

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.506.018>

## DNA Quantification Studies of *Selaginella bryopteris* (L.) Bak. Genotypes in Telangana, India

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### ABSTRACT

#### Keywords

*Selaginella bryopteris* (L.) Bak., CTAB method, DNA isolation and Telangana.

#### Article Info

Accepted:  
11 May 2016  
Available Online:  
10 June 2016

*Selaginella bryopteris* (L.)Bak. is a resurrection plant with unique desiccation tolerance character. It is lithophytic, heterosporous, first vascular and potent medicinal herb belonging to the family Selaginellaceae. *Selaginella bryopteris* (L.) Bak. genotypes were identified, collected and preserved from various hilly regions of Rangareddy, Medak, Mahaboobnagar, Karimnagar and Warangal districts of Telangana, India. Collected plant genotypes were used to screen the genomic DNA, quantification and their analysis. For the DNA isolation modified CTAB method of Murray and Thomson was used. Quantity and quality of genomic DNA of *Selaginella bryopteris* (L.)Bak. genotypes were assessed by spectrophotometry and gel electrophoresis methods. The range of DNA Quantification values (251 ng/μl to 879ng/μl) and DNA Purity index values (1.13 to 2.37) of *Selaginella bryopteris* (L.) Bak. indicated the diversity among genotypes.

### Introduction

Arthur Garfit Alston was the first botanist to report *Selaginella* species from China and Philippines in the early years of 1930. *Selaginella* is the first vascular plant on the earth (Swetha Singh 2015). It has been existing from 300 million years (Reena Antony and Rini Thomas, 2011). *Selaginella bryopteris* (L.) Bak is an endemic pteridophytic species of India and Nepal distributed in warm hilly slopes between 400-1000 m altitudes (Swetha Singh 2015) and regions of Arawali Mountain (Sunitha Lahkar *et al.*, 2015). It is commonly called as Sanjeevani booti.

*Selaginella bryopteris* (L.) Bak. is also called as spike moss, it grows in rocks, litter and their own dead tissue of organic matter (Sunitha Lahker *et al.*, 2015). The plant has shown pharmacological activities such as anti bacterial (Agarwal and Singh, 1999), anti protozoal (Kunert *et al.*, 2008), anti inflammatory (Jitender malviya *et al.*, 2012), anti stomachache (Pandey *et al.*, 1993), anti stress and growth promoting activity (Sah *et al.*, 2005).

Isolation of the DNA is the pre requisite step for molecular and genetic diversity studies. Several protocols available for DNA

isolation especially for *Sellaginella* species are Sodium Dodecyl Sulphate method (SDS) of Dellaporta et al., 1983, Cetyl Tri Methyl Ammonium Bromide (CTAB) method of Murry and Thomson 1980, Doyle and Doyle (1987) and modified CTAB method of Rogers and Bendich 1994 (Sanyantani Das *et al.*, 2012).

CTAB method is simple, effective and most commonly used method (Pratibha Devi, 2005) to isolate the genomic DNA from different plant tissues like cotton (Chandra shekar and Lakshmi Bhavani, 2012), Red sandar (Jyothi Chaithanya *et al.*, 2013), ferns (Dempster *et al.*, 1999) and *Sellaginella* species like *S.delicatula*, *S.repanda*, *S.bryopteris*, *S.plana* and *S.monospora*. (Sanyantani Das *et al.*, 2012).

## Materials and Methods

Fresh and healthy *Selaginella bryopteris* (L.) Bak. plant materials obtained from various hilly regions of Telangana state especially in the regions of Rangareddy, Medak, Mahaboobnagar, Karimnagar and Warangal districts in various seasons of the year was wrapped in aluminum foils and stored at 4°C of temperature for future usages. For the present study fresh as well as frozen plant materials were used (table 1).

Total genomic DNA was extracted from *Selaginella bryopteris* (L.) Bak. plant material by CTAB method given by Murray & Thompson (1980) with some modifications. Approximately 1gm of the sample was masticated to fine suspension with CTAB buffer (100mM Tris, 20mM EDTA, 1.4M NaCl, 2% CTAB, pH 8.0). The fine suspension was taken into 2ml eppendorf tubes and incubated in a water bath at 65°C for 40 minutes with intermittent mixing for every 5 minutes.

The incubated samples were allowed to attain room temperature and an equal volume of chloroform: isoamyl alcohol was added gently, mixed for 15 minutes and centrifuged at 10,000rpm for 10 minutes. The aqueous layer obtained is transferred to 1.5ml eppendorf tubes. To these tubes containing supernatant, an equal volume of chilled isopropanol was added and gently mixed by inverting the tube, incubated for 10 minutes in refrigerator and centrifuged at 12,000rpm for 10 minutes. The supernatant obtained is discarded and the pellet is washed with 70% ethanol followed by centrifugation at 8000rpm for 5 minutes. This ethanol and centrifugation step is repeated twice. The pellet is air dried and dissolved in sterile distilled water/ T<sub>10</sub>E<sub>1</sub> buffer, where T<sub>10</sub>E<sub>1</sub> buffer facilitates for longer storage whereas sterile distilled water for shorter storage. The samples were stored in -20°C freezer for further use.

DNA quantification has been made by spectrophotometer which is a simple accurate and most popular method. Spectrophotometric measurements indicate the amount of ultra violet irradiation absorbed by the bases of the nucleic acid (Pratibha Devi, 2005). Purines and pyrimidines in nucleic acid show absorption maximum around 260nm (dATP: 259nm, dCTP: 272nm, dTTP: 247nm) if the DNA sample is pure without significant contamination from proteins or organic solvents (Sambrook, 1989).

The amount of DNA can be quantified using the formula:

DNA concentration (µg/ml) =

$$\frac{OD_{260} \times 100 (\text{dilution factor}) \times 50 \mu\text{g/ml}}{1000}$$

Gel quantification is a technique used to estimate the size and quantity of DNA

fragments in an agarose gel. The technique involves the comparison of a known quantity of DNA to an unknown quantity of DNA on an agarose gel.

**Results and Discussion**

The DNA quantification values of all the genotypes are calculated and presented in table 2 and plotted as graph and represented in graph (fig1). DNA purity was determined

by using of the ratio of OD260/OD280 and presented in table 3. Pure samples of DNA and RNA have value of 1.8 and 2 respectively (Pratibha Devi, 2005). The gel is stained with ethidium bromide and photographed. The brightness of a particular fragment, as well as the distance travelled, can be used to estimate the quantity and size of the DNA fragments (fig 2).

**Table.1** The sampling locations and their GPS (Global Position System)

Sampling site no.	Sampling site code	Location of Sampling	Latitude	Longitude
1	KNR-1	Shanigharam, Karimnagar(D)	18 <sup>0</sup> 11'32.84"N	79 <sup>0</sup> 00'32.57"E
2	KNR-2	Lingannapet, Karimnagar(D)	18 <sup>0</sup> 17'10.87"N	78 <sup>0</sup> 35'05.48"E
3	MBNR-1	Ippalpalle, Mahabubnagar(D)	16 <sup>0</sup> 40'59.43"N	77 <sup>0</sup> 58'34.08"E
4	MDK-1	Pullur, Medak(D)	18 <sup>0</sup> 10'33.95"N	78 <sup>0</sup> 48'44.77"E
5	MDK-2	Chandlapur, Medak(D)	18 <sup>0</sup> 10'07.13"N	78 <sup>0</sup> 49'25.43"E
6	R.R-1	Ekambbari, Rangareddy(D)	17 <sup>0</sup> 82'09.31"N	77 <sup>0</sup> 27'10.22"E
7	R.R-2	Kulakcharla, Rangareddy(D)	17 <sup>0</sup> 01'13.37"N	77 <sup>0</sup> 54'44.12"E
8	WL-2	Komaravelli, Warangal(D)	17 <sup>0</sup> 55'51.41"N	78 <sup>0</sup> 53'51.04"E

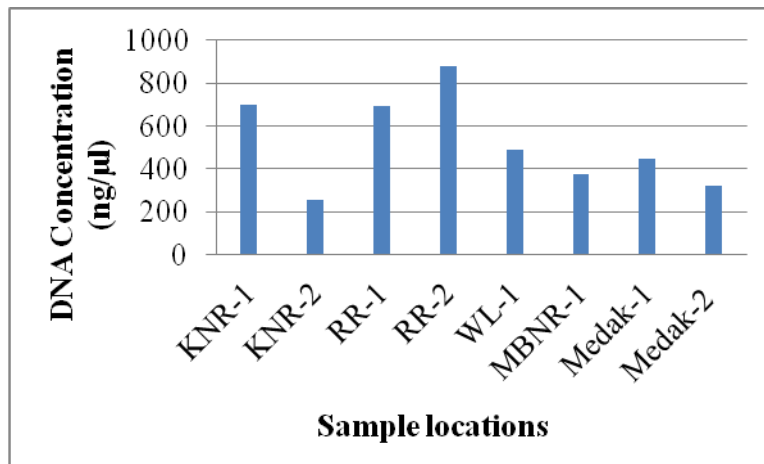
**Table.2** DNA Quantification values of *Selaginella bryopteris* (L.) Bak. genotypes obtained by U.V Spectrophotometer.

S.No.	Samples	Concentration
1	KNR-1	699ng/μl
2	KNR-2	257ng/μl
3	RR-1	691ng/μl
4	RR-2	879ng/μl
5	WL-1	487ng/μl
6	MBNR-1	377ng/μl
7	Medak-1	445ng/μl
8	Medak-2	325ng/μl

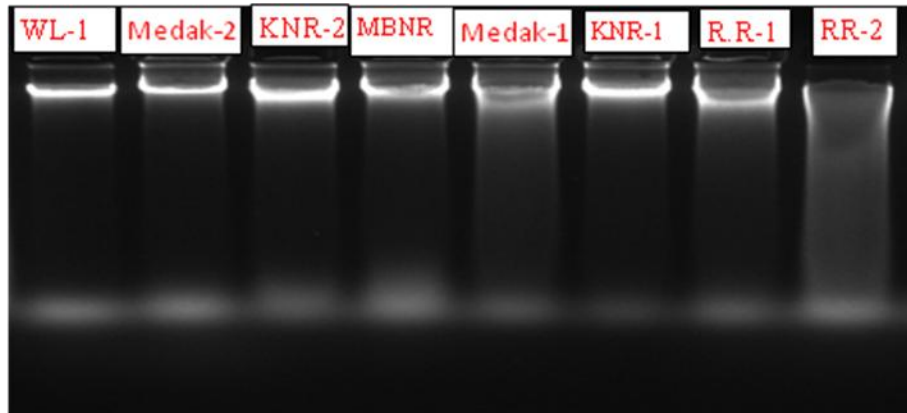
**Table.3** DNA Purity index (OD260/280) values of *Selaginella bryopteris* (L.) Bak. genotypes.

S.NO	SAMPLES	OD260/280
1	KNR-1	1.85
2	KNR-2	1.52
3	RR-1	1.68
4	RR-2	1.13
5	WL-1	1.49
6	MBNR-1	1.83
7	Medak-1	1.79
8	Medak-2	2.37

**Fig.1** Graphical representation of DNA Quantification values of *Selaginella bryopteris* (L.) Bak. genotypes.



**Fig.2** Gel pictures of *Selaginella bryopteris* (L.) Bak. Genotypes.



In conclusion, the main objective of the present study is the isolation, quantification and purity check of the genomic DNA of eight genotypes from various districts in Telangana region of *Selaginella bryopteris* (L.) Bak. The DNA quantification studies emphasized that the yield of DNA ranged from 251 ng/μl to 879ng/μl. The highest (879ng/μl) and the lowest (251ng/μl) DNA yield are achieved by R.R-2 and KNR-2 respectively. Purity index was checked by taking the ratio of OD260/OD280. The present study revealed that DNA purity of *Selaginella bryopteris* (L.)Bak. genotypes ranged from 1.13 to 2.37. The quantity and purity of DNA of *Selaginella bryopteris* (L.) Bak. genotypes have shown greater variation from one place to another.

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**How to cite this article:**

Rupa, P. and Lakshmi Bhavani, N. 2016. DNA Quantification Studies of *Selaginella bryopteris* (L.) Bak. Genotypes in Telangana, India. *Int.J.Curr.Microbiol.App.Sci.* 5(6): 144-149. doi: <http://dx.doi.org/10.20546/ijcmas.2016.506.018>