J. Phytol. Res. **27** (1 & 2) : 1-5, 2014 **ISSN 0970-5767**

STREAMLINED EXTRACTION PROTOCOL FOR HIGH QUALITY RNA FROM *PROSOPIS JULIFLORA* AND *SALVADORA PERSICA* AND ITS SUITABILITY FOR DOWNSTREAM PROCESSING

MOHAMMAD SHOYAB and TARUN KANT*

Molecular Biology Laboratory, Forest Genetic and Tree Breeding Division, Arid forest Research Institute, Jodhpur-342005, Rajasthan, India.

* Corresponding author : E-mail: tarunkant@icfre.org

The extraction of high quality RNA is a vital technique in plant molecular biology, particularly functional genomics. The quality of RNA determines the reliability of downstream process like real time PCR, RNA Sequencing etc. In the present study, we have reported a high quality RNA extraction protocol for *Prosopis juliflora* and *Salvadora persica*. Quality and quantity of RNA was determined by using spectrophotometer and formaldehyde gel electrophoresis. The extracted RNA was thereafter used for cDNA synthesis. The primers were designed through an *in silico* approach using Primer-blast tool. The designed primers were successfully used to amplify the cDNA derived from mRNA from *P. juliflora* and *S. persica*. The results indicate that RNA was of good quality and fit for RT-PCR. This high throughput plant RNA extraction protocol can be used for extracting high quality RNA from these two plant species for real time PCR and other downstream applications.

Keywords: cDNA; Electrophoresis; Nucleic acid; PCR amplification; Primer

Introduction

High quality, clean and high concentration of genetic material is required for gene expression analysis or next generation sequencing work. Extraction of sufficient quantity of pure RNA from a plant or a tree is even more challenging. Plant samples contain large amount of polysaccharides, high level of RNases, various kinds of secondary metabolites and/or fibrous tissues such as lignin (wood) that are challenging to break up and remove[1]. Various protocols for RNA extraction from plant species rich in polyphenolics or polysaccharides exist[2-9]. However, these methods have been developed for specific plant tissues, and are generally time consuming. *P. juliflora* has become a prominent woody species in agro-ecosystems of arid and semi-arid regions of India. With its tremendous ability to adapt to arid and semi-arid environments, and its fast growth and multiple utility, it has long been recognised by foresters as a versatile species for afforestation[10]. On the other hand, due to its survivability under conditions of drought, high

temperature and aggressiveness of its growth characteristics, it has also been classified as an alien invasive species. Nonetheless, this very character makes it an extremophilic tree species it is that can act as a source of novel genes for abiotic tolerance. *Salvadora persica* Garc, [Salvadoraceae] popularly known by the names – Saltbush, '*Khara jal*' or '*Miswak*' is an ever green medium size facultative halophytic tree that predominantly occurs as natural vegetation on saline soils where the salt concentration of the soil would inhibit the growth of most other crops[11]. Saltbush is a very robust species (92.8% survival 30 months after planting) that can survive extreme harsh conditions including high salinity, heat stress and drought conditions[12]. The tree is able to tolerate very dry environment with mean annual rainfall of less than 200 mm. Highly salt tolerant, it can grow on coastal regions and inland saline soils[13]. he optimized and generally applicable protocols for total RNA extraction from *P. juliflora* and *S. persica* have been developed and are being reported here. The RNA extracted was found suitable for downstream molecular biology techniques.

Materials and Methods

(i) *RNA Extraction:* For *P. julifora* plant material (young leaves) was collected from mature plant and frozen immediately in liquid nitrogen. Ribozol (Amresco, USA) – a plant RNA extraction reagent was used for RNA extraction and the manufacturer's instructions were followed for effective use of the Plant RNA extraction reagent. All solutions were prepared with sterile RNase-free water, and all supplies and handling materials were cleaned with DEPC treated water and 70% alcohol prior to storage. For 1 ml of extraction reagent approximately 0.1 g of plant tissue was used. In case of *Salvadora persica* along with Ribozol (Amresco, USA), HiPuraA (HiMedia, India) plant and fungal RNA Miniprep purification kit was used for isolation of RNA.

The yield and purity of RNA were determined spectrophotometrically at wavelength of 260 nm for RNA and 280 nm for proteins using nano-scale spectrophotometer (DeNovix DS-11, USA) and in addition to this 1% formaldehyde gel was also used to check integrity of isolated RNA.

(ii) *cDNA synthesis:* cDNA was synthesized from 1µg of isolated total RNA by using Verso cDNA synthesis kit (Thermo-fisher, India). Reaction was carried out in 20µl reaction mixture (containing 1X cDNA synthesis buffer, 0.5 mM dNTP Mix, oligo dT primer, RT enhancer, Verso Enzyme Mix and 1ng Template [RNA]).

Table 1: Condition for cDNA synthesis

Steps	***Temp.***	***Time***	***Number of cycles***
cDNA synthesis	42 °C	30 min.	1
Inactivation	95 °C	2 min.	1

(iii) *Primer Designing:* Primer for actin gene was designed using reference genome of *Prosopis fracta* and *Populus trichocarpa* using Primer-blast tool from NCBI (http://www.ncbi.nlm.nih.gov/tool/primer-blast). Primer3 was also used for cross checking (http://frodo.wi.mit.edu/cgibin/primer3/primer3.cgi). Selected sequences were sent for synthesis.

(iv) *PCR amplification:* PCR amplification reactions were performed in 25µl mixture containing 1µl cDNA, 1x PCR buffer (including 15 mM $MgCl_2$), 0.2 mM of each of the dNTPs (Merck Biosciences,

India), 0.5µM of each primer (Sigma Aldrich, India) and 1.0 U of Taq polymerase (Taq DNA polymerase, Merck Biosciences, India). PCR was performed in thermal cycler (Surecycler, Agilent Technologies, USA) with a heated lid (Table 2).

Table 2: PCR programme showing temperature and cycling condition:

Steps	Temperature	Time	Number of cycle
Initial denaturation	94°C	3 min.	1
Denaturation	94°C	1 min	35
Annealing	60°C	1 min	
Elongation	72°C	2 min	
Final Elongation	72°C	5 min	1

Result and Discussion

RNA of *P. juliflora* was extracted by using Ribozol and Hi-pura RNA extraction kit and its integrity was determined by denaturing formaldehyde gel electrophoresis. For *P. julifolra*, Ribozol and Hi-pura RNA extraction kit gave high quality RNA with concentration of 1-2 ng/µl and 1.2-2 ng/µl and 260/280 ratio were 1.98 – 2.10 and 1.96 – 2.00 obtained, respectively. In case of *S. persica*, Ribozol method yield low quality RNA with low purity levels. Concentration ranged from 0.3 – 0.8 ng/µl and purity (260/280 nm) ratio was 1.2 – 1.6 respectively. RNA extracted using Hi-pura kit gave high quality RNA with concentration ranging from 1.5 – 2.5 ng/µl and purity ratio (260/280 nm) ratio from 1.98 – 2.10 (Fig. 1 and Fig. 2).

The accession number XM_006370951.1 (*Populus trichocarpa*) and KX151705.1 (*Prosopis farcta*) of the actin gene were retrieved from NCBI database. The coding sequence of actin gene in FASTA format was used in primer blast for designing of primers. It gave a list of several oligonucleotide sequence capable of amplifying the gene of interest but only one specific pair of each species was selected consisting of a forward and a reverse primer. This RNA was used for cDNA synthesis and PCR amplification using the finalised primer pair. These primers were found to amplify the fragment of actin genes of *P. juliflora* and *S. persica* and generated a band of 201 bp and 473 bp respectively (Fig. 3). Primer designed from *Prosopis fracta* gave specific single band in *P. juliflora* only and primer from *P. trichocarpa* gave single band in *S. persica* only.

Primer sequence selected for this study:

(A) From *Populus trichocarpa*
Forward:
AAGGTTGTTGCACCACCAGA
Reverse:
CATCTGCTGGAAGGTGCTGA

(B) From *Prosopis farcta*
Forward:
ACTTCCAAGACCAGCTCTGC
Reverse:
ACCGTACAGGTCCTTCCTGA

Conclusion

In this report we present a method for the extraction of functional RNA from tissues rich in disturbing polyphenolic substances for two species – *P. juliflora* and *S. persica*. This method is optimized and less time consuming. Moreover, it resulted in intact RNA, which proved to be suitable for common molecular biological techniques. Primer designing by *in silico* approach is an important area in bioinformatics. It has many implications in molecular biology experiments. The primers designed, successfully amplified the actin gene segment from cDNA template obtained from

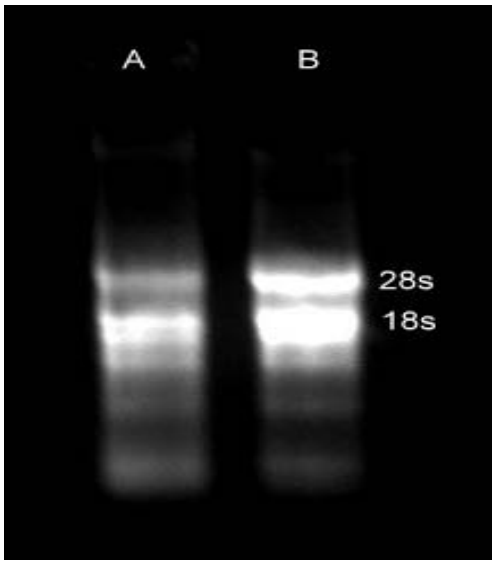

Fig.1. Denaturing RNA gel electrophoresis image of extracted RNA from leaf of (A) *P. juliflora* and (B) *S. persica* using Hi-pura RNA extraction kit.

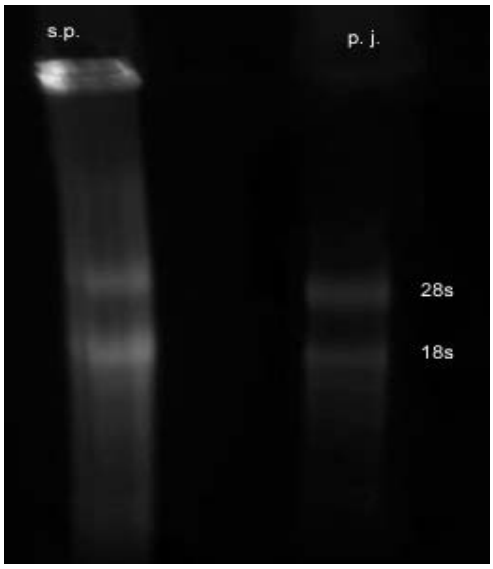

Fig. 2. RNA of *S. persica* (s.p.) and *P. juliflora* (p.j.) isolated using Ribozol extraction buffer.

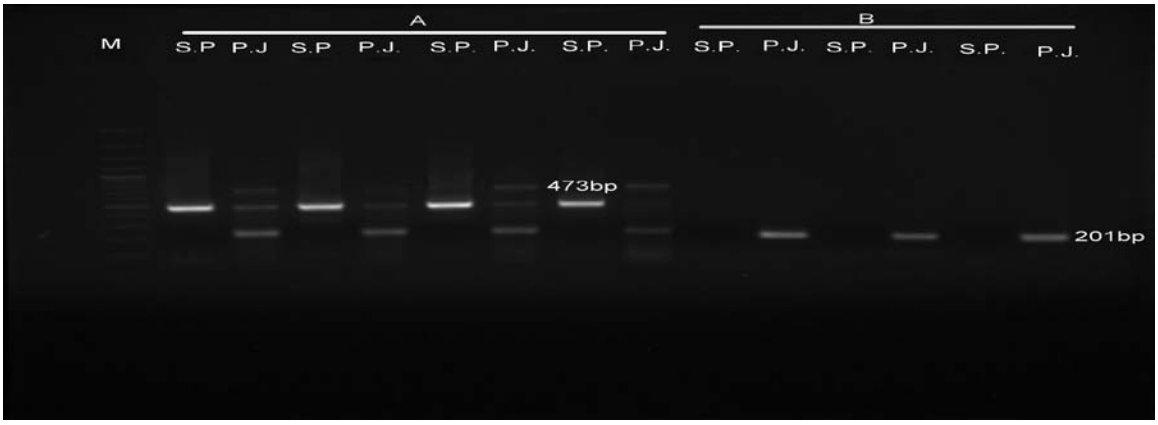

Fig.3. (A) *S. persica* gave a single 473 bp band with primer designed from *P. trichocarpa* while *P. julifora* gave no specific band. (B) *P. julifora* gave a single 201 bp band with primer designed from *P. farcta* and *S. persica* gave no band with this primer.

mRNA extracted from both species.

Acknowledgment

M.S. is grateful to Department of Biotechnology (DBT) for fellowship grant.

Reference-

1. MacRae E 2007, Extraction of plant RNA. In E. Hilario and J. Mackay [eds.], Methods in molecular biology, vol. 353: Protocols for nucleic acid analysis by nonradioactive probes, 2nd ed. Humana Press, Totowa, New Jersey, USA.
2. Ainsworth C 1994, Isolation of RNA from floral tissue of *Rumex acetosa*

(Sorrel). *Plant Molecular Biology Reporter* **12** 198-203.
3. Chang S, Puryear J and Cairney J 1993, A simple and efficient method for isolating RNA from pine trees. *Plant Molecular Biology Reporter* **11** 113-116.
4. Claros MG and Canovas FM 1998, Rapid high quality RNA preparation from pine seedlings. *Plant Molecular Biology Reporter* **16** 9-18.
5. Dong JZ and Dunstan DI 1996, A reliable method for extraction of RNA from various conifer tissues. *Plant Cell Reports* **15** 516-521.
6. Geuna E Hartings H and Scienza A 1998, A new method for rapid extraction of high quality RNA from recalcitrant tissues of grapevine. *Plant Molecular Biology Reporter* **16** 61-67.
7. Grosskopf E, Wegener-Strake A, Sandermann H and Ernst D 1994, Ozone-induced metabolic changes in Scots pine: mRNA isolation and analysis of in vitro translated proteins. *Canadian Journal of Forest Research* **24** 2030-2033.
8. John ME 1992, An efficient method for isolation of RNA and DNA from plants containing polyphenolics. *Nucleic Acids Research* **20** 2381.
9. Schneiderbauer A, Sandermann H and Ernst D 1991, Isolation of functional RNA from plant tissues rich in phenolic compounds. Analytical Biochemestry **197** 91-95.
10. Tewari, JC, Harris, PJC, Harsh, LN, Cadoret, K and Pasiecznik, NM 2000, Managing *Prosopis juliflora* (Vilayati babul)-A technical manual. CAZRI, jodhpur, India and HDRA, Coventry, UK. 96 pp. ISBN 0 905343 27 1.
11. Zodape, ST and Indusekhar, VK 1997, Salvadorapersica—a boon to wasteland development. *Journal of Scientific & Industrial Research* **56** 657–661.
12. AFRI 2010, Annual report 2009-10. Arid forest research institute, Jodhpur.
13. Orwa C, A Mutua, Kindt R, Jamnadass R and Anthony S 2009, Agroforestree Database: a tree reference and selection guide version 4.0 (http://www.worldagrofroestry.org/sites /treedbs/treedatabases.asp).