seconds, an annealing temperature optimize differently range 50-55ºC for 45 second, an

extension step at 72ºC for 2 minutes, and an additional extension cycle for 5 minutes at 72ºC in the final cycle. The amplification products were then resolved by electrophoresis in 1.8% agarose gel containing ethidium bromide (0.5µg / ml) in 1X TAE buffer at 110 volts for 35-45 minutes, then PCR products were visualized on UV light and photographed using Gel documentation, and the size of markers was estimated by comparing to the standard marker included in the gel.

Data Analysis

The amplified bands were scored as 1 (present) and 0 (absent), SSR data were clustered and dendrograms based on similarity matrices were generated using the paired group method by using NTSYS 2.10j (Rohlf, 1998). Polymorphic information content (PIC) was calculated using the following formula: PIC = $1 - \Sigma (P_i)^2$, where P_i *is* the proportion of genotypes carrying the *i*th allele (Botstein *et al.,* 1980*)*.

RESULTS AND DISCUSSION

Microsatellite markers were isolated and characterized in *Berberis microphylla* Varas *et al.*, 2013 are used in this study to evaluate genetic diversity *in B. baluchistanica* an endemic specie of Balochistan. Results revealed the clear genetic diversity of selected genotypes. The product size of SG ranged from 90bp to 110 bp. Where as in Site ZR1-4 it ranged from 90 to 220 bp. DNA Amplification of all genotypes were studied by using SSR markers. Out of seven primer pairs of SSR markers, four amplified 27 allelic variants with 72 polymorphic bands (81%). Polymorphic bands (%) with an average of 5.5 amplified per primer (Table 4). The average similarity coefficient among the genotypes of *B. baluchistanica* of two different populations ranged from (0.32-0.77) (Table 5). The UPGMA tree produced by the SSR marker also revealed the more genetic diversity within population of site B than site A. Morphological characteristics of the taxon also recorded that is in relation to our molecular data. Similar results were earlier reported by Nazar and Mahmood 2011.

Overall our result revealed that maximum genetic diversity is estimated in Zarghoon (semi-conserved) regions population. Whereas less diversity observed among Sra gurgai population in juniper track (Figure 1). It is assumed that this might be due to over collection of the plant for its medicinal value. Roots are considered as an important source of antioxidant and healing of wounds of cattle so its conservation is highly recommended otherwise this important endemic medicinal plant would be near to extinction in future.

ACKNOWLEDGEMENTS

Author is thankful to Taj Muhammad chief conservator Forest Department, Balochistan, for field surveys. Author also would like to thank Dr. G.M. Ali director National Institute of Genomics and Advanced Biotechnology NIGAB, NARC Islamabad for facilitating this research.

Conflict of Interest: The authors declare that they have no conflict of interest.

Table 4: Primers analyses and number of DNA polymorphic bands produced.

Primer	**T_A (°C)**	**T**	**P**	**(%) P**	**PIC**
BmLP-05	55	6	6	100%	0.95
BmLP-07	52	10	5	50%	0.84
BmLP-09	55	5	5	100%	0.96
BmLP-11	55	6	6	100%	0.95
TOTAL		**27**	**22**	**81%**	**0.72**

Note: T_A(°C) (annealing temperature), T (Total bands), P (Number of polymorphic bands), %*P*, percentage of polymorphism, PIC (Polymorphic Information Content).

Table 5: Similarity index of *B. baluchistanica based* on SSR data analysis.

	A1	**A2**	**B1**	**B2**	**B3**	**B4**
A1	*					
A2	0.55	*				
B1	0.55	0.77	*			
B2	0.38	0.55	0.32	*		
B3	0.55	0.33	0.33	0.33	*	
B4	0.44	0.66	0.44	0.66	0.44	*

Figure 1: *B. baluchistanica* in Zarghoon.

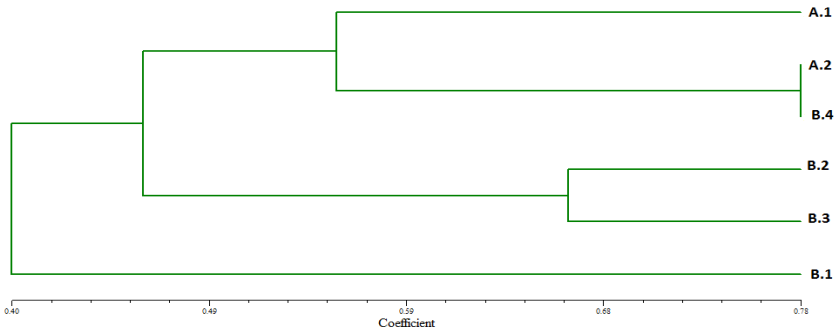

Figure 2: UPGMA dendrogram based on data generated from SSR primers, showing the genetic distance among the studied populations (listed in Table 1).

REFERENCES

Abbasi, M.A., S.S.H. Naqvi, A. Rehman and R.B. Tareen. 2013. B*erberis baluchistanica* Assdesment of Natural antioxidant to reprieve from oxidative stress. Int. Res J. Pharm. 4(5):101-105.

Allen, J.M., G. Samuel, Obae , H. Mark, Brand, A. John, Silander, L. Kenneth, Jones, O. Schyler, Nunziata, L. Stacey and Lance. 2012. Development and characterization of microsatellite Markers for *Berberis thunbergii* (berberidaceae). A. J. B. 220-222.

Badr, A., Z. Abo-El-Khier, A. Ghada, A. Hegazi, Abd-El-Kawi and A. El-Sawy. 2012. Genetic variation in seven natural populations of *Artemisia judaica* L. in South Sinai using RAPD Markers. WA Sci J. 18(10):1475-1480.

Botstein, D., R.L. White, M. Skolnick and R.W. Davis. 1980. Construction of genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32:314-331.

Khan, A.A., N.U. Baloch, S. Nabi, M.J. Khan, M.S. Jamali and Y.M.S.A. Al-Kahraman. 2012. In vitro antimicrobial and insecticidal activity of methanolic extract and its fractions of B*erberis baluchistanica* roots. WJPR. 2(1):219-226.

Nazar, N. and T. Mahmood. 2011. Morphological and molecular characterization of selected Artemisia species from Rawalakot, Azad Jammu and Kashmir. Acta Physiol Plant. 33:625-633.

Rohlf, J. 1998. NTSYS pc numerical taxonomy and multivariate analysis system version 2.0 User Guide F. Department of Ecology and Evolution State University of New York Stony Brook, NY. 11794-5245.

Varas, B, M.H. Castro, R. Rodriguez, D. Von baer, C. Mardones and P. Hinrichsen. 2013. Identification and characterization of microsatellites from Calafate (*Berberis microphylla*, berberidaceae). App in Plant Sci. 1(7):1-5.