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ORIGINAL ARTICLE

Fabrication of silica magnetic beads suitable for genomic DNA isolation from desert specific trees for their genetic analysis

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ABSTRACT
A cost effect and fast method of PCR usable genomic DNA isolation is reported here. Leaf samples of some desert specific tree species were used as experimental material. Silica coated magnetic bead were synthesized by chemical co-precipitation of Fe^{2+} and Fe^{3+} ions in an alkaline solution. The concentration of isolated gDNA was tested by absorbance ratio (A_{260}/A_{280}).DNA amplification using RAPD primers were also performed for further validation of proposed method of DNA isolation.Agarose gel electrophoresis resolution reflect the rich quality of isolated genomic DNA for downstream molecular analysis.
Keywords: *Cordiamyxa*,DNA Isolation, Magnetic particle, Tetraethyl orthosilicate (TEOS), polyphenol, etc
Abbreviations: MPs: Magnetic particle; DDW: Double distilled water

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INTRODUCTION

DNA profiling, a well-known technique for diversity assessments can efficiently aid in conservation of tree species in arid environments plant biodiversity is crucial to buffer negative effects of climate change and desertification in drylands [1, 16].For that a cost effective and time intensive method for rich quality DNA isolation is always be requisite. Isolation of genomic DNA from hot arid tree species, of sufficiently rich-quality/ PCR usable, is still challenging because of accumulated secondary metabolites. The traces of secondary metabolites (polysaccharides, phenolic compounds) may inhibit the DNA polymerase activity in PCR reaction for amplification [8, 20, 5]. A significant attention has been made on the synthesis of magnetic particles for fast and cost-effective separation of PCR usable genomic DNA. The magnetism/magnetic properties of magnetic particle have ideas for efficient separation techniques from chemical or biological suspension. [10, 17]. DNA separation through magnetic particle were first reported by [7]. The technique of magnetic separation involves the use of particles constituting iron oxide core coated with silane or carboxylic acid molecules which have ability to bound with DNA [2, 6].In present study we were able to synthesize the silica coated magnetic particles for isolation of high-quality genomic DNA of some desert specific tree species collected from Rajasthan (N-W state of India).

MATERIAL AND METHODS

Collection of experimental material

Fresh leaf of average size and vigor were harvested from field grown plants of *Cordia myxa* L., *Tecomella undulata* (Sm.) Seem., *Prosopis cineraria*(L.) Druce., *Acacia nilotica* (L.) Delile., *Acacia senegal* (L.) Willd., *Terminalia bellirica* (Gaertn.) Roxb., *Balanitesa egyptiaca* (L.) Delile. Collected leaf sample were immediately fixed in liquid nitrogen(−196 °C) and stored in deep freezer(-20˚C) for further analysis.

Synthesis of magnetite particles

Magnetic particle (MPs)of metal oxides and ferrites were synthesized using co-precipitation principle. Aqueous salt solutions of $FeSO_4.7H_2O$ and $FeCl_3$(98% chemically pure) were prepared separately by

dissolving 2.7g and 5.7g in 10 ml of double distilled water (DDW) respectively. Both the freshly prepared aqueous solutions were mixed by pouring $FeSO_4.7H_2O$ solution to the $FeCl_3$ container following to this a double volume of NH_4OH(10M) solution were added with constant stirring at 25ºC.The mixture was stirred vigorously using a magnetic stirrer at 80ºC in water bath for 5-6-hour,pH (9-10) were maintained during reaction. Consequently, the formation of black precipitation was visualized. The precipitate was washed repeatedly (5-6 time) with DDW to remove excess of ammonium ions got accumulated in upper aqueous layer. Resultant humid suspension was evaporated at water bath and oven dried at 25ºC.After drying process, magnetic particles were ground to remove aggregates and to get manageable MPs (Fe_3O_4).

Silica Coating of MPs

1g of MPs were added in 160ml of absolute alcohol (Jai Chemical Ltd, Jaipur)to produce suspension by stirring at for 10-15min. To this suspension 24ml of DDW and 12ml of NH_4OH (25% Himedia)solution were added. Subsequently 1.6ml of tetraethyl-orthosilicate (TEOS, 98%, Sigma-Aldrich)were added with continuous stirring. pH of reaction mixture was maintained between 9-10 and was stirred continuously for 5hr. washing of silica coated magnetic beads were performed using ethanol and DDW for six to eight times and subsequently followed by and drying under vacuum. All the reactions were performed at room temperature.

DNA isolation and quality assessment

200mg of pre-fixed leaf sample were ground to fine powder using silica gel-60(Merck Ltd). About 100-120 mg of leaf powder was taken in a centrifugation tube (2 ml, Tarson) and 1 ml of freshly prepared andpre heated(50 ºC) extraction buffer[(2M) NaCl (Merck, India), (100mM) TrisHCl (Art. 6390, Loba Chemie, India), EDTA (0.5M), (3%) CTAB (Himedia, India), (4%) PVP (Loba Chemie, India)]+5µl of β-mercaptoethanol (0.2%) were added. The mixture was gently swirled, incubated at 65ºC for 30min and brought back to room temperature. Eventually equal volume of chloroform and isoamyl alcohol (24:1v/v) were added to mixture and centrifuged for 10min at 10,000rpm at room temperature. Supernatant were carefully taken in to another centrifuge tube, to this800µl of binding buffer [NaCl (5M), PEG(8000Mw, 13%)]+200µl silica coated magnetic bead solution (20 µgin 200 µl of DDW)were added and kept undisturbed to react for 10min then tube was placed on magnetic platform to get settled-pellet at bottom. Discard the supernatant and attentively wash (2 to 3 times)the pellet with washing buffer [NaCl (100mM), TrisHCl (10mM pH 7.5) EDTA (1mM), Ethanol (70%)]. Thoroughly dried pellet was resuspended in 100µl of TE buffer (10 mMTris-HCl pH 8.0, 1 mM EDTA)and incubated at 60ºC for 10min. following the incubation, centrifugation (3000 rpm for 2 minutes) step were performed and supernatant were collected in fresh centrifuge tube (2 ml, Tarson. The concentration of isolated gDNA was tested by absorbance ratio (A_{260}/A_{280}) using UV–VIS Spectrophotometer (Elico Hyderabad, India)/ nanodrop spectrophotometer (DeNovix, DS-11, USA) and quality was assessed by horizontal agarose gel (0.8%, Sigma) electrophoresis (AE - 6125/6133 ATTO, Japan). Ethidium bromide (0.25 µg/ml)was used as stain to visualize the gel under (Syngene's gel documentation system, Synoptics Ltd., UK).

Suitability of isolated g DNA for PCR based marker analysis

PCR (Eppendorf 5331) based DNA amplification using RAPD primers were achieved in 25 µl volumes of mixture as 1X PCR buffer [100mM Tris (pH 9.0),500 mMKCl, and 1% Triton X-100 with 15mM MgCl2],75 ng of template DNA, 2 U Taq DNA polymerase (Bangalore Genei, India),0.2 mMdNTP mix (Bangalore Genei, India), 1.5 mM $MgCl_2$, and RAPD primer (0.3 µM) (Table 1).Thermal cycler programme was adjusted for initial denaturation (5 min at 94 °C,) 40 cycles of denaturation (30 s at 94 °C), RAPD primer annealing (80 s at Ta°C), elongation (90 s at 72 °C) and final extension 10min at 72 °C). The annealing temperature (Ta)was maintained 2 °C lower than Tm of that particular primer sequence. OligoAnalyzer tool of IDT website was used to calculate Tm. Primers were synthesized from IDT(Integrated DNA Technologies Inc.). The amplification products for *Cordia myxa* samples were resolved on agarose (Sigma) gel (1.4%).

RESULTS AND DISCUSSION

Selection of leaf sample of average size and age was proved good for isolation of genomic DNA. We observed that beyond marginal young and old leaf was not suitable for PCR usable DNA isolation. Traditional approach of plant DNA extraction[9] takes more time and many steps of centrifugation, along with additional purification methods for removal of high content of secondary metabolite such as alkaloid, phenolics and polysaccharide compound [11]. Here we preferred to fabricate silica magnetic bead for genomic DNA isolation that proved cost effective, fast [12,13,14] and suitable for further DNA based downstream applications such as assessment of genetic diversity [17,4,] and/or genetic homogeneity [3].

A magnetite fluid is prepared by using conventional coprecipitation of Fe^{3+} and Fe^{2+} ions by OH^- at room temperature, here maintaining of temperature and pH throughout the reaction was crucial step for synthesis of appropriately sized particles[15].Reports are available that the naked magnetic particle have least adsorbing capacity for DNA as compared to Silica coated magnetic beads as modification / coating of these necked magnetic particles with TEOS, improved the chemical stability of the magnetic particles and provide more biocompatibility, and functionalize surface[21].Reports are also available for DNA isolation under high sodium chloride salt with PEG concentration through modified cobalt ferrite particle [15]. In present study we preferred Silica coated magnetic beads for genomic DNA isolation. From this method *Cordia myxa, Acacia nilotica, Terminalia bellerica* and *Tecomella undulata* to achieved DNA extraction and also, as seen in gel picture (Fig. 1). Results obtained from twelve accession of *C. myxa* of genomic DNA were nanodropspectrophotometric assessment showed the suitability of method used as by this we were able to get rich quality DNA yield ranges from 30.39ng/µl to 206.55ng/µl. The range for A260/A280 ratio (1.49 to 1.83) reflects the purity of the isolated gDNA, here higher value indicates lesser impurities of RNA and proteins(Table 1).Further validation by agarose gel electrophoresis not revealed other low molecular weight band indicated no visible RNA impurity (Fig.2).Various reports in favor of present study indicates that silica coated magnetic beads particle thermodynamically support the adsorption of DNA in presence of high-salt conditions or chaotropes or PEG (8000 Mw) but not favored the adsorption of protein and single-stranded RNA [7, 19, 2]. Electrophorogram image for RAPD primers (OPQ07, OPH05 and OPG07) based DNA amplification for from *C. myxa* sample is also presented here to reflect quality of isolated genomic DNA of for further molecular analysis (Fig. 3)

Table 1:- Quality assessment of isolated genomic DNA from leaf sample of *Cordia myxa* from various locations.

S. No	Accession of *Cordia myxa*	Location	Coordination	Quantity of DNA (ng/µl)	260/280 ratio	260/230 ratio
1	CM1	Chokha Jodhpur	72.6351Lon, 26.2862La	127.77	1.51	1.94
2	CM2	Chokha Jodhpur	72.63Lon, 26.28La	206.55	1.55	1.96
3	CM3	Balesar Jodhpur	72.4791Lon, 26.3974Lat	30.39	1.83	2.02
4	CM4	Dheerpura, Jodhpur	72.4317Lon, 26.4713Lat	38.28	1.46	1.84
5	CM5	Osian, Jodhpur	72.9047Lon, 26.7245Lat	120.27	1.82	2.20
6	CM6	Ghumati, Pali	73.2578Lon, 25.8118Lat	162.05	1.49	1.92
7	CM7	Bagawas, Pali	73.5606Lon, 25.8749Lat	84.56	1.63	1.93
8	CM8	Marwar Junction, Pali	73.6095Lon, 25.7228La	67.63	1.80	1.99
9	CM9	Jojawar, Pali	73.7404Lon, 25.5292La	55.44	1.55	1.86
10	CM10	Siyat-Sojat, Pali	73.7345Lon, 25.880La	197.28	1.79	1.91
11	CM11	Sadri, Pali	73.4534Lon, 25.1326La	145.11	1.76	1.99
12	CM12	Sadri, Pali	73.4534Lon, 25.1326La	162.44	1.77	2.12

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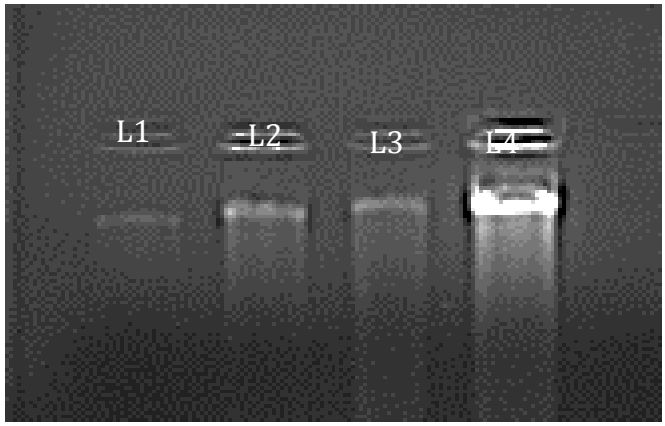

Fig 1. DNA isolation of genomic DNA resolved on 0.8% of agarose gel. L1- *Acacia nilotica*, L-2 *Cordiamyxa*, L3- *Terminalia belerica*, L4- *Tecomella undulata*

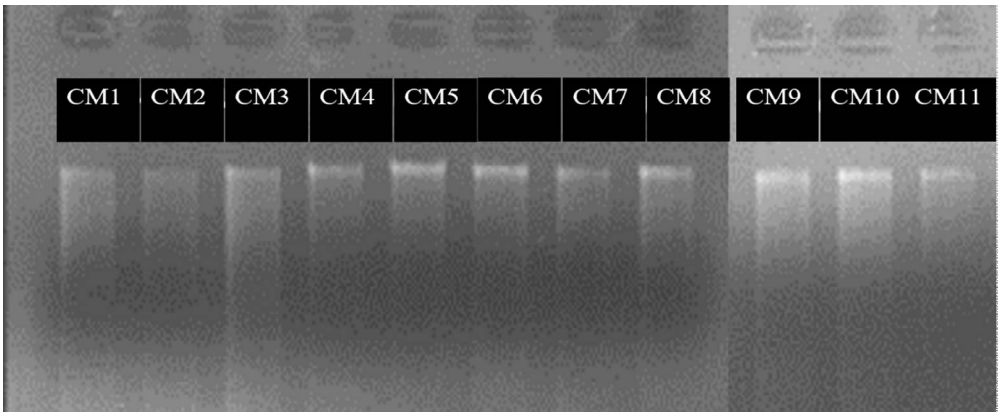

Fig 2. Isolation of genomic DNA from *Cordia myxa* leaf sample collected from different locations.

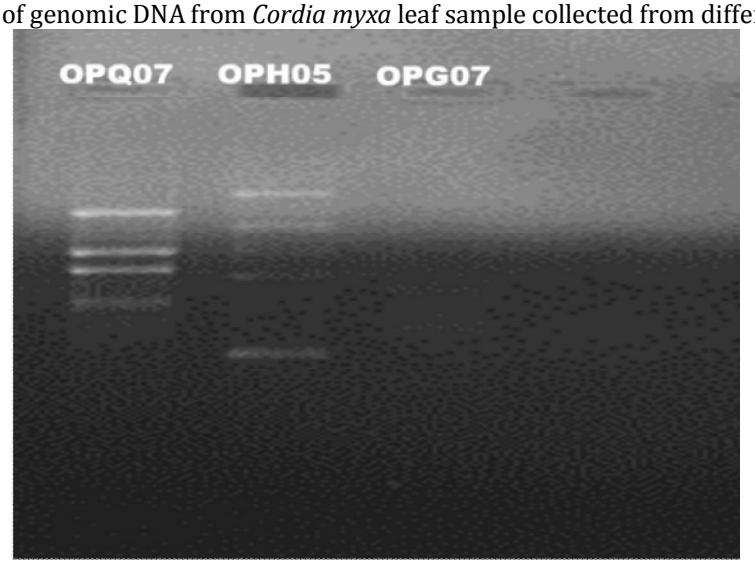

Fig 3. Amplified of RAPD Marker of genomic DNA from *Cordia myxa* through magnetic bead particle

CONCLUSION

Present study reveals the development of a cost effective and time intensive method for rich quality DNA isolation from some desert specific tree species using silica coated magnetic beads. Spectrophotometric assessment A260/A280 ratio (1.49 to 1.83) showed the suitability of method. PCR (Eppendorf 5331) based DNA amplification using RAPD primers were performed to validate the method for its downstream application based on genomic analysis.

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