Research Article

Use of an optimal and distinctive method for the extraction of genomic DNA from *Juniperus excelsa* of the Zarghoon Juniper ecosystem in Balochistan, Pakistan

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Citation

Zahoor Ahmed Bazai, Rasool Bakhsh Tareen, Muhammad Younas Khan Barozai, Humma Batool, Abdul Kabir Khan Achakzai and Basira Akhtar. Use of an Optimal and Distinctive Method for the Extraction of Genomic DNA of *Juniperus excelsa* at the Zarghoon Juniper Ecosystem in Balochistan, Pakistan. Pure and Applied Biology. Vol. 4, Issue 4, 2015, pp 659-663. <http://dx.doi.org/10.19045/bspab.2015.44026>

Received: 15/09/2015

Revised: 13/11/2015

Accepted: 24/11/2015

Abstract

This exploration was carried out through field inspection in immediate forest neighborhoods of two settlements entitled Killi Tor Shore (K.T.S.) and Killi Shaban (K.S.) in revision region Zarghoon Juniper ecosystem, three districts named Quetta, Sibi and Ziarat, province Balochistan, Pakistan. The leaf samples of *Juniperus excelsa* trees were collected from these areas and subjected for the DNA isolation. As no genetic analysis is reported for this area Juniper, thus this study is aimed to optimize a protocol for the extraction of DNA. In order to isolate high aggregate of pure and intact DNA would serve great base for the downstream genetic analyses. The impurities of plants predominantly the pine trees are owing to the existence of high magnitude of secondary metabolites, polyphenols and polysaccharides. After the application of different acknowledged protocols for the DNA extraction of *Juniperus excelsa* trees it was perceived none of the protocols extracted pure and high yield DNA. Therefore, with the adaptation in technique an optimized protocol abetted in isolating pure DNA chiefly for tree *Juniperus excelsa*. This optimized protocol applied multiple times consequently made possible the isolation of pure DNA of Juniper.

Key words: Ziarat Juniper; DNA extraction; Secondary metabolites.

Introduction

Fortication and Conservation of forests is a significance constituent of environmental policies in numerous countries. Genetic

diversity is intact quantity of innate features in the genome of a species or a population. Populations in different surroundings and species within populations may have

different genetic characteristics [1, 2].

Consequently, to study genetic interactions of species within populations and amongst populations in dissimilar environment is the substance for administration of flora. Biotechnology involvement to affluence in scrutinizing genetic mannerisms of an individual species in different environmental conditions is prodigious. Seedlings planted in arid or semi-arid areas should have a well-developed root system for better absorption of water lost from the soil in shoots and leaves [3, 4]. Juniper forest functioning as an essential source of wood for construction, food preparation and fuel over ages but they are subjected to over exploitation, substantial grazing and fires; while silvi-cultural practices or other management practices are lacking. Juniper is the most prominent type of vegetation at or above 1600 m elevation [5, 6]. Junipers can endure deprived soils and persist at exceedingly high and low temperatures in contrast to other forest trees. In addition, they are extremely resilient and they are the latest species that abandon areas in the

course of deforestation [7]. Afforestation revisions on juniper documented that seedlings with low shoot/root fresh or dry weight provided healthier results, chiefly in scorched areas [8, 9]. Biodiversity has three components and genetic diversity is one of the portions of biodiversity, and therefore is significant in conservation. Conservation of forests is important content of biodiversity conservation [10]. The objective of this study was to test the hypothesis that *Juniperus excelsa* did not endorse intact and high yield of DNA from one isolation protocol. Therefore, numerous protocols for DNA extraction were applied to many plant species [11 - 13]. These were modified and a suitable method formulated which is discussed below.

Materials and Methods

In Zarghoon Juniper forest leaf samples of total eight trees as per four samples from growth vicinity named Killi Tor Shore and Killi Shaban collected for DNA isolation (Table 1).

Table 1. List of location, altitude, collection date, and University of Balochistan Botany Herbarium (UoBBH) voucher number of *Juniperus* samples used in this study.

SPECIES	LOCATION	Alti (m)	COLLECTD	VOUCHER #
<i>J. excels</i>	Killi Tor Shor	2400	20-7-11	UoBBH201
<i>J. excels</i>	Killi Tor Shor	2400	20-7-11	UoBBH202
<i>J. excels</i>	Killi Tor Shor	2400	20-7-11	UoBBH203
<i>J. excels</i>	Killi Tor Shor	2400	20-7-11	UoBBH204
<i>J. excels</i>	Killi Shaban	3200	27-7-11	UoBBH205
<i>J. excels</i>	Killi Shaban	3200	27-7-11	UoBBH206
<i>J. excels</i>	Killi Shaban	3200	27-7-11	UoBBH207
<i>J. excels</i>	Killi Shaban	3200	27-7-11	UoBBH208

DNA isolation protocol

Fresh leaf samples were used for DNA isolation by the modified PVP method [14] owing to its appropriateness to *Juniperus excelsa*. Eight gram of leaf sample from each tree were pulverized in liquid nitrogen and grinded using pestle and mortar. An

extraction buffer consisting of 2% CTAB (w/v), NaCl (2 M), 150 mM Tris-HCl pH 8.0 and 30 mM EDTA pH 8.0 was prepared. After being autoclaved for 20 min, 3% PVP (w/v), 2% (v/v) 2-mercaptoethanol and 2% SDS (w/v) were added to the extraction buffer. Almost 100mg grinded tissue was

taken in 1.5ml tubes with 1.2 ml of extraction buffer. Eppendorf tubes were mixed by vortex and incubated for 10 min at 60°C and centrifuged at 12,000 rpm for 15 min at RT (room temperature). The supernatant was transferred to a new 1.5ml Eppendorf tube and extracted with equal volume of chloroform: isoamyl alcohol (24:1, v/v). Carefully the supernatant was carried out into a new 1.5 ml tube and an equal volume of basic Phenol: Chloroform: Isoamyl alcohol (PCI 25:24:1, v/v/v) was added and vigorously mixed. The tubes were centrifuged for 15min at 12000 rpm. To remove the entire proteins and other organic phase soluble contaminants the PCI extraction was repeated once more. Then took supernatant into new tubes and added twice volume of 100% ethanol. To precipitate the genomic DNA 20 µl of 5M sodium acetate was added to each tube and kept overnight at -20°C. After overnight incubation the tubes were centrifuged for

10min at 12000rpm. The pellet was then washed with 70% ethanol and air dried. The pellet was dissolved in 30 µl double distilled deionized water and kept at -20°C.

Confirmation on gel electrophoresis and spectrophotometer

The quantitative and qualitative analyses of DNA was performed by measuring optical density at 260 nm and 280 nm using Genova Nano spectrophotometer. Polysaccharide contamination was determined by maximum absorbance measurement at 230 nm. Ratio measurements at wavelengths 230, 260 and 280 indicated degree of DNA purity. The absorption ratio A260/230 indicated polysaccharide/polyphenolic contaminants and A260/280 indicated protein contaminates. The DNA were loaded on a 1% agarose gel, stained with ethidium bromide (EtBr) electrophoresed and visualized under UV light to assess the integrity of DNA [15, 16].

Figure 1. Total Genomic DNA was extracted from the leaf of Juniper tree (*Juniperus excelsa*) and run on 1% agarose gel, stained with ethidium bromide, showing intact DNA bands

Results & discussions

In the beginning, the isolation of DNA was conducted using the available procedures from the survey of literature, but found that these methods could not remove

polyphenolics from the Juniper tree tissues, and that no DNA was extracted [11]. In this study a modified method was used to isolate the genomic DNA from *Juniperus excelsa* containing high amounts of polysaccharides

and phenolic compounds. Similar study was done by Michiels et al., [17] in latex-containing plants and Japelaghi et al., [18] in different plant tissues that contain large amounts of polysaccharides and polyphenol compounds. Compared with the existing method, we have isolated DNA from Juniper in considerable quantity and in intact form (Fig-1). The isolated DNA was of high quality as it showed a reading in between 1.7 to 1.9 after calculating the ratio of absorbance 260/280 nm. Later on all of these DNAs were used for PCR analysis in a separate study. This DNA preparation method yielded a predominance of high molecular weight DNA. Considering all factors involved, this method appears to be the most efficient, reliable and labor-effective DNA isolation procedure from Juniper tree containing a high content of polyphenolics.

Acknowledgment

The Research was conducted in Biotech Lab, Department of Biotechnology, University of Balochistan, Pakistan. The Research Team is thankful for the provision of lab facility. The Financial support by Higher Education Commission (HEC), Pakistan is highly acknowledged.

Authors' contributions

Conceived and designed the experiments: RB Tareen & ZA Bazai. Performed the experiments: MYK Barozai & ZA Bazai. Analyzed the data: AKK Achakzai & H Batool. Contributed reagents/ materials/ analyses tools: B Akhtar. Wrote the paper: ZA Bazai.

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