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Optimization of quality DNA isolation protocol from various mucilage rich cultivated and wild *Abelmoschus sp.* and its validation through PCR amplification

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Abstract

Okra is such a genomically less explored crop that, many labs are still trying for the optimization of quality DNA isolation protocol for the efficient use in the contemporary genomic studies. In this study, we have optimized a quick and reproducible DNA isolation protocol for the isolation of quality genomic DNA from different tissues of two cultivated (*Abelmoschus esculentus* and *A. caillei*) and seven wild okra species (*A. manihot, A. moschatus, A. tetraphyllus, A. tuberculatus, A. ficulneus, A. rugosus* and *A. angulosus*), including related species *Hibiscus cannabinus*. The quality of isolated DNA was also confirmed using PCR amplification of various DNA markers like Inter Simple Sequence Repeat (ISSR), Random Amplified Polymorphic DNA (RAPD), Start Codon Targeted (SCoT) and Expressed Sequence Tag-Simple Sequence Repeat (EST-SSR). This is probably the first report wherein the DNA isolation protocol has been optimized for nine *Abelmoschus* species along with a related species *Hibiscus*. Thus, good quality and quantity of DNA can be isolated in okra, with the care that it should be performed using appropriate plant tissue, either very young leaves or etiolated seedlings, and well optimized DNA isolation protocol as prescribed in this study.

Key words: Okra, DNA isolation, Marker validation, Wild-relatives, Quantification

Introduction

Okra (*Abelmoschus esculentus* L.) is a vital member of Malvaceae family which also includes some important fiber crops like cotton (*Gossypium spp*.) and kenaf (*Hibiscus cannabinus*). The present accepted binomial for okra is *Abelmoschus esculentus* (L.) Moench and earlier it was referred as *Hibiscus esculentus* L. It is primarily a warm season vegetable crop, requiring warm nights (>20°C) and mostly grown in tropical and subtropical parts of the world (Seth et al. 2017). Annually, okra is grown globally in approximately 2.16 m ha land, yielding around 8.9 mt, having average yield of nearly 4.12 t ha^{-1}. From over 0.48 m ha area with 11.3 t ha^{-1} productivity, India is first in world ranking having of 5.5 mt production (62% of world production). Although, more than 99% okra farming is solely done in Asian and African countries but its productivity is very poor in African countries (1.82 t ha^{-1}) compared with any other part of the world (FAOSTAT 2016). The centres of genetic diversity of *Abelmoschus* are India, West Africa and Southeast Asia (Mishra et al. 2017). Although the genus *Abelmoschus* consisted of 34 species (30 old world and 04 new world), but *A. esculentus* and *A. caillei* are the two cultivated species. It is an allopolyploid crop with substantial disparity in its chromosome numbers and ploidy levels. The lowest known chromosome number is 2n=56 in *A. angulosus*, whereas the highest goes up to 200 in *A. caillei*. Even within *A. esculentus*, a range of 2n chromosome numbers including 72, 108, 120, 132 and144 are recorded which are actually derived as a series having basal chromosome number n=12 (Mishra et al. 2017). Okra contains thick and slimy mucilaginous substance in various plant parts except seeds. In India, okra mucilage from its stem and roots are used as an additive for clarifying sugarcane juice; whereas, in Malaysia and China it is used for sizing of paper. Chemically, mucilage is an acidic polysaccharide associated with proteins and minerals and is potentially used in various food and non-food products including medicines (Mishra et al. 2017).

In okra, during photosynthesis large amounts of polysaccharides, secondary metabolites and viscous glue like mucilaginous substances are produced which

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hinders the isolation of good quality DNA. Further, secondary metabolites including polysaccharides, alkaloids, flavonoids, phenols and terpenes cause major hindrance in the extraction of genomic DNA of excellent quality. These also creates problem during pipetting as well as during amplification as it inhibits *Taq* polymerase action (Bayer et al. 1999). Major obstacle of okra DNA isolation are very-less DNA yield, poor amplification and restriction digestion for high end genomic studies (Kumar et al. 2011). Although okra is an export earning vegetable but unfortunately very little work has been done at molecular level due to the unavailability of perfect DNA isolation protocol. Researchers are still facing problems in the isolation of high quality genomic DNA. Although several DNA isolation methods are reported for cultivated okra, but none of these gives very promising results. Besides, no report is available for the optimization of DNA isolation protocol especially from the wild okra species. Thus, optimization of an efficient DNA isolation protocol from both cultivated and wild okra species is urgently required. In this backdrop, present investigation was designed to isolate good quality genomic DNA from different *Abelmoschus sp.* for its use in various molecular biology applications.

Materials and Methods

Plant materials: A set of forty diverse genotypes of cultivated and wild *Abelmoschus* species, consisting of *A. esculentus* (22), *A. manihot* (02), *A. moschatus* (01), *A. tetraphyllus* (02), *A. tuberculatus* (01), *A. ficulneus* (01), *A. rugosus* (01), *A. angulosus* (01), *A. caillei* (02) and a related species *Hibiscus cannabinus* (07) collected and maintained at ICAR-IIVR were used for the present study.

DNA Extraction and quantification: The seeds are sown in pre-soaked germination paper and kept for germination in the germinator under dark conditions. Two grams of 7 to 10 days old etiolated seedlings, tender leaves form seedlings, and mature green leaves from the plants grown under field conditions were used for the DNA isolation (Figure 1). Extraction and purification of okra DNA both from etiolated seedlings and fresh leaves were performed using three protocols *viz.*, (i) Modified CTAB method with liquid N_2 (Doyle and Doyle 1990); (ii) Modified CTAB method without liquid N_2 and (iii) DNA isolation kit (QIAGEN Ltd., Crawley, UK) as per manufacturers' protocol.

The plant tissues (leaves and seedlings) were first rinsed with autoclaved distilled water and dried using sterilized blotting paper. The plant tissue (~2.0 g) was crushed in liquid nitrogen to fine powder which was then added in 3 volume of freshly prepared CTAB buffer. In another

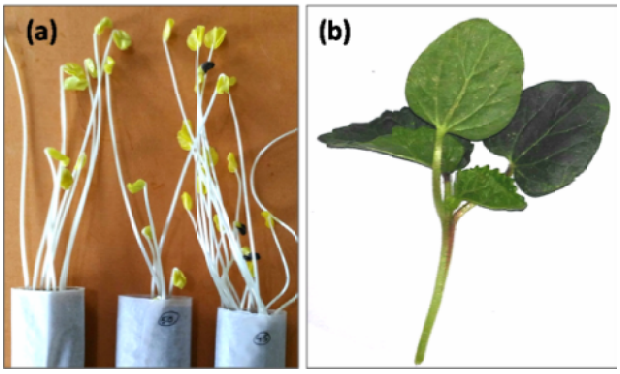

Figure 1: Representative picture showing (a) Etiolated seedlings and (b) Young seedlings, which are used for the isolation of DNA from okra.

case, where no liquid nitrogen was used, the tissue was directly crushed in the pre-warm CTAB extraction buffer. The crushed tissue in the buffer was incubated at 65°C for 1.0 h with intermittent mixing. Then, chloroform: isoamyl alcohol (24:1) was added in equal volume, mixed well, centrifuged (11000 rpm; 20 min) and supernatant was collected. After that, chilled isopropanol was added in equal volume and precipitate was collected as pellets after centrifugation which was then washed using 70% ethanol, dried at room temperature and dissolved in double distilled water.

RNaseA (10 µg/100 µl of DNA) was added and kept at 37°C (1 h), then equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added followed by centrifugation (10,000 rpm; 5 min). Then upper aqueous phase was collected in a new tube and extracted twice in chloroform: isoamyl alcohol (24:1) with recurring centrifugation (10,000 rpm; 5 min). Again upper aqueous phase was separated, mixed with 1/10 volume of 3M sodium acetate and DNA was precipitated by adding two volume chilled absolute alcohol and pelleted by centrifugation (5000 rpm, 3 min). The pellet was dried and dissolved in double distilled water and quantity of genomic DNA was assessed by NanodropND 8000 (Thermo Scientific, USA), while its quality was checked by running 10 iL of DNA on 0.8% agarose gel at 80-100V for 2 h in 0.5X TBE buffer. Staining of the gel was done with ethidium bromide (0.5ig/mL) and observed on UV-transilluminator. Quality of DNA samples was studied based on whether the DNA sample formed a single high molecular weight band or a smear.

DNA amplification and visualization: PCR reactions were performed in 25 µl mixture containing genomic DNA (50 ng), 10x PCR buffer (2.5 µL), dNTP mix (0.2 mM), primers (2 µM each) and Taq polymerase (1 unit). The DNA which was isolated using various extraction protocols was then used for PCR amplification using four marker systems, viz., Inter

Simple Sequence Repeat (ISSR), Random Amplified Polymorphic DNA (RAPD), Start Codon Targeted (SCoT) and Expressed Sequence Tag-Simple Sequence Repeat (EST-SSR) markers (Bosamia et al. 2015; Alam et al. 2009; Kumar et al. 2009). The primers were synthesized from Sigma Aldrich (India) and PCR amplification was performed in a gradient thermal cycler (Applied Biosystems, USA) using separate programmes for different primers. For RAPD the thermal cycler was programmed as follows: Initial denaturation at 94°C (4 min) followed by 36 cycles each of 94°C (1 min), 36°C (1 min), 72°C (2 min) and 72°C (10 min); whereas for ISSR, SSR and SCoT the PCR programme has a bit higher annealing temperature than the RAPD primers. The PCR programme for these primer was as follows: initial denaturation at 94°C (4 min) followed by 36 cycles each of 94°C (1 min), 55±3°C (1 min), 72°C (2 min) and 72°C (10 min). Further, the amplified products were stored at 4°C till electrophoresis. For electrophoresis, PCR product was first mixed with 6X loading dye and then electrophoresed on agarose gel (2%) having ethidium bromide (0.5ìg/mL) in 0.5X TBE buffer at 100V for about 2.5 h. The size of amplified product was determined by running of 100 bp ladder. Further, the amplified products were visualized using UV transilluminator and photographed using Gel documentation system (Alfa Innotech, Alpha Imazer^{PM} 3400).

Results and Discussion

Isolation of DNA using different plant parts and isolation methods: The major impediment which hinders the extraction and purification of good quality DNA from okra leaves is the presence of huge concentration of mucilaginous polysaccharides, having polygalacturonic acid and its association with a range of minerals (Ahmed et al. 2013; Singh and Kumar 2012). Further, during cell-lysis, the DNA gets bound with these polysaccharides and polyphenols get oxidized resulting in formation of a brown gelatinous substance and ultimate reduction in both quantity and quality of extracted DNA (Aljanabi et al. 1999). Therefore, a total of eight DNA isolation combinations of fully grown leaves, immature leaves (shoot tips) and etiolated *Abelmoschus sp.* seedlings were used in combination with CTAB (with and without liquid nitrogen) and commercially available DNA isolation kit (Table 1; Figure 2a-h).

While isolating the DNA using CTAB method, a slight modification was done by adding 4.0g polyvinylpyrrolidone (PVP) and 50µl 6M potassium iodide in 100 mL of DNA extraction buffer. PVP was added to reduce the amount of mucilage and polyphenols present in the leaves, by forming a complex with polyphenols via hydrogen bonding; while potassium iodide was added to denature the secondary metabolites. Besides, samples were also kept for digestion in the extraction buffer at bit higher temperature 65°C for 60 min. instead of standard 60°C for 30 min. Finally, good quality DNA was obtained for its ultimate use in PCR and other applications (Kumar et al. 2011). On the similar note, Adiger and Sridevi (2014) also reported slight modification of CTAB method in which they have added 100 mg PVP and 2.5M potassium acetate in the extraction buffer and increased the incubation time and temperature (65°C for 45min) for the isolation of DNA from okra fresh leaves. Some other researchers have also reported slight modification in the CTAB protocol

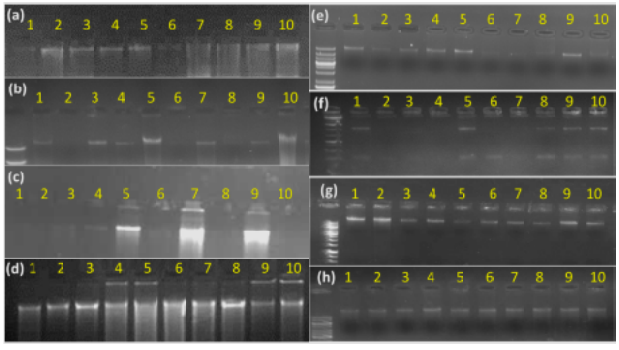

Figure 2: Representative picture showing the quality of DNA isolated using (i) Modified CTAB method from (a) Fully grown leaves; (b) Very young leaves with liquid N_2; (c) Very young leaves without liquid N_2, (d) Etiolated seedlings without liquid N_2; (e) Etiolated seedlings with liquid N_2; (ii) DNA isolation using kit from (f) Fully grown leaves; (g) Very young leaves; (h) Etiolated seedlings.

Table 1: Comparison of various DNA isolation protocols as reported by different researchers

S. No.	Plant material	DNA quality obtained	Reference
1.	Dark-grown seedlings	+++	Kochko and Hamon,1990
2.	Leaves (both fresh and mature)	+	Khanuja, 1999
3.	Fresh leaves (used PVP)	++	Singh and Kumar, 2012
4.	Fresh leaves (No liquid nitrogen)	+++	Ahmed et al. 2013
5.	Leaves (DNeasy-Qiagen DNA extraction kit)	++	Narendran et al. 2013
6.	Dried leaves (No liquid nitrogen)	++	Meena et al. 2014
7.	Fresh leaves	+++	Adiger and Sridevi, 2014

Where: + : Poor quality; ++ : Good quality; +++ : Very good quality DNA

for DNA isolation from fresh okra leaves (Jie et al. 2008). Further, Singh and Kumar (2012) have reported a DNA isolation protocol from the fresh leaves of okra in which modification involved use of more volume of DNA extraction buffer (1.5 mL/sample), less sample quantity (50-60mg), more salt concentration (5M) and PVP.

The experimental results showed poor quality DNA when isolated from fully grown leaves using both modified CTAB method with liquid N_2 and from DNA isolation kits (Figure 2a and 2f). Likewise, Schafleitner et al. (2013) used second fully expanded leaf of okra from the top and CTAB method with liquid nitrogen for DNA isolation. The problem was also faced even during pipetting of the isolated DNA due to the presence of high concentration of polysaccharides, secondary metabolites and brown mucilaginous substances. It was reported that, at the time of cell-lysis, DNA make contact with various polysaccharides. Further, polyphenols in oxidized form bind covalently and irreversibly to proteins and nucleic acids which reduce both content and quality of isolated DNA (Aljanabi et al. 1999).

However, DNA isolated using very young leaves from the shoot tip (1-2 cm) using CTAB method (with or without liquid N_2) showed relatively less smearing and fragmentation of isolated DNA during agarose gel electrophoresis (Figure 2b and 2c). This may be due to comparatively less concentration of polysaccharides, polyphenols and other secondary metabolites in the young leaves than in the mature leaves (Zhang and Stewart, 2000). Moreover, the quantity of DNA obtained in this study was also very less (Table 2). Bayer et al. (1999) also stated such complexity during DNA isolation from the plants belonging to Malvaceae family. Fang et al. (1992) also reported viscous polysaccharides making it very cumbersome to handle the DNA both during pipetting and amplification. The DNA isolation protocol as reported by Ahmed et al. (2013) and Meena et al. (2014) yielded good quality DNA from the highly mucilaginous okra green and dried leaves respectively, which also does not require liquid nitrogen. However, in this investigation we obtained good quality DNA only with etiolated seedlings when no liquid nitrogen was used for the crushing of the samples (Figure 2d). Moreover, best quality and quantity of DNA could be isolated when etiolated seedlings were used either with modified CTAB method (with liquid N_2) or using DNA isolation kits (Figure 2e and 2h). Further, the agarose gel electrophoresis also showed very distinct and intact high molecular weight DNA bands without any smearing or fragmentation (Figure 2g and 2h). Likewise, previous workers have also used various DNA isolation kits and plant tissues for the isolation of DNA from the okra. Further, Narendran et al. (2013) used QIAGEN DNeasy® Kit (QIAGEN Ltd., Crawley, UK), and obtained good quality DNA, suitable for the PCR ampliûcation from fresh okra leaves. However, the quality of DNA was found best when commercially available DNA isolation kit was used along with etiolated seedlings. On the similar note, Kochko and Hamon (1990) have also used dark-grown seedlings which yielded restrictable genomic DNA from okra. The better quality of DNA from the etiolated or dark grown seedlings is due to the presence of very low concentration of secondary metabolites. Moreover, the quality of DNA was equally good for cultivated okra (*A. esculentus* and *A. caillei*) and other wild relatives (*A. manihot, A. moschatus, A. tetraphyllus, A. tuberculatus, A. ficulneus, A. rugosus,* and *A. angulosus*), including related species *Hibiscus cannabinus*, which are used in this investigation.

Comparison of other available DNA isolation protocols in okra: A total of seven DNA isolation protocols which are already reported by different researchers (Kochko and Hamon, 1990; Khanuja, 1999; Singh and Kumar, 2012; Ahmed et al. 2013; Narendran et al. 2013; Meena et al. 2014; Adiger and Sridevi, 2014) in okra were used for the comparison with protocols optimized in this study (Table 1 and Table 2). Among various protocols studied, best quality and quantity of DNA was obtained when etiolated seedlings were used

Table 2: Quality and PCR amplification results of DNA isolated by using different protocols and plant tissues.

Markers/ Parameters	Fully grown leaves	Very young leaves		Etiolated seedlings		DNA Isolation Kit		
		With Liq. N_2	Without Liq. N_2	With Liq. N_2	Without Liq. N_2	Fully grown leaves	Very young leaves	Etiolated seedlings
Quantity (ng/µL)	20-30	250-280	150-200	350-450	200-250	100-130	150-200	230-270
Quality	+	+++	++	++++	+++	++	+++	++++
RAPD	?	?	?	?	?	?	?	?
ISSR	?	?	?	?	?	?	?	?
EST-SSR	×	?	?	?	?	×	?	?
SCoT	×	?	?	?	?	×	?	?

Where: + : Poor quality; ++ : Good quality; +++ : Very good quality; +++: Best quality DNA; ": PCR Amplified; ×: No PCR amplification.

using either CTAB method (liquid N_2) or DNA extraction kits (DNeasy-Qiagen). The isolated genomic DNA was found very pure and suitable for a range of PCR amplifications (Figure 2e and 2h). Similar results were also reported by Fougat et al. (2015) in which they have isolated the genomic DNA from 10-14 day old etiolated seedlings using the protocol of Kochko and Hamon (1990).

Amplification of isolated DNA using four marker systems: The DNA which was isolated using different protocols and also from different plant tissues were then used for the PCR amplification using four robust marker systems viz. RAPD, ISSR, EST-SSR and SCoT. Although, ISSR and RAPD primers showed amplification with the DNA samples which was isolated using different protocols (Figure 3a, 3b), but EST-SSR and SCoT primers did not amplified when fully grown leaves were used for DNA isolation (Table 2). This was supported by the findings of Sahu et al. (2012) who reported that even the DNA which gets dissolved in presence of various polysaccharides, still inhibit various genomic studies. The amplification of any kind of DNA using RAPD and ISSR maker can be explained by the low annealing temperature and abundance of primer binding sites across the genome for these markers. Further, EST-SSR and SCoT primers produced good results (Figure 3c, and 3d) when DNA of very young leaves and etiolated seedlings were used for PCR amplification, which was isolated using CTAB method (with liquid nitrogen). But, the best PCR amplification of various *Abelmoschus sp*. and related species was recorded when DNA was isolated from etiolated seedlings using commercially available DNA isolation kit (DNeasy-Qiagen).

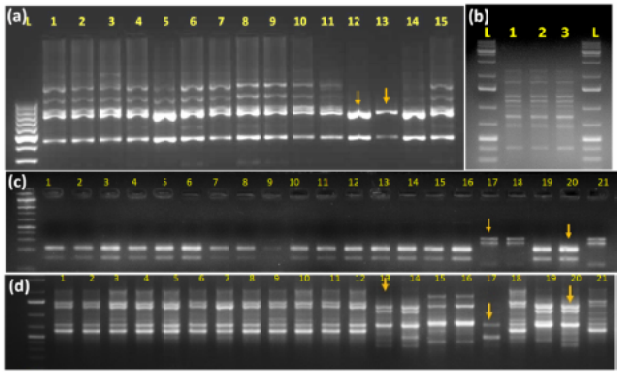

Figure 3. Representative picture showing PCR amplification of various *Abelmoschus* genotypes using (a). ISSR primer (UBC-818; CAC ACA CAC ACA CAC AG), (b) RAPD primer, (c) SSR primer (*Okra141532;* Fwd: GTG GAG AAG AGA AAA CAA CG; Rev: CCA CGT AGG AAC TCT CAT TC) and (d). SCOT primer (SCoT_35; CATG GCT ACC ACC GGC CC). Where, arrow indicates the presence of polymorphic bands.

Conclusion

The okra plants have high concentration of polysaccharides and other secondary metabolites which gets associated with the nucleic acid during DNA isolation which in turn hampers quality DNA yield. The DNA isolation protocol optimized in this study proved quite efficient and reliable for the isolation of high quality pure genomic DNA from various tissue samples of different *Abelmoschus* species. The method developed in this study, has solved the problems generally associated with quality DNA isolation in okra such as, DNA contamination, degradation, and poor-yield due to binding and/or co-precipitation of starch and polysaccharides. Further, the technique optimized in this study is quite fast, reproducible, and can be used for the quality DNA isolation from not only cultivated and wild *Abelmoschus sp*. but also from a related species *Hibiscus* species. Thus, isolation of best quality DNA is now not a problem in okra or its related species, provided it should be done using a combination of appropriate sample tissue and DNA isolation protocol as discussed in this study.

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भिण्डी आज भी जीनोमिक रूप से कम शोध फसल मानी जाती है विभिन्न प्रयोगशालाओं में समकालीन जीनोमिक अध्ययनों हेतु उच्च–गुणवत्ता वाले डीएनए पृथक्करण प्रोटोकॉल हेतु प्रयासरत हैं। इस अध्ययन में *एबलमास्कस* की विभिन्न प्रजातियों से उच्च–गुणवत्ता वाले डीएनए का विभिन्न उत्तकों से पृथक्करण की एक त्वरित और प्रतिलिपि प्रस्तुत करने योग्य प्रोटोकॉल का अनुकुलन किया गया है। इसके लिये दो खेती योग्य *(एबलमास्कल एस्कूलेन्टस* एवं *ए. कैली*) तथा भिण्डी की जंगली प्रजातियों (*ए. मैनीहाट, ए. मास्चैटस, ए. टेट्राफाइलस, ए. ट्यूबर कुलैटस, ए. फिकलनिस, ए. रूगोसस* एवं एवं *ए. एन्युलोसस*) के साथ सम्बन्धित प्रजाति *हिबिस्कस कैनाबिनस* को भी सम्मिलित किया गया तत्पश्चात् पीसीआर विसंयोज्य विगलित गुणवत्तायुक्त विभिन्न डीएनए मार्कर जैसे कि आईएसएसआर, आरएपीडी, एससीओटी और ईएसटी–एसएसआर द्वारा निकाले गए डीएनए की गुणवत्ता की भी पुष्टि की गयी। यह सम्भवतः विश्व की पहली रिपोर्ट है, जिसमें डीएनए पृथक्करण प्रोटोकाल को नौ एबलमास्कस प्रजातियों सहित एक संबंधित प्रजाति हिबिस्कस के लिए एक साथ अनुकूलित किया गया है। इस प्रकार, उच्च–गुणवत्ता एवं मात्रात्मक वाले डीएनए को भिण्डी की विभिन्न प्रजातियों द्वारा अब बहुत ही आसानी से निकाला जा सकता है, इसके लिए यह जरूरी है कि पौधे के उचित ऊतकों वो जो बहुत छोटी पत्ती वाले या पीली नर्सरी पौध से लिए गए हो तथा अवस्था का उपयोग इस अध्ययन के निर्देशानुसार किया गया।

Reference

Adiger S and Sridevi O (2014) Isolation of DNA from mucilage-rich okra (*Abelmoschus esculentus* L.) for PCR analysis. Trends Biosci 7(16): 2306-2309.

Ahmed N, Nawaz S, Iqbal A, Mubin M, Butt A, Lightfoot DA and Maekawa M (2013) Extraction of high-quality intact DNA from okra leaves despite their high content of mucilaginous acidic polysaccharides. Biosci Methods 4(4): 19-22.

Alam A, Naik PK and Mishra GP (2009) Congruence of RAPD and ISSR markers for evaluation of genomic relationship among 28 populations of *Podophyllum hexandrum* from Himachal Pradesh. Turk J Bot 33:1-12.

Aljanabi SM, Forget L and Dookun A (1999) An improved and rapid protocol for the isolation of polysaccharide and polyphenol free sugarcane DNA. Plant Mol Biol Rep 17:1-8.

Bayer C, Fay MF and DeBruijn AY (1999) Support for an expanded family concept of Malvaceae within a recircumscribed order Malvales: a combined analysis of plastid atpB and rbcL DNA sequences. Bot J Linnean Soc 129:267-303.

Bosamia TC, Mishra GP, Radhakrishnan T and Dobaria JR (2015) Novel and stress relevant EST derived SSR markers developed and validated in peanut. PLoS ONE 10(6): e0129127.

Doyle JJ and Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 1(9):11-15.

Fang G, Hammar S and Grumet R (1992) A quick and inexpensive method for removing polysaccharides from plant genomic DNA. Biofeedback 13:52-53.

FAOSTAT (2016) http://www.fao.org/faostat/en/#data/QC (Accessed on 26th March, 2018).

Fougat RS, Purohit AR, Kumar S, Parekh MJ and Kumar M (2015) SSR based genetic diversity in *Abelmoschus* species. Indian J Agr Sci 85(9):1223-8.

Jie W, Xiaobing C, Hualan Y and Guodao L (2008) Okra genomic DNA extraction and identification. Chin Agric Sci Bull 24:99-103.

Khanuja SP, Shasany AK 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 Mol Biol Rep 17:1-7.

Kochko AD and Hamon S (1990) A rapid and efficient method for the isolation of restrictable total DNA from plants of the genus *Abelmoschus*. Plant Mol Biol Rep 8:3-7.

Kumar J, Mishra GP, Naik PK, Murkute AA and Srivastava RB (2011) Genomic DNA isolation from *Artemisia* species grown in cold desert high altitude of India. Afr J Biotechnol 10(37):7303-7307.

Kumar M, Mishra GP, Singh R, Kumar J, Naik PK and Singh SB (2009) Correspondence of ISSR and RAPD markers for comparative analysis of genetic diversity among different apricot genotypes from cold arid deserts of trans-Himalayas. Physiol Mol Biol Plants 15(3):225-236.

Meena RK, Chhatterjee T and Thakur S (2014) An efficient method of genomic DNA isolation from mucilage-rich okra leaves for molecular biology studies. Indian J Appl Res 4(1):57-59.

Mishra GP, Singh B, Seth T, Singh AK, Halder J, Krishnan N, Tiwari SK and Singh PM (2017). Biotechnological advancements and begomovirus management in okra (*Abelmoschus esculentus* L.): Status and perspectives. Front Plant Sci 8(360).

Narendran M, Shirale D, Parimi S, Deole SG, Nanote A, Char BR, Harkude S, Bihani P and Zehr UB (2013) Efûcient genetic transformation of okra (*Abelmoschus esculentus* (L.) Moench) and generation of insect-resistant transgenic plants expressing the *cry1Ac* gene. Plant Cell Rep 32:1191–1198.

Sahu SK, Thangaraj M and Kathiresan K (2012) DNA extraction protocol for plants with high levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol. ISRN Mol Biol 1-6. doi:10.5402/2012/205049

Schafleitner R, Kumar S, Lin C, Hegde SG and Ebert A (2013) The okra (*Abelmoschus esculentus*) transcriptome as a source for gene sequence information and molecular markers for diversity analysis. Gene 517: 27-36.

Seth T, Chattopadhyay A, Dutta S, Hazra P and Singh B (2017) Genetic control of yellow vein mosaic virus disease in okra and its relationship with biochemical parameters. Euphytica 213:30.

Singh V and Kumar V (2012) An optimized method of DNA isolation from highly mucilage-rich okra (*Abelmoschus esculentus* L.) for PCR analysis. Adv Appl Sci Res 3(3):1809-1813.

Zhang J and Steward JM (2000) Economical and rapid method for extracting cotton genomic DNA. J Cotton Sci 4(3):193–201.