Available online at http://www.journalijdr.com

IJDR

International Journal of
DEVELOPMENT RESEARCH

ISSN: 2230-9926

*International Journal of Development Research*
*Vol. 06, Issue, 04, pp. 7661-7667, April, 2016*

## *Full Length Review Article*

# A REVIEW ON ISOLATION OF DNA FROM BOTANICAL SAMPLES AND THEIR DNA FINGERPRINTING

**Priyanka Singh, *Mishra, M. K., Gupta, A. K., Vichar Mishra and Nidhi Sharma**

Department of Forensic Science, Sam Higginbottom Institute of Agriculture Technology and Sciences, India

**ARTICLE INFO**

***Article History:***

Received 26th January, 2016
Received in revised form
22nd February, 2016
Accepted 01st March, 2016
Published online 27th April, 2016

***Key Words:***

CTAB, Genomic plant DNA,
PCR, RAPD, SSR.

**ABSTRACT**

Isolation of plant genomic DNA is very difficult as plant cell contains many cell organelles such as chloroplast, mitochondria etc. Many plants contain high level of polysaccharides, essential oils and metabolites that make DNA isolation a bit tough. Isolation of such plants has been done by many people and successful isolation has been reported. PCR amplification of plant DNA has also been successfully carried out with RAPD primers, SSR primers etc. This paper presents a review on isolation of genomic DNA from different plants and their fingerprinting.



## INTRODUCTION

A plant cell consists of cell organelles such as chloroplast, mitochondria, plastids etc. that contains their own DNA instead of nuclear DNA. Therefore, a method should be followed that only lyses the nucleus of the cell not the cell organelles. As plants are very good source of energy, food and have medicinal properties also, some plants have very large amount of polysaccharides, essential oils and metabolites such as alkaloids, flavanoids, terpenoids, tannins etc. that interfere while isolating DNA from botanical samples. PCR is an amplification technique that amplifies a nanogram DNA into thousands and millions of copies. When DNA is isolated from the botanical samples is the amplified by PCR by using various primers such as RAPD primers, SSR primers etc.
Isolation of DNA from botanical samples and their PCR amplification have been successfully done by various researchers. Some such works have been described in the reviews below

Khanuja *et al.,* (1999) described a DNA isolation protocol which can be used for medicinal and aromatic plants that produces essential oils and metabolites which interfere in DNA isolation and reactions. They collected plant materials (leaves, stem, whole flowers or their parts) from *Allium sativum*, *Artemisia annua*, *Bacopa monnieri*, *Catharanthus roseus*, *Cymbopogon winterianus*, *Pelargonium graveolens*, *Mentha arvensis*, *Ocimum kilmandscharicum*, *Taxus wallichiana* and *Vetiveria zizanioides*. They dried half of the material at 50°C for 48 hours and rest was frozen in liquid nitrogen. They ground the plant material in liquid nitrogen and transferred the crushed material to 10 ml polypropylene tube and 3 ml freshly prepared extraction buffer (100 mM Tris-Cl, 25 mM EDTA, 1.5 M NaCl, 2% CTAB, 0.2% β-mercaptoethanol and 1% PVP) was added and mixed by inversion to a slurry. The tube was incubated at 60°C in a shaking water bath (100 rpm) for 1-2 hours for fresh samples and was incubated overnight at 37°C for dry samples. 3 ml CIA (24:1) was added and mixed by inversion for 15 minutes and centrifuged at 8000 rpm for 10 minutes at 25-30°C. The upper clear aqueous layer was transferred to new 10 ml tube. 1.5 ml 5 M NaCl was added and mixed properly. 0.6 volume Isopropanol was added and kept at room temperature for 1 hour. After 1 hour, it was mixed slowly and centrifuged at 10,000 rpm for 10 minutes at 25-30°C.

The supernatant was discarded and the pellet was washed with 80% ethanol. The pellet was dried in vacuum for 15 minutes and was dissolved in 0.5 ml high salt TE buffer. 5 µl RNase A was added and incubated at 37°C for 30 minutes and extracted with equal volume of CIA (24:1). The aqueous layer was transferred to a fresh 1.5 ml microfuge tube and 2 volumes of cold ethanol was added and centrifuged at 10,000 rpm for 10 minutes at 25-30°C. The pellet was washed with 80% ethanol and dried in vacuum and dissolved in 200 µl sterile double

***Corresponding author: Mishra, M. K.,***
*Department of Forensic Science, Sam Higginbottom Institute of Agriculture Technology and Sciences, India.*

distilled water. The quality of DNA was checked by running 2 µl DNA sample on 0.7% agarose gel. 2 µg DNA was restricted with EcoRI according to the guidelines provided by the supplier. PCR was carried out in 25 µl volume reaction mixture which contained 25 ng DNA, 0.2 U Taq DNA polymerase, 100 µM each dNTP, 1.5 mM $MgCl_2$ and 5 pmol decanucleotide primers. The amplified product was electrophoresed on 1.2% agarose gel containing 5 µg/ml ethidium bromide and was photographed. The protocol achieved good yield of high quality DNA from fresh as well as dry leaves. The DNA yield of different plants was between 15-85 µg/g tissues. The isolated DNA proved amenable to PCR amplification and restriction digestion.

Storchova *et al.,* (2000) presented a simple method for isolation of genomic DNA from wild plants sampled in remote field areas. They used leaves of Hieracium L. subgen. Hieracium sect. Alpina F.N. Williams and subgen. Pilosella (Hill) Gray (Asteraceae). They preserved the leaves in NaCl/CTAB solution in a micro-centrifuge tube using a thin glass rod. The tubes were closed, wrapped with paraffin and stored at ambient temperature until used. The microtubes with the preserved leaves were thawed at room temperature and 0.1 g of tissue was placed in a sieve and rinsed briefly with tap water. The leaves were ground in pestle and mortar with 1.0 ml extraction buffer (0.34 M sorbitol, 0.1 M Tris HCl (pH-7.6), 5 mM EDTA and 0.2% (v/v) β-mercaptoethanol). The crushed leaves were incubated for 20 minutes at room temperature and centrifuged at 8,800 g for 10 minutes at 4°C. The supernatant was removed and the pellet was suspended in 0.35 ml lysis buffer (0.2 M Tris-HCl (pH-7.6), 2 M NaCl, 0.05 M EDTA, 2% CTAB) was added and mixed and incubated at 64°C for 20 minutes, then extracted by shaking with 0.5 ml of CIA (24:1) and centrifuged at 8,200 g for 10 minutes at 20°C. The upper phase was transferred into a new microtube. Ice-cold Isopropanol (0.67 volumes) was added and extract was stored at -20°C for 10 minutes and centrifuged at 13,700 g at 4°C for 15 minutes. The pellet was washed with 0% ethanol to remove residual salt and CTAB and centrifuged again at 13,700 g at 4°C for 15 minutes and dried. The DNA was dissolved in 10-40 µl TE buffer. The concentration and OD ratio 260/280 of DNA were determined spectrophotometrically.

Quality of DNA was checked on agarose gel by comparison with a high molecular weight DNA marker. For comparison, DNA from fresh leaves was purified using a column procedure (DNeasy plant kit, QIAGEN) according to the manufacturer's instructions. 2-4 ng of purified genomic DNA was used per RAPD reaction in a final volume of 25 µl (200 µM dNTP, 2 mM $MgCl_2$, 0.2 µM ABA 19 primer (5'-CAAACGTCGG-3'), magnesium-free reaction buffer AND 1 U Taq DNA polymerase). PCR conditions were initial heating at 94°C, 5 minutes; 42 cycles (94°C, 20 sec; 39°C, 20 sec; 72°C, 1 minute) and final extension at 72°C, 4 minutes. They did not used liquid nitrogen was not used because leaves from saturated NaCl/CTAB solution can be well ground in the sorbitol extraction buffer. Polyphenols and polysaccharides are released into this extraction buffer and DNA remains in the pellet, after centrifugation. This extraction step removes secondary compounds which may contain inhibitors of Taq DNA polymerase. The sorbitol extraction of DNA from NaCl/CTAB preserved leaves is a fast, reliable and cheap alternative to commonly used procedures employing silica gel drying of plants and column extraction.

Nalini *et al.,* (2004) developed a simple method for isolation of DNA from plant tissues (leaf or seed) which is suitable for long term storage. They used fresh and frozen (-70°C) segregating $F_2$ population obtained from a cross Sonalika X Kalyansona (bread wheat: *Triticum aestivum*). The leaves were washed with tap water and then with distilled water, dried and weighed. 0.5 g of leaf tissue was taken in mortar and crushed with 2 ml extraction buffer (100 mM Tris, 20 mM EDTA, 0.5 M NaCl, 7 M urea, 0.1% β-mercaptoethanol, 2% SDS). The homogenate was transferred to a 2 ml microfuge tube. Equal volume of Phenol:Chloroform:isoamyl alcohol (25:24:1) was added to the tubes and mixed by gentle shaking and were centrifuged at room temperature for 15 minutes at 15,000 rpm. The upper aqueous phase was transferred in another tube and equal volume of CIA (24:1) was added and mixed and centrifuged at 15,000 rpm for 10 minutes. The upper aqueous phase was collected into a new tube and 0.1 volume of 3 M sodium acetate (pH-7.0) and 0.7 volume isopropanol were added and incubated for 15 minutes at room temperature and centrifuged at 15,000 rpm for 15 minutes at 4°C. The pellet was washed twice with 70% ethanol and then with 100% ethanol and air-dried and was dissolved in TE buffer and 5 µl DNase free RNase A was added to remove RNA. The amount of DNA was estimated by measuring fluorescence emission using dye Hoechst at emission maximum of 546 nm using fluorimeter. The amount of DNA was checked by comparing the emission intensity obtained using a known amount of standard DNA (100 µg/ml). The quality of DNA was checked by electrophoresis on 2% agarose gel using 1X TBE buffer and staining was done with ethidium bromide. The gel was visualized under UV light and was photographed. 100 ng/µl λHindIII was used as a marker. AP-PCR amplification was carried out in a 25 µl reaction mixture containing 100 ng template DNA, 2 mM $MgCl_2$, 25 pmol primer, 10X assay buffer, 0.2 mM each dNTP and 1 U Taq DNA polymerase.

The PCR conditions were 1 cycle of 5 minutes at 94°C, 5 minutes at 45°C, and 5 minutes at 72°C and 35 cycles of 1 minute at 94°C, 1 minute at 45°C and 1 minute at 72°C, followed by a final 10 minutes extension at 72°C. PCR amplification of template DNA was also done using 3' anchored ISSR primer which was carried out in a 25 µl reaction mixture containing100 ng template DNA, 2 mM $MgCl_2$, 25 pmol primer, 10X assay buffer, 0.2 mM each dNTP and 1 U Taq DNA polymerase. The PCR conditions were 1 cycle of 5 minutes at 94°C, 5 minutes at 50°C, and 5 minutes at 72°C and 45 cycles of 1 minute at 94°C, 1 minute at 50°C and 1 minute at 72°C, followed by final 10 minutes incubation at 72°C. PCR products obtained by AP-PCR and ISSR were fractioned on 2% agarose gel by electrophoresis using 1X TBE buffer. Ethidium bromide was used for staining. Gel was visualized under UV light and was photographed. DNA isolated by the protocol was reliable and sufficiently pure to be used in AP-PCR analysis to study genetic segregation and ISSR profiles were consistent and reliable. The advantages of this protocol are its simplicity, rapidity of isolation and reduction in cost.

Zidani *et al.,* (2005) described a modified procedure based on the CTAB method to isolate DNA from tissues containing high levels of polysaccharides. The method is applicable to both dry and fresh leaves of Pearl millet. The leaves of Pearl millet were harvested and frozen immediately in liquid nitrogen. 0.3 g of leaf sample was ground in liquid nitrogen using a mortar and pestle. The crushed leaves were quickly transferred to liquid nitrogen. 2% CTAB buffer (1ml) containing 1% (v/v) β-mercaptoethanol and 1% PVP was added to the micro-centrifuge tube (2 ml) and stirred with a glass to mix and was incubated at 60$^{o}$C for 30 minutes with frequently swirling. Equal volume of CIA (24:1) was added and centrifuged at 10,000 rpm at 4$^{o}$C for 15 minutes to separate phases. The supernatant was transferred to a new tube. The CIA step was repeated twice. The supernatant was then precipitated with 2/3 volume of ethanol. The precipitated nucleic acids were collected and washed twice with the buffer (75% ethanol, 3 M sodium acetate, TE). The pellet was air dried and re-suspended in TE. The dissolved nucleic acids were brought to 1.4 M NaCl and re-precipitated using 2 volumes of 75% ethanol. The pellets were washed twice using 100% ethanol, dried and re-suspended in 100 µl of TE buffer. The tube was incubated at 37$^{o}$C for 30 minutes to dissolve genomic DNA and RNase was then added. The DNA yield per gram of leaf tissue extracted was measured using a UV-VIS Spectronic Genesys 5 (Milton Roy) spectrophotometer at 260 nm. Purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. DNA samples from the leaf tissues were digested with Sau3A and electrophoresed on a 0.8% agarose gel. The primer used for PCR was $(GACA)_5$: 5'-GACAGACAGACAGACAGACA-3' and its specific annealing temperature (Ta) was 62$^{o}$C. 25 µl of reaction mixture was prepared which contained 2.5 µl reaction buffer (10X), 2.5 µl $MgCl_2$ (25 mM), 2 µl dNTP mixture (2.5 mM), 4 µl of primer (10pmol$^{-1}$), 0.5 µl Taq DNA polymerase and 1 µl of DNA (40 ng). PCR consisted of one cycle of 94$^{o}$C, 2 minutes, which was followed by 27 cycles of 94$^{o}$C, 1 minute; 62$^{o}$C, 1 minute; 72$^{o}$C, 2 minutes, and finally one cycle of 72$^{o}$C, 7 minutes. The PCR products were analyzed by electrophoresis using a 2% agarose gel in TBE buffer. DNA was stained by soaking the gel in a 0.5 mg/µl ethidium bromide solution. This method solved the problems of DNA degradation, contamination and low yield due to binding and co-precipitation with starches and polysaccharides. The technique is fast, reproducible and can be applied for SSR-PCR markers identification.

Deshmukh *et al.,* (2007) modified the DNA isolation method of *Terminalia arjuna* because current protocols have limitations due to the presence of high content of gummy polysaccharides and polyphenols. DNA isolated by these protocols is contaminated with yellowish, sticky and viscous matrix. In this study, they used juvenile leaves of T. chebula and T. tomentosa, T. arjuna and T. bellerica. The leaves were ground in liquid nitrogen. The crushed leaves were transferred to 1.5 ml centrifuge tube and 1000 µl of wash buffer (100 mM HEPES, 0.1% (w/v) PVP, 4% β-mercaptoethanol (v/v)) was added. The sample was vortex for 5 minutes to remove polyphenols. It was centrifuged at 12,000 g for 3 minutes and the supernatant was removed. The washing step was repeated four to five times to remove sticky residues. 1000 µl extraction buffer was added to the precipitant and centrifuged at 8,400 g for 5 minutes. The supernatant was removed and 450 µl of re-suspension buffer (20 mM Tris-Cl (pH-8.0), 10 mM EDTA (pH-8.0)) was added to the precipitant along with 80 µl of 10% SDS and incubated at 70$^{o}$C for 15 minutes. The sample then allowed to cool at room temperature and 300 µl of 7.5 M ammonium acetate was added and the sample was placed on ice for 30 minutes and centrifuged at 12,000 g for 15 minutes. The upper clear aqueous layer was transferred to another tube and equal amount of ice-cold Isopropanol was added and centrifuged at 12,000 g for 15 minutes. The supernatant was discarded and the pellet was washed with 70% ethanol twice. Pellet was dried and dissolved in 100 µl TE buffer. 10 µl of RNase (10 µg/ml) was added and incubated at 37$^{o}$C for 1 hour. Equal amount of CIA (24:1) was added and centrifuged at 12,000 g for 10 minutes. The aqueous layer was transferred to a fresh tube and 2 volumes of ice-cold ethanol was added and centrifuged at 12,000 g for 5 minutes at room temperature. The pellet was washed with 70% ethanol. The pellet was dried in vacuum and dissolved in 100 µl TE buffer or sterile double distilled water and stored at -20$^{o}$C until used. The DNA content was checked by running 0.7% agarose gel or by taking absorbance at 260 nm. 2 µg DNA was used for restriction digestion and 25 ng for PCR amplification. The DNA was digested with 5 to 10 U of EcoRI restriction enzyme for 1 hour. The digested DNA fragments were fractioned on 0.7% agarose at 5 V/cm. PCR was carried out in 25 µl volume reaction mixture containing 25 ng DNA, 2.5 U Taq DNA polymerase, 100 mM dNTP, 1X buffer and 10 mM decamer primer. The PCR conditions were initial denaturation at 94$^{o}$C for 5 minutes, followed by 45 cycles, denaturation at 94$^{o}$C for 1 minute, annealing at 38$^{o}$C for 1 minute and extension at 72$^{o}$C for 3 minutes and final extension at 72$^{o}$C for 7 minutes. PCR products were fractioned on 1.2 % agarose gel using 1X TAE buffer containing 5 µg/ml ethidium bromide. This protocol yielded a high molecular weight DNA isolated from fresh as well as dry leaves of T. arjuna, which was free from contaminations and colour. On amplification using RAPD primer, the isolated DNA shows high intensity bands.

Ogunkanmi *et al.,* (2008) presented a protocol for isolation of genomic DNA from different tissues of pepper (*Capsicum annuum*). They reviewed the protocol of Dellaporta *et al.,* (1983) and modified the protocol for isolation of DNA from preserved tissues of Capsicum which contains high levels of polysaccharides. They used healthy matured fruits with red/green colour which were preserved in Dellaporta buffer and were refrigerated at 4$^{o}$C. The preserved fruit tissues were washed with 70% ethanol. They ground the washed sample (400 mg) in preheated (65$^{o}$C) mortar with Dellaporta buffer (1600 µl) mixed with 200 µl of 20% SDS. 4 ml RNase was added and mixed and incubated at 65$^{o}$C for 30 minutes. 1000 ml ice-cold isopropanol was added and incubated at -20$^{o}$C overnight without mixing. They washed with 100 ml of 70% ethanol after second precipitation with ice-cold isopropanol by discarding two-thirds of the supernatant carefully without dislodging the DNA and 2-3 times invert mixing was done and were centrifuged at 9,000 rpm for 10 minutes to pellet the DNA. DNA was checked on 0.8% agarose gel along with standard DNA. It was stained with ethidium bromide and visualized under UV transilluminator for quality. The quality of DNA was further determined by measuring the OD 260 and OD 280 and calculating the absorbance ratio. PCR

amplification was done with RAPD primer in 12.5 µl reaction mixture containing 2.5 µl template DNA, 10X buffer, 2.5 mM dNTPs, 25 mM $MgCl_2$, 5% Tween20, 0.2 µl Taq polymerase and 0.5 µl RAPD. PCR conditions were initial denaturation at 94°C for 3 minutes followed by 45 cycles of 94°C for 20 seconds, 37°C annealing for 40 seconds and 72°C for 1 minute and final extension at 72°C for 7 minutes and stored at 4 °C. Amplified products were fractioned on 1.5% agarose gel stained with ethidium bromide. This modified protocol yielded a high quality DNA and was found to be suitable for PCR and RAPD analysis. The absorbance ratio was between 1.7 and 1.9.

Choudhary *et al.,* (2008) developed a simple and efficient protocol for isolating genomic DNA from fresh and dry leaves of *Vigna aconitifolia* and *V. trilobata. V. aconitifolia* (moth bean) is grown for food in the arid and semi-arid regions of India. It is an important pulse crop in semi-arid regions adjoining tropical dry regions. *V. trilobata* is a wild species, commonly called as African gram, Chidi moth (Hindi) etc. had a higher content of crude protein than commonly consumed Indian pulses. Genus Vigna have high amount of polyphenols, orthohydroxyphenols and polysaccharides. They used fresh juvenile leaves that were harvested from in vitro grown plantlets raised from seeds of V. aconitifolia and V. trilobata maintained in growth rooms. The leaves were washed with sterilized distilled water and then by 80% alcohol. The samples were divided into two- one was kept at 4°C and the other was dried at 50°C. The leaves were cut into small pieces of size approx. 1 mm with sterile blade. The fresh (2 g) and dried (1 g) leaf samples were crushed in a pre-chilled pestle and mortar. The powder was transferred in 8 ml extraction buffer (3% CTAB (w/v), 100 mM Tris HCl (pH-8.0), 2 M NaCl, 25 mM EDTA (pH-8.0), 4% β-mercaptoethanol (v/v), 5% PVP (w/v)) into a 30 ml centrifuge tube. The mixture was incubated at 70°C for 30 minutes. After 30 minutes, mixture was cooled at room temperature and equal volume of CIA (24:1) was added. They purified DNA by adding 500 ml CIA and mixed the contents by shaking for 15 minutes and centrifuged at 12,000 rpm for 15 minutes. The aqueous phase was transferred to a new tube and 200 ml 1 M NaCl-TE added to the old tube and shaken for 15 minutes. Old tube was centrifuged for 15 minutes at 12,000 rpm. The aqueous phase was transferred to new tube and mixed and centrifuged at 12,000 rpm for 15 minutes to settle down any remaining debris. The supernatant was transferred to a new tube. 700 ml ice-cold Isopropanol was added to the sample and was mixed gently and centrifuged at 10,000 rpm for 5 minutes and the supernatant was discarded. Cold 75% ethanol and 5 M NaCl were added to the pellet to wash it thrice and contents were centrifuged at 5,000 rpm for 5 minutes. Ethanol was discarded and pellet was air dried. Pellet was re-suspended in 200 ml 1X TE buffer and incubated overnight at 55°C. RNase (10 µl of 10 mg/ml) was added to 100 µl of re-suspended DNA pellet and was incubated at 37°C overnight. Equal volume of ice-cold absolute ethanol was added to each sample and was centrifuged at 10,000 rpm for 10 minutes to re-precipitate the DNA. This was done twice. The supernatant was poured off and DNA pellet was air dried and finally DNA pellet was dissolved in 50 µl 1X TE buffer. They determined DNA concentration by using bio spectrophotometer by measuring optical density at 230, 260 nm, 280 nm and 320 nm. Total DNA purity was tested by a ratio of optical density values at 230:260:280. They also checked the quality of DNA by agarose gel (1% (w/v)) containing 0.05 µl $gml^{-1}$ in which 2 µl DNA, 7µl sterile distilled water and 1 µl 10X loading dye were loaded in a lane. Hind III digested lambda DNA was used as a size marker. Electrophoresis was carried out for ~1 hour at 50 V. The gel was visualized and photographed under UV light. The purity of isolated DNA was checked with restriction digestion of the purified DNA samples with EcoRI and Hind III (4 unit per µg) for 5 h incubated periods and restricted DNA run on ethidium bromide stained gel (1.2%) with EcoRI digested lambda DNA as marker. PCR was performed to check the quality of isolated DNA from the root samples of Vigna species. PCR were carried out in a 25 µl reaction mixture containing 20 ng template DNA, 100 µM each dNTP, 20 ng random primers, 1.5 mM $MgCl_2$, 1X buffer, 1U Taq polymerase. PCR temperatures were preliminary 4 min denaturation step at 94°C, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 37°C for 1 minute and extension at 72°C for 2 minutes and finally at 72°C for 10 minutes. Amplified products were separated alongside EcoRI/Hind III double digested lambda genome as marker by electrophoresis on 1% agarose gel run in 0.5X TAE buffer, stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through Gel Doc System (Vilber Lourmat, Germany) and the amplified product sizes were evaluated by using software (Vision Capt Version 14.3). The result indicated in V. aconitifolia, the quantity of the genomic DNA was about 46-48 $\mu gg^{-1}$ from fresh leaves and 51-53 $\mu gg^{-1}$ from dried leaves. In V. trilobata the total DNA yield was about 46-48 $\mu gg^{-1}$ of fresh young leaf and 55-57 $\mu gg^{-1}$ of dried leaves. The yield in both species was higher than methods suggested by Dellaporta *et al.,* (1983) and Doyle and Doyle (1990).

Shahzadi *et al.,* (2010) tested and modified various standard protocols to isolate high quality DNA from different plant tissues of *Tagetes minuta*. *T. minuta* contains high concentration of essential oils and metabolites which interfere with DNA isolation and PCR. They used sun-dried, shade-dried and fresh leaf tissues and seeds of *T. minuta*. Fresh leaf tissue was ground in a 1.5 ml centrifuge tube with a micropestle and 800 µl warm freshly prepared extraction buffer (0.1 M Tris-Cl (ph-9.5), 20 mM EDTA (pH-8.0), 1.4 M NaCl, 2% CTAB, 1% β-mercaptoethanol) was added to the tube. Seeds, sun-dried and shade-dried leaf samples were grounded to fine powder with a pestle and mortar and transferred to a 1.5 ml centrifuge tube and 800 µl extraction buffer was added. The tubes were incubated at 65°C for 35-45 minutes with invert mixing during incubation. Equal volume of CIA (24:1) was added and invert mixing was done 8-10 times and centrifuged at 13,000 rpm for 15 minutes. The supernatant was transferred into a new tube. The CIA step was repeated until precipitation was removed. Equal volume of ice-cold absolute isopropanol was added and centrifuged at 13,000 rpm for 10 minutes. The supernatant was discarded and the pellet was washed with 70% ethanol and air-dried for 1 hour at room temperature and dissolved in 100 µl TE buffer. 2 µl (1µg/µl) RNase A was added and incubated at 37°C for 1 hour. For further purification, CIA step was repeated. DNA was precipitated by adding $1/10^{th}$ volume of 3 M sodium acetate (pH-5.2) and 2.5 volume ice-cold ethanol. Invert mixing was

done and this mixture was maintained for 30 minutes at -20°C and centrifuged at 13,000 rpm for 10 minutes. The supernatant was discarded and the pellet was washed with 70% ethanol and was dried and dissolved in 100 µl TE buffer. The quality of DNA was checked by electrophoresis on 0.8% agarose gel and DNA concentrations were measured spectropho tometrically by reading absorbance at 260 nm. DNA samples were stored at -20°C till further use. PCR amplification of DNA was carried out in 20 µl reaction mixture containing 25 ng template DNA, 0.125 U Taq DNA polymerase, 1.6 mM dNTPs, 3.75 mM $MgCl_2$, 1X buffer and 2 mM primer. PCR conditions were- initial denaturation at 94°C for 30 seconds, annealing at 50°C for 1 minute, and extension at 72°C for 1 minute and final extension at 72°C for 7 minutes. The PCR products were separated on 0.8% agarose gel using 1X TBE buffer containing 10 mg/ml ethidium bromide and were visualized by UV light and the gels were photographed. The DNA obtained by this protocol was good in quality which was without contaminants and coloured pigments. Good quality DNA was obtained without using liquid nitrogen. DNA isolated from sun0dried and shade-dried tissues were large in amount but quality was not good as compared to seeds. DNA isolated from seeds and fresh leaves were successfully amplified by PCR using arbitrary RAPD primers.

Xin and Chen (2012) presented a low cost, high yield, high quality method to isolate DNA from Sorghum (Sorghum bicolour (L.) Moence) leaves and dry seeds. They used freeze dryer to dry the leaves and seeds. They added one tungsten ball to the lyophilized leaf tissue and dry seeds in microfuge tubes and placed the tubes in the grinding racks. The tissues and seeds were ground and 750 µl extraction buffer was added to each tube. Mixing was done and tubes were incubated at 60°C for 1 hour. After 1 hour, tubes were then cooled down at room temperature for 5 minutes and 750 µl CIA (24:1) was added to each tube and mixed well and centrifuged at 3000g for 15 minutes. The aqueous layer was transferred to a new set of labelled tubes and 1 ml dilution buffer was added to this aqueous phase. It was mixed properly and again incubated at 60°C for 30 minutes and centrifuged at 3000g for 15 minutes and the supernatant was discarded. 1ml washing buffer was added to the pellet and soaked at room temperature for 30 minutes to remove excess CTAB and again centrifuged at 3000g for 15 minutes and supernatant was discarded. The DNA pellet was re-suspended in 100µl high salt TE with RNase A and incubated at 60°C for 30 minutes. The TE DNA solution was transferred to 96-well microtiter plate, one sample per well and 5µl MagAttract suspension G solution was added to each well. 120µl 100% ethanol was added to each well. The microtiter plate was tightly covered with silicone plate sealing mat and mixed gently and incubated at room temperature for 5 minutes to allow DNA adhere onto the surface of beads. The DNA plate was then placed on Magnet B to hold the MagAttract beads and poured off the ethanol solution. The beads were washed three times with 200µl washing buffer and air-dried the beads for 10 minutes at room temperature. 100µl TE to each sample well was added to re-suspend DNA and incubated at 60°C for 5 minutes to allow the bound DNA release into TE solution. Plate was placed on Magnet B and transferred DNA solution to a new 96- well plate and quantification of DNA was done on a Nanodrop spectrophotometer or TECAN plate reader. With this extraction method, DNA yield was increased by an average of 30 folds with consistently high purity. The hand on time for preparing one 96-well plate DNA samples is less than 2.5 hours. They also tested this method on various plant species to check its applicability and they found out that the DNA yield of those plant species were also very high.

Roychowdhury *et al.,* (2012) standardized a DNA isolation protocol for rice which is simple, cost-efficient, high throughput, PCR compatible and require a less amount of plant tissue without using liquid nitrogen. They used germinating seedlings, dehusked kernels, rice straw and callus tissue grown in MS-media supplemented with NAA and kinetin of rice (*Oryza sativa* L.) var. Gorah which is a drought tolerant traditional rice of Rarh Bengal. The plant material was washed thoroughly and was soaked in nuclear extraction buffer (15% Sucrose, 50 mM Tris-HCl (pH-8.0), 50 mM Sodium-EDTA and 250 mM NaCl) except for seedlings, callus and kinetin. The samples were weighed and homogenised in pre-chilled (-20°C) mortar and pestle with 850 µl of nuclear extraction buffer. The homogenate was centrifuged at 10,000 rpm for 8 minutes at 4°C. The supernatant was discarded and the pellet was suspended in 700 µl of lysis buffer (10 mM Tris-HCl (pH-8.0) and 1 mM Sodium-EDTA). 200 µl of 10% SDS solution was added to lyses the nuclei and incubated at 70°C for 15 minutes in water bath and was cooled to room temperature and 200 µl 7.5 M ammonium acetate solution was added and invert mixing was done and incubated at 4°C for 2-5 hours and centrifuged at 10,000 rpm for 10 minutes at 4°C. 800 µl pre-chilled isopropanol was added to the supernatant and incubated overnight at 0°C and centrifuged at 10,000 rpm for 10 minutes at 4°C. The pellet was washed twice with 70% ethanol and vortexed and air-dried and dissolved in 80 µl TE buffer at 4°C for 12 hours and preserved at -20°C. 5 µl of 10 µg/ µl RNase A was added to the dissolved DNA and incubated at 50°C for 30 minutes in water bath to remove RNA. The mixture was cooled to room temperature and volume was made up to 400 µl by adding TE buffer and equal volume (400 µl) CIA was added to remove proteins and invert mixing was done. The mixture was centrifuged at 8,000 rpm for 8 minutes at room temperature. The upper aqueous phase was removed and $1/10^{th}$ volume of 3 M Sodium acetate solution (pH-5.2) was added. 500 µl pre-chilled isopropanol was added and incubated for 12 hours at 4°C and centrifuged at 10,000 rpm for 10 minutes. DNA pellet was washed twice with 70% ethanol and air-dried and dissolved in 80 µl TE buffer. Quantitative analysis of both non-purified (before RNase treatment) and purified (after RNase treatment) genomic DNA was done by using UV spectrophotometer and the ratio of absorbance at 260 nm to that of 280 nm was calculated. The isolated DNA was fractioned on 0.8% agarose gel. PCR amplifications were carried out in 25 µl reaction mixture containing 10X Taq buffer, 50 mM $MgCl_2$, 2.5 mM dNTPs, 10 pmole/µl of forward and reverse rice microsatellite primer (RM315), 5 U/ µl Taq DNA polymerase, 100 ng DNA sample and PCR-grade water. PCR conditions for first cycle were 97°C for 5 minutes and 55°C for 2 minutes, followed by 35 cycles of 95°C for 1 minute, 55°C for 1 minute and 72°C for 2 minute and final extension at 72°C for 10 minutes. The amplified products were separated on 6% polyacrylamide gel and documentation was done in gel documentation system. 100 bp DNA ladder was used. Molecular weights of amplified

DNA bands were analyzed using AlphaEaseFC software. Highly pure DNA was obtained from callus tissue (1.77 µg/µl), and minimum was found in straw (1.68 µg/µl). Maximum DNA concentration was found in seedlings (9.52 µg/ml) and minimum in straw (1.68 µg/ml). The yield was highest in seedlings (0.57 µg) and lowest in straw tissues (0.1 µg). PCR amplification gave clear, sharp and uniform bands. The advantages of this protocol are non-requirement of liquid nitrogen and phenol, which are hazardous.

Iqbal *et al.,* (2013) presented a simple and cost effective protocol for isolation of genomic DNA from dry parts of Berberis and Mentha. These two genera are of important medicinal plants. They used five species of Berberis, i.e., *B. kunwarensis*, *B. lyceum*, *B. orthobotrys*, *B. pachyacantha* and *B. perkeriana* and four species of Mentha, i.e., *M. arvensis*, *M. longifolia*, *M. royleana* and *M. spicata*. They sun-dried the fresh plant samples. They grounded 0.026 g dry part of plant in pestle and mortar without liquid nitrogen. The powder material was transferred to 1.5 ml eppendorf tube and 500 µl CTAB buffer (Sodium EDTA, Tris-HCl, NaCl, 2% CTAB), 1% PVP and 0.5 µl β-mercaptoethanol were added. The mixture was incubated at 65$^{o}$C for 35 minutes and cooled down at room temperature and one volume of CIA was added and mixed for 1-2 minutes and centrifuged at 6,000 rpm for 15 minutes. The supernatant was transferred to new tube. CIA step was repeated if impurities are present. 0.5 volume of 5 M NaCl and one volume of ice-cold pure ethanol were added to the supernatant and kept at 6-7$^{o}$C for 15-20 minutes or 12 hours and centrifuged at 3,000 rpm for 3 minutes and again at 8,000 rpm for 5 minutes. The supernatant was discarded and the pellet was washed with 70% ethanol and dried and dissolved in 15 µl TE buffer and kept for 1 hour or overnight at 6-7$^{o}$C to dissolve DNA completely. The quality and quantity of DNA was checked on 1% TBE agarose gel. The gel was observed under UV light. If RNA is present then 1 µl RNase A was added and incubated at 35$^{o}$C for 15 minutes to remove RNA. PCR amplification was done with a primer. Pure and sufficient DNA was isolated by this method and was suitable for PCR amplification and Southern blotting hybridization analysis.

Aliyu *et al.,* (2013) presented a DNA isolation protocol for rice species. They compared three published protocols (Protocol A- Dellaporta *et al.,* (1983), Protocol B- Doyle and Doyle (1990), Protocol C- Wang *et al.,* (1993)) with the proposed 'STI' (Sodium hydroxide, Tris-HCl, Isopropanol) (Protocol-D) protocol and the basis of evaluation was duration of extraction, purity, yield and cost of protocol. They used rice seeds of ten genotypes (2 *Oryza sativa*, 2 interspecific hybrids and 6 *Oryza glaberrima*) which were cleaned and dried at 50$^{o}$C in an oven for five days and were surface sterilized with 0.1% $HgCl_2$ and were rinsed with distilled water. The sterilized seeds were soaked in water and incubated for 48 hours at 30$^{o}$C. These seeds were sown in loamy soil. After ten days, young leaf tissues were harvested and placed on ice in eppendorf tube. They extracted DNA from fresh young leaves by Protocol A, B and C and D. In 'STI' protocol, they grounded the samples in geno grinder and 100 µl sodium hydroxide and 400 µl 100 mM Tris-HCl were added to the crushed sample. Invert mixing was done for 5 minutes and was centrifuged at 13,000 rpm for 10 minutes. The supernatant was transferred to a new eppendorf tubes. 20 µl TE-RNase solution was added and incubated for 20 minutes at 37$^{o}$C. 300 µl sodium hydroxide and 300 µl isopropanol were also added to the tube and centrifuged at 13,000 rpm for 10 minutes. The supernatant was discarded and the pellet was washed with 200 µl of 70% ethanol and air-dried for 20 minutes.

The pellet was re-suspended in 100 µl low salt TE buffer. DNA concentration was determined by ND-1000 spectrophotometer. The purity of DNA was calculated by absorbance ratio of 260 nm to that of 280 nm and the duration of extraction for 96 samples was estimated. PCR amplification was carried out with SSR primer in 10 µl reaction mixture containing 1X PCR buffer, 25 mM $MgCl_2$, 10 mM dNTPs, 20 pmole primer pair, 0.5 µl Taq polymerase, milu-Q water and 10 ng/µl DNA sample. PCR conditions were 2 minutes preheating at 94$^{o}$C, denaturation for 30 seconds at 94$^{o}$C, 34 cycles of 30 seconds at 94$^{o}$C, 30 seconds annealing at 55$^{o}$C and 67$^{o}$C depending on the marker used and 30 seconds initial extension at 72$^{o}$C and final extension at 72$^{o}$C for 2 minutes. The amplified products were fractioned on 2% agarose gel in 1X TBE buffer at 78-80 V for 2-3 hours. The gel was stained with ethidium bromide and was visualized under UV light. DNA extracted using Protocol A and Protocol D yielded good quality DNA. DNA obtained from Protocol B and C were coloured (yellowish or dark green). Protocol A was laboured intense. Protocols B, C and D were cost effective as compared to Protocol A. DNA yielded from Protocol B and C were higher than Protocol A and D. PCR amplification with SSR primer was good.

**Conclusion**

Remarkable work has been done in the field of plant DNA isolation by various researchers using different protocols. These protocols have been successfully isolated DNA and their PCR amplification have been carried out with various primers.

## REFERENCES

Aliyu, R. E., Wasiu, A. A., Dangora, D. B., Ademola, J. O. and Adamu, A. K. 2013. "Comparative Study Of Genomic DNA Extraction Protocols In Rice Species", *International Journal Of Advancements In Research & Technology,* Vol 2, Issue 2, ISSN- 2278-7763.

Choudhary, K., Mathur, N., Choudhary, O. P. and Pillai, U. 2008. "Protocol for Isolation of Genomic DNA from Dry and Fresh Leaves Of *Vigna* Species Suitable For RAPD and Restriction Digestion", *Advances In Biological Research,* 2 (5-6): 83-89, ISSN 1992-0067.

Deshmukh, V. P., Thakare, P. V., Chaudhari, U. S. and Gawande, P. A. 2007. "A Simple Method For Isolation Of Genomic DNA From Fresh And Dry Leaves Of *Terminalia Arjuna* (Roxb.) Wight and Argot", *Electronic Journal of Biotechnology,* Vol.10 No.3, ISSN: 0717-3458.

Iqbal, A., Ahmad, I., Ahmad, H., Nadeem, M.S., Nisar, M. and Riaz, H. 2013. "An Efficient DNA Extraction Protocol for Medicinal Plants", *International Journal of Biosciences,* Vol. 3, No. 7, P. 30-35.

Khanuja, S. P. S., Shasany, A. K., Darokar, M. P. and Kumar S. 1999. "Rapid Isolation Of DNA From Dry And Fresh

Samples Of Plants Producing Large Amounts Of Secondary Metabolites And Essential Oils", *Plant Molecular Biology Reporter,* 17: 1–7.

Nalini, E., Jawali, N. and Bhagwat, S. G. 2004. "A Simple Method for Isolation of DNA from Plants Suitable For Long Term Storage and DNA Marker Analysis", *Bhabha Atomic Research Centre Newsletter,* Issue No. 249.

Ogunkanmi, A. L., Oboh, B., Onifade, B., Ogunjobi, A. A., Taiwo, I. A. and Ogundipe, O. T. 2008. "An Improved Method Of Extracting Genomic DNA From Preserved Tissues Of *Capsicum Annuum* For PCR Amplification", *Eurasian Journal of Biosciences,* 2, 115-119.

Roychowdhury, R., Karmakar, J. and Dey, N. 2012. "PCR-Compatible Genomic DNA Isolation from Different Tissues of Rice (*Oryza Sativa*) For SSR Fingerprinting", *Eurasian Journal of Biosciences,* 6: 85-90.

Shahzadi, I., Ahmed, R., Hassan, A. and Shah, M. M. 2010. "Optimization of DNA Extraction from Seeds and Fresh Leaf Tissues of Wild Marigold (*Tagetes Minuta*) For Polymerase Chain Reaction Analysis", *Genetics and Molecular Research,* 9 (1): 386-393.

Storchova, H., Hrdlickova, R., Jr. J. C., Tetera, M., Fitze, D. and Fehrer, J. 2000. "An Improved Of DNA Isolation From Plants Collected In The Field And Conserved In Saturated NaCl/CTAB Solution", *Taxon,* 49, 79-84, ISSN-0040-0262.

Xin, Z. and Chen, J. 2012. "A High Throughput DNA Extraction Method with High Yield and Quality", *Plant Methods,* 8:26.

Zidani, S., Ferchichi, A. and Chaieb, M. 2005. "Genomic DNA Extraction Method From Pearl Millet (*Pennisetum Glaucum*) Leaves", *African Journal Of Biotechnology,* Vol. 4 (8), Pp. 862-866.

*******