Gene Reports 10 (2018) 157–161

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

Genetic diversity analysis of *Leptadenia pyrotechnica* in Jodhpur region of India

Shari Nair[a], Juleri M. Upendra[a,*], Satyawada R. Rao[b], Harchand R. Dagla[a]

[a] Plant Biotechnology and Molecular Biology Laboratory, Department of Botany, Jai Narain Vyas University, Jodhpur, India
[b] Plant Biotechnology Laboratory, Department of Biotechnology and Bioinformatics, North-Eastern Hill University, Shillong, India

ARTICLE INFO

Keywords:
Arid environment
Leptadenia pyrotechnica
Molecular markers
Genetic diversity analysis

ABSTRACT

Genetic diversity of *Leptadenia pyrotechnica* were analysed by RAPD, ISSR and ISJ genetic markers from Jodhpur region of India in present investigation. Cumulative data of three SPAR markers revealed 37.6% polymorphism. Maximum 73% genetic variability was observed within population than 27% between populations. Genetic similarity and differences based on UPGMA dendrogram and PCo Analysis are closely related to their geographical distances in natural habitats of *Leptadenia pyrotechnica*. Geographically Agolai and Rohat populations are distantly located than Bilara and Soila populations in natural habitats and is also evidenced in UPGMA dendrogram and PCo Analysis. Jodhpur city may be a major physical barrier of cross pollination and genetic exchange between Agolai population from remaining four population of *L. pyrotechnica* in present investigation.

1. Introduction

The danger of plant genetic resources erosion was recognized in 1974 and International Board for Plant Genetic Resources (IBPGR) was established to develop global network to ensure collection, conservation and evaluation of genetic resources of plants and to make available to plant breeders, anywhere in the world. The United Nations prepared the Convention on Biological Diversity (CBD) and adopted it in 1992, recognizing that the biodiversity of organisms in the wild should be maintained not only for their own intrinsic value, but also on practical grounds. Forestry genetic resources have or may have an economic, scientific or social value for people (FAO, 1993). Genetic and epigenetic mechanisms and diversity analysis may help in basic understanding of habitat fragmentation and implications in stable ecosystems (Fahrig, 2003; Henle et al., 2004). Indiscriminate use, habitat fragmentation due to human settlement and other man made activities are some of the major threats to genetic diversity loss in the natural habitats. Variations in gene expressions due to abiotic and biotic stresses in the natural habitat have been used to assess the potential of genome flexibility and intrinsic adaptable evolution mechanisms in dynamic environments. Characterization, propagation and conservation of the plant genetic resources of the arid and semi-arid regions are required for ecological balance and prospects (Dagla and Shekhawat, 2005a, b; Dagla and Shekhawat, 2006; Dagla et al., 2007, 2012, 2014; Gehlot et al., 2014; Nair and Dagla, 2016; Upendra and Dagla, 2016). The PCR based Single Primer Amplification Reaction (SPAR) methods are being used as versatile tools for genetic diversity studies in plants and they collectively provide a comprehensive description of the nature and extent of the diversity (Gupta et al., 1994). SPAR methods are generally more reliable than allozyme's and other techniques to analyse genetic diversity (Ayres and Ryan, 1999). Random Amplified Polymorphic DNA (RAPD) (Williams et al., 1990), Inter Simple Sequence Repeats (ISSR) (Zietkiewicz et al., 1994) and Intron-exone Spliced Junction (ISJ) (Weining and Langridge, 1991) markers have been successfully used for identification and determination of the relationship at the species, population and cultivar levels. (ISJ) system is universal for fingerprinting of plants (Rafalski et al., 1997). Present studies were aimed at investigating effect of geographical distances and barrier on genetic diversity of *Leptadenia pyrotechnica* around Jodhpur city of Rajasthan, India using RAPD, ISSR and ISJ molecular markers.

L. pyrotechnica is a di or tri-chotomously branched keystone shrub of arid and semiarid environment of India and Africa and belongs to family Asclepiadaceae. It is a cross pollinated species and insects are main pollinators and carriers of pollinia (pollen masses) from one plant to another. Plant is known as Kheemp in Jodhpur region of India. Plant is a good source of medicine, fodder, fruits (Kheempoli; eaten raw), fibers, cord and thatching materials for huts. Plant also contains bioactive constituents such as steroidal glycosides, cardenolides, alkaloids,

Abbreviations: CTAB, Cetyl Trimethyl Ammonium Bromide; PCR, polymerase chain reaction; RAPD, Random Amplified Polymorphic DNA; ISSR, Inter Simple Sequence Repeat; ISJ, Intron Splice Junction; UPGMA, Unweighted Pair-Group Method with Arithmetic Averages; PCoA, Principle Coordinate Analysis

* Corresponding author.
E-mail address: juleri.marutiupendra@gmail.com (M.U. Juleri).

https://doi.org/10.1016/j.genrep.2017.12.006
Received 17 September 2016; Received in revised form 9 November 2017; Accepted 23 December 2017
Available online 27 December 2017

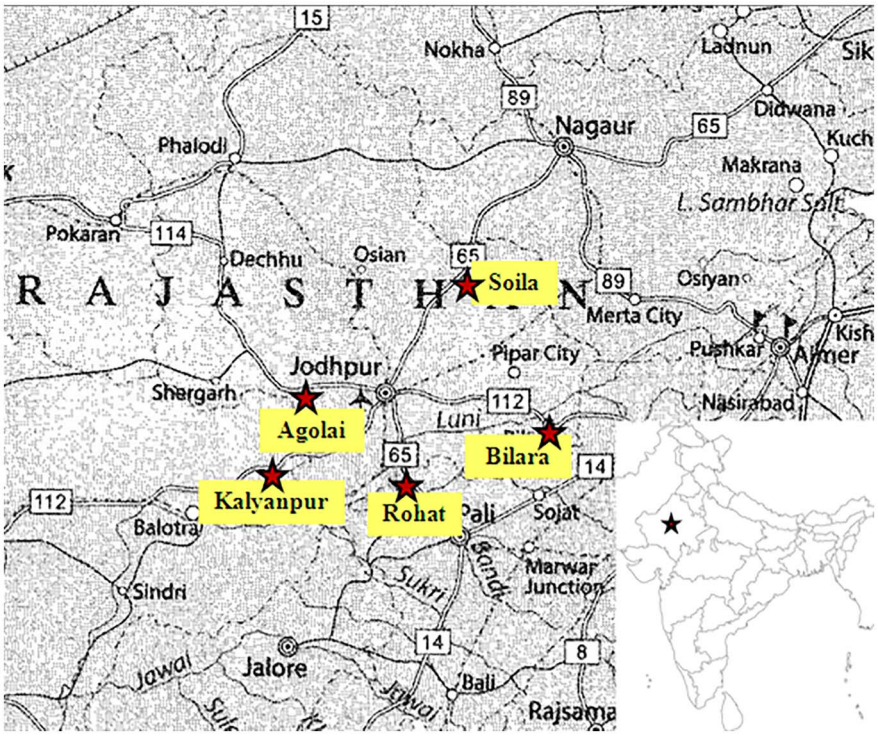

Fig. 1. Collection sites of *Leptadenia pyrotechnica* in Jodhpur region of Rajasthan.

flavonoids, triterpenes and polyoxypregnane derivatives (Cioffi et al., 2006).

2. Materials and methods

2.1. Plant material

Young shoot tips of *L. pyrotechnica* were collected from 5 sites around (60–80 km) the Jodhpur city of Rajasthan, India (Fig. 1, Table 1). These samples were frozen in liquid nitrogen, for DNA extraction and further genetic diversity analyses.

2.2. Extraction and purification of DNA

One gram of preserved young shoot tips were cleaned with 70% alcohol, powdered in a chilled mortar and pestle using liquid nitrogen. The DNA was extracted by Murray and Thompson (1980) method. The extraction buffer prepared using 2% CTAB, 1.4 M NaCl, 100 mM Tris-HCl, 20 mM EDTA and 3% 2-β-mercaptoethanol. The DNA was precipitated with chilled isopropyl alcohol and subsequently dissolved in TE buffer. Extracted DNA was treated with RNase A (Medox-Bio) to remove RNA. DNA was further purified by saturated phenol, chloroform: isoamyl alcohol (24:1) and precipitated in chilled absolute ethanol. Quality and quantity of the DNA were analysed by ELICO SL 164 double beam UV–Visible Spectrophotometer as well as with 0.85% (w/v) Agarose (Low EEO HIMEDIA, India) using Medox-Bio midi submarine gel electrophoresis in 1 × TAE buffer to ensure compatibility for PCR amplification.

Table 1
Collection sites of *Leptadenia pyrotechnica* and their geographical location.

S no	Sample code	Population location	Latitude (°N)	Longitude (°N)
1	LP Ago-02	Judia, Agolai	26° 17′ N	72° 38′ E
2	LP Ago-04	Jalan Nagar, Agolai		
3	LP Ago-05	Dhadhaniya Sasan, Agolai		
4	LP Bil-02	Ransi, Bilara	26° 10′ N	73° 42′ E
5	LP Bil-03	Pichiyak, Bilara		
6	LP Bil-04	Luni River, Bilara		
7	LP Kal-02	Sarvadi Purohitan, Kalyanpur	26° 01′ N	72° 34′ E
8	LP Kal-03	Sarvadi, Kalyanpur		
9	LP Kal-04	Nimbakhera, Kalyanpur		
10	LP Roh-02	Nimbla, Rohat	25° 57′ N	73° 08′ E
11	LP Roh-03	Kharabera Purohitan, Rohat		
12	LP Roh-06	Gandav, Rohat		
13	LP Soi-02	Kajnau Khaurd, Soila	26° 49′ N	73° 20′ E
14	LP Soi-04	Chantaliya, Soila		
15	LP Soi-05	Todiyana, Soila		

2.3. Screening of RAPD, ISSR and ISJ primers

The RAPD primers procured from Eurofins Genomics India Pvt. Ltd., Bangalore, India and ISSR, ISJ primers were procured from Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, India. Five each of RAPD, ISSR and ISJ primers were used for PCR amplification of *L. pyrotechnica* DNA template. The selection of primers for amplification of DNA was based on the clearness and reproducibility of fragments. PCR were performed in a 20 µl reaction mixture containing 50 ng DNA template, 2 µl of primer (10 µM/µl), 4 µl of dNTP's mix (2 mM/µl) (GeNei™, Bangalore, India), 2.5 µl of 10 × assay buffer (10 mM Tris HCl, pH 8.0 with 1.5 mM $MgCl_2$), 1 U of Taq DNA polymerase (3 U/µl) (GeNei™, Bangalore, India).Reaction mixtures were thermal cycled at 94 °C for 5 min followed by 35 cycles at 94 °C for 1 min, 37 °C (in case of the ISSR and ISJ amplification, annealing temperature was varied according to primers, Tm ranging from 45 to 55 °C) for 1 min and at 72 °C for 1 min 30 s, with a final extension at 72 °C for 7 min for polymerase chain reaction in BIO-RAD T100 Thermal Cycler. After amplification, 3 µl of 6 × tracking dyes (Bromo phenol blue, Xylene cyanol FF) were added to the amplified product and was resolved on 1.5% (w/v) agarose gel in 1 × TAE buffer with 1% EtBr. Amplified PCR products were run at 100 V current. The gel images were documented by BIO- RAD Gel Doc™ XR^+ System (Fig. 2).All the analyses were repeated thrice.