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An Improved and Modified Technique for Isolation of Genomic DNA from Okra (*Abelmoschus esculentus L.*)

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Abstract

The DNA isolation process started with the selection of twenty different genotypes of okra. However, due to the presence of a high concentration of acidic polysaccharides (mucilage) and polyphenols in okra, it becomes problematic during DNA extraction. For the collection of superior-quality genomic DNA, healthy and juvenile leaves from okra genotypes were collected and rinsed thoroughly with distilled water. Thereafter, a cost-effective, swift, and modest technique was standardized for DNA isolation during the study. Hence, the extracted DNA through the modified method reported a significantly higher yield (20-30 percent more than other methods) and better quality than the traditional methods and was sufficiently pure for approximately 275-300 PCR reactions to study subsequent genetic diversity.

Keywords: *Abelmoschus esculentus, CTAB, Genomic DNA, and modified technique*

Introduction

Okra (*Abelmoschus esculentus* L Moench) is a highly versatile crop often referred to as vendi, lady's finger, gumbo, and other names. It is a member of the Malvaceae family, reported to be cultivated globally in different tropical, subtropical, and even in the warmer parts of the

temperate regions throughout the globe (Kabir *et al*., 2016; FAOSTAT, 2021). India being the world leader in okra production, accounts for approximately 72 percent (6.4 million tonnes) of the total world's production just from a 0.53-million-hectare area with an average productivity of 12.17 metric tonnes per hectare (FAOSTAT, 2021). Being a highly multi-faceted crop, okra is also regarded as the powerhouse of valuable nutrients such as fiber, protein, Vitamin C, Vitamin A, iron, calcium, etc. (Wang *et al*., 2023). Further, besides, being an important nutritious vegetable crop, the mature and ripened seeds of the species can be roasted, and ground and are used as a substitute for coffee in several countries. Also, the seeds are taken as an alternative source of edible oil due to their higher oil (40%) content (Zhan *et al*., 2019).

However, due to increasing population pressure, lack of better resources, and an increasing number of problematic soils, such as saline soils, lack of irrigation water, alkalinity, etc. An urgent need is required for the advancement of more highly productive and superior cultivars for improving productivity besides maintaining nutritional security & livelihood generation (Chaudhari *et al*., 2023). Therefore, to counter and overcome the stresses, occurring due to abiotic factors breeding for elite varieties and development of superior cultivars is urgently needed.

Hence, marker-assisted selection can work as an important technique in assessing the genetic diversity and subsequent selection of elite genetic material for the development of hybrid vigor. Molecular-based markers provide a feature for genome screening at the DNA level thereby, reducing the environmental effects to a minimum. However, for the estimation of genetic diversity among the genotypes, isolation, purification, and quantification of high-quality DNA plays a vital role (Seth *et al.*, 2018). Several protocols have been developed for the extraction of high-grade genomic DNA from okra plant parts (Jeyaseelan *et al.*, 2019). However, mucilage being an important barrier deteriorates the quality as well as quantity of the extracted DNA making it unfit for further PCR reactions. Therefore, taking into consideration all the above-mentioned facts an attempt has been made through the paper for the extraction & quantification of the highest-quality genomic DNA from Okra to carry out further breeding and hybridization work (Kalkan *et al*., 2023).

Material & Methods

Plant collection

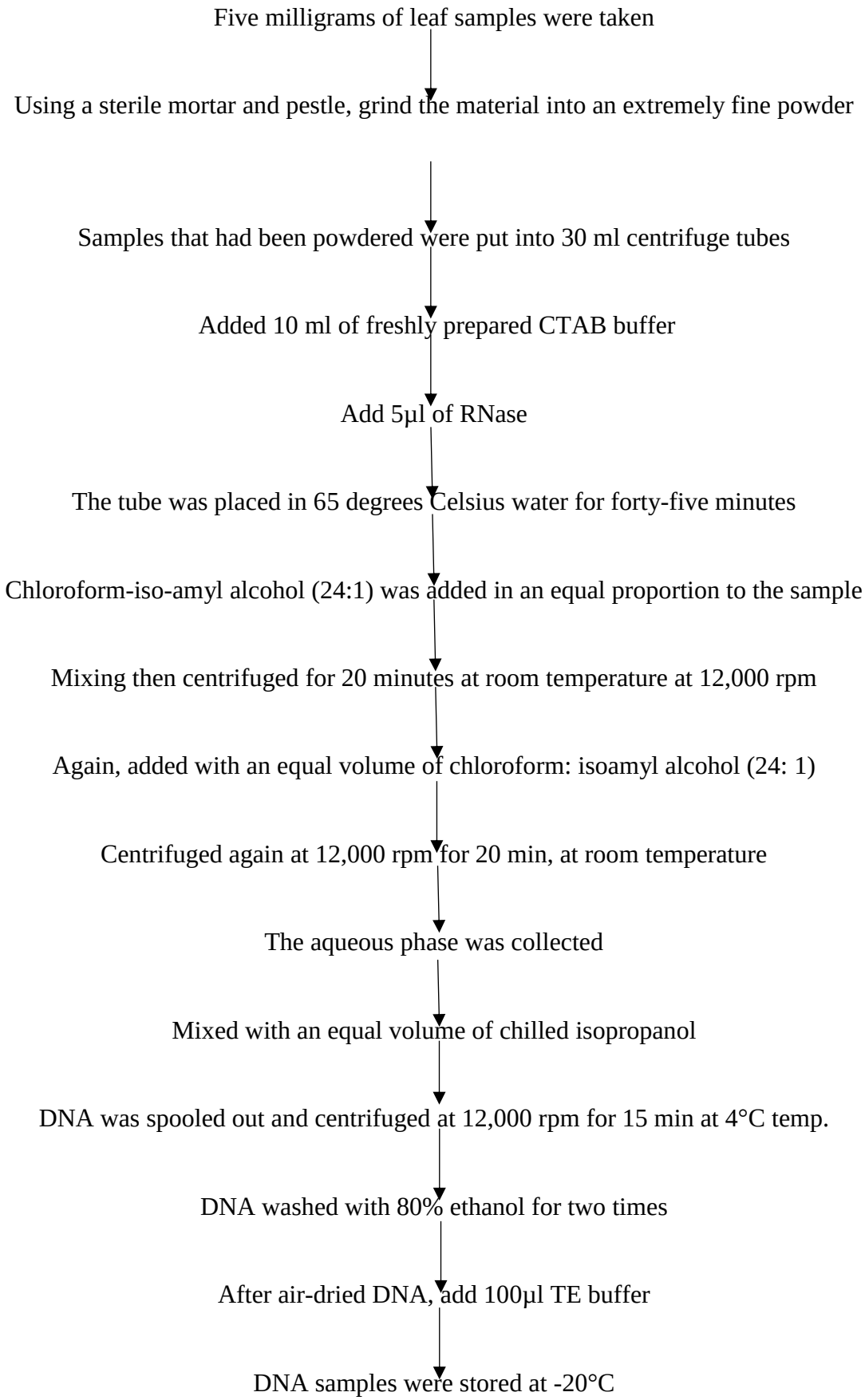
Twenty different genotypes of okra were collected from different ecological locations based on their desirable traits, high-yielding ability, and ability to survive under problematic

soil conditions (Table 1). Okra belonging to the Malvaceae family is easily available throughout the year, rendering no difficulty for continuous availability of the plant material.

DNA Extraction and Isolation

Fresh and juvenile leaves from individual genotypes were collected and kept in an icebox to maintain the leaf freshness and to prevent the degradation of the DNA molecules. The leaves were first rinsed with sterilized distilled water and ethanol and then were dried using sterilized blotting paper. Using a sterile mortar & pestle, five milligrams of leaf samples were ground into an extremely fine powder to isolate high-quality genomic DNA. After crushing, the fine powdered material was poured into 30 ml centrifuge tubes each of which contained 10 ml of pre-heated freshly prepared CTAB (cetrimonium bromide) buffer consisting of 3.5% CTAB, 200 mM tris, 1.4 mM NaCl pH 8.0, 22 mM EDTA, pH 8.0, 2% w/v PVP and 2% B-mercaptoethanol. Further, 5µl of RNAase was added to mixture in the anticipation of the contaminations that may occur due to RNA in the sample which would hamper the PCR reactions. thereafter, the tubes were kept in a water bath at 65 degrees Celsius for 45 minutes while being shaken and mixed periodically. Subsequently, the samples were then mixed with an equal amount of chloroform: iso-amyl alcohol (24:1), centrifuged at 12,000 rpm for 20 minutes at room temperature and allowed to settle into the separation of phases. After transferring the aqueous supernatant to fresh Eppendorf tubes, it was once again added and extracted using the same volume of chloroform: isoamyl alcohol (24:1). Thus, after centrifugation, the aqueous phase obtained was mixed with an equal amount of chilled isopropanol, and kept at a temperature of -20°C for the entire night. To create DNA pellets, the precipitated DNA was spooled out using a micropipette and centrifuged for 15 minutes at 12,000 rpm and 4°C. The precipitated pellets of DNA were twice washed with the help of 80 percent ethanol and were air dried for 2-4 hours. Thereafter, the recovered DNA pellets were dissolved in 100 µl TE buffer and were stored at -20°C before use (Fig.1).

Fig. 1. Review of Plant genomic DNA isolation



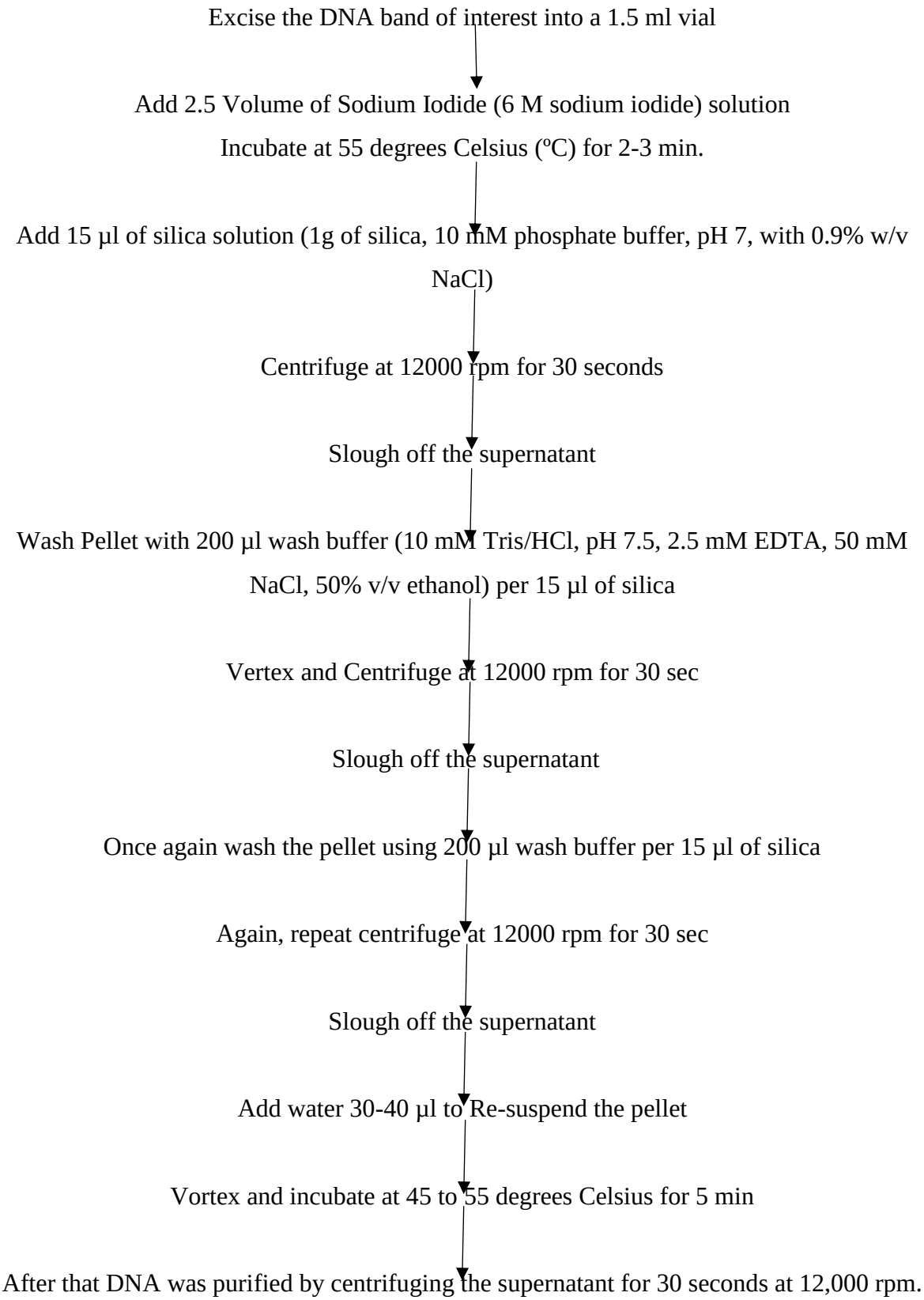
Purification of Isolated DNA

To obtain superior and high-quality genomic DNA from okra, the isolated DNA was transferred to 1.5 ml tubes and added to Sodium Iodide (6 M) (2.5 times the volume of isolated material) solution. The mixture was mixed well and was incubated for two to three minutes at 55 degrees Celsius in a water bath. Thereafter, the mixture was added with 15 µl of silica solution (1g of silica, 10 mM phosphate buffer, pH 7, with 0.9% w/v NaCl) and was centrifuged at 12000 rpm for 30 seconds to separate the silica-bound DNA and other impurities from the solution. After incubation the supernatant was removed and discarded with caution, being careful not to damage the DNA pellet. The obtained DNA pellet was further washed with the help of 200 µl of wash buffer containing Tris/HCl, EDTA, NaCl, and ethanol followed by centrifugation at 12000 rpm for 30 sec to form a clear pellet. The process was repeated 3-4 times until a clear DNA pellet was obtained. Thereafter, the pellet was added with 30-40 µl of water to re-suspend the purified DNA and the vial was placed over a vortex to ensure thorough mixing. After mixing the mixture was incubated at 45 to 55 ºC for 5 minutes to ensure the even dissolution of the DNA pellets which can be used for downstream applications such as PCR amplification (Fig.2).

DNA Quantification

With the aid of a 0.8 percent agarose gel, the extracted DNA was assessed using an agarose gel electrophoresis to ascertain the degree of DNA degradation and contamination that may be brought on by the presence of RNA. The gel was prepared by mixing 0.800 g of the agarose powder in 100 ml of solution and boiled for up to 8-10 minutes. Thereafter, the gel solution was allowed to cool down to 50ºC, and 5µl of ethidium bromide was added. The mixture was poured into a gel casting tray and the comb was carefully placed in the casting tray after the pouring of the gel mixture. After, the mixture was allowed to solidify the comb was removed carefully, and the gel was transferred to the gel electrophoresis tank assembly containing 1x TAE buffer. To evaluate the DNA purity 5µl of DNA sample was mixed with 2µl of gel loading dye (bromophenol blue) and the samples were loaded in the gel wells. The results after electrophoresis were evaluated over a gel documentation system (Bio-Rad, USA) to visualize the presence of superior-quality DNA bands. The data obtained using electrophoresis was further verified using a NanoDrop spectrophotometer (NanoDrop™ 2000/2000c, Thermo-Scientific, USA) at an absorbance ratio ofA260/280to determine the quantity of DNA obtained during the isolation process (Kidane *et al.*, 2020) (Table 2).

Fig.2 Review of Purification of Isolated DNA



Results & Discussion

The major hindrance in the isolation, purification, and quantification of good quality DNA from okra is the occurrence of higher concentration of mucilaginous polysaccharides consisting of poly galacturonic acid and its associates carrying a wide range of minerals (Ahmed *et al.,* 2022; Singh and Kumar, 2012). Furthermore, during the cell-lysis, the DNA gets bounded with the presence of these polysaccharides whereas, the polyphenols may get oxidized resulting in the formation of a brown color gelatinous substance and ultimately resulting in inferior quantity and quality of the isolated DNA (Dhankhar *et al.*, 2020). Therefore, during the study juvenile leaves collected from the okra seedlings from different sources were used for the isolation of genomic DNA with the help of a modified CTAB method without using liquid nitrogen. While isolating the DNA using the modified CTAB method, lysis buffer was added with 2 percent poly vinyl pyrrolidone (PVP), 4 percent β-Mercaptoethanol, and 45µl 6M potassium iodide for 100 mL of DNA extraction buffer. PVP was used to check the quantity of mucilage and polyphenols present in the okra leaves, by developing a complex with polyphenols with the help of hydrogen bonding whereas, potassium iodide helped to denature the secondary metabolites present in the samples. However, β-Mercaptoethanol was used as a strong reducing agent that, in addition to helping to deactivate RNase, efficiently cleaves and reduces disulfide bonds, speeding up the DNA isolation process.

Furthermore, for digestion in the extraction buffer, the samples were kept at 65 degrees Celsius for 45 min. resulting in better lysis and digestion of the samples as compared to 60°C for 30 min as a standard procedure (Doyle & Doyle, 1991). Similarly, Adiger and Sridevi (2014) also reported a slight modification of the CTAB method where the buffer was added with 100 mg PVP and 2.5M besides increasing the incubation time and temperature (65°C for 40 min) for the isolation of DNA from okra fresh leaves. Concomitantly, different modification in the CTAB protocol for DNA isolation from okra leaves was also reported by Zhang *et al*. (2023). Further, Singh and Kumar (2012) reported the use of a higher volume of DNA extraction buffer (1.5 mL/sample), lesser sample quantity (50-60mg) with higher salt concentration (5M), and PVP concentrations.

The results revealed that the DNA isolated using very young leaves from the shoot tip (1-2 cm) using modified CTAB without liquid nitrogen resulted in relatively lesser smearing and fragmentation of isolated DNA during gel electrophoresis (Fig. 3). This may be due to comparatively lower concentration of polysaccharides, polyphenols and other secondary metabolites in the juvenile leaves as compared to the mature leaves (Sharma *et al*., 2013). Also,

the DNA quantity obtained during the present study was considerably sufficient to carry out 275 – 350 PCR reactions (Table 2). Similarly, Xie *et al.* (2023), reported the complexity observed during the DNA extraction from the species belonging to the Malvaceae family. Further, Elhefnawi *et al.*, 2023, also observed the presence of viscous polysaccharides that make DNA handling very cumbersome during both pipetting and amplification. Furthermore, the DNA extraction protocols reported by Ahmed *et al.*, (2022) and Meena *et al.* (2015), also resulted in excellent quality DNA extracted from the dried, green, and extremely mucilaginous leaves of okra, respectively. However, during the present study superior quality DNA was isolated from the seedlings etiolated without the use of liquid nitrogen during the crushing of samples this may be due to the presence of a very low concentration of secondary metabolites. The data obtained during the present study can be confirmed using agarose gel electrophoresis that resulted in very distinct and intact high molecular weight DNA bands without any smearing or fragmentation (Fig. 3).

Conclusion: -

The study successfully established a cost-effective, swift, and modest technique for DNA isolation, overcoming the limitations associated with mucilage and polyphenols. The modified method produced a significantly higher quantity of DNA (20-30% more than other methods) and showed superior quality as compared to traditional methods. The extracted DNA was found to be sufficiently pure enough for approximately 275-300 PCR reactions, which made it easier to analyze the genetic diversity. The optimized technique presented in this study not only enhances efficiency but also provides a groundwork for further genetic diversity investigations in okra genotypes. Overall, this research work advances DNA isolation methodologies, especially in the context of overcoming challenges that come with okra mucilage and polyphenols in okra.

Declarations

We hereby declare that the research paper entitled "An improved and modified technique for isolation of genomic DNA from okra (*Abelmoschus esculentus* L.)" is the result of my PhD dissertation work and has not been submitted elsewhere for publication. All sources of information used in this research have been duly acknowledged, and proper citations have been provided where necessary. No part of this work has been plagiarized, and all data and results presented in the manuscript are authentic. All experiments were conducted following ethical guidelines and regulations. Any third-party tools, software, or datasets utilized in this research are appropriately credited in the acknowledgments section. Furthermore, all co-

authors have been actively involved in the research process and have contributed significantly to the development of the manuscript.

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Availability of Data and Materials

The datasets supporting the conclusions of this article are provided within the article. Additionally, all materials used in this study, including software tools, databases, and experimental reagents, are adequately described in the Methods section.

Ethics approval and consent to participate

This research does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

All authors have given their consent to publish the manuscript

Declaration of Competing Interest

The authors declare that they do not have any competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Reference

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

Ahmad, I., Rana, R. M., Hassan, M. U., Khan, M. A., & Sajjad, M. (2022). Association mapping for abiotic stress tolerance using heat-and drought-related syntenic markers in okra. *Molecular Biology Reports*, 49(12), 11409-11419.

Chaudhari, S., Talavia, B. & Meena, A. (2023). Effect of saline irrigation water on biochemical parameters of okra (*Abelmoschus esculentus* L.) varieties. *The Pharma Innovation Journal*; 12(3): 3629-3632.

Dhankhar, S.K. & A. V. V., Koundinya. (2020). Accelerated Breeding in Okra. In book: *Accelerated Plant Breeding*, Volume 2, Vegetable Crops. Pp.6-12.

Doyle & Doyle. J. (1991). DNA protocols for plants. In *Molecular techniques in taxonomy*, 283-293.

El-Sherbeny, G. A. R., Khaled, A. G. A., Obiadalla-Ali, H. A., & Ahmed, A. Y. M. (2018). ISSR markers linked to agronomic traits in okra. *Int. J. Mod. Agric*, 7(1), 9-15.

Esan & A. M. (2019). Influence of bioregulators and salinity stress on some biochemical, nutritional and antidiabetic properties of *Abelmoschus esculentus* Linn. (Moench.). Thesis. Pp. 1-118.

Jeyaseelan, T. C., Jeyaseelan, E. C., De Costa, D. M., & Shaw, M. W. (2019). Selecting and optimizing a reliable DNA extraction method for isolating viral DNA in okra (*Abelmoschus esculentus*). *Vingnanam Journal of Science*, 14(1), pp.7–14.

Kabir, M. R., Ahmed, S., & Akhond, M. A. Y. (2016). Organogenesis in okra (*Abelmoschus esculentus* L. *Moench*.): a plant recalcitrant to tissue culture. *Bangladesh Journal of Agricultural Research.* 41(3):521-528.

Kalkan, E., & Maskan, M. (2023). Mucilage in okra: extraction, modelling, optimization and application. *Journal of Food Measurement and Characterization*, 17(5), 4812-4822.

Kidane, L., Kalousova, M., & Demissie, H. (2020). Comparison and optimization for DNA extraction of okra (*Abelmoschus esculentus* L. *Moench*). *African Journal of Biotechnology*, 19(6), 353-361.

Kumar, S., Parekh, M. J., Fougat, R. S., Patel, S. K., Patel, C. B., Kumar, M., & Patel, B. R. (2017). Assessment of genetic diversity among okra genotypes using SSR markers. *Journal of plant biochemistry and biotechnology*, 26, 172-178.

Kyriakopoulou, O. G., Arens, P., Pelgrom, K. T., Karapanos, I., Bebeli, P. & Passam, H. C. (2014). Genetic and morphological diversity of okra (*Abelmoschus esculentus* [L.] *Moench*.) genotypes and their possible relationships, with particular reference to Greek landraces. *Scientia Horticulturae*, 171, 58-70.

Meena, R. K., Verma, A. K., Kumar, M., Chatterjee, T. & Thakur, S. (2015). Evaluation of okra (*Abelmoschus esculentus*) germplasm against yellow vein mosaic disease. *Indian Phytopathology*, 68(2), 226-228.

Seth, T., Mishra, G. P., Singh, B., Kashyap, S., Mishra, S. K., Tiwari, S. K., & Singh, P. M. (2018). Optimization of quality DNA isolation protocol from various mucilage rich cultivated and wild *Abelmoschus* sp. and its validation through PCR amplification. *Vegetable Science*, 45(01), 1-6.

Sharma, K., Bhattacharjee, R., Sartie, A., & Kumar, P. L. (2013). An improved method of DNA extraction from plants for pathogen detection and genotyping by polymerase chain reaction. *African Journal of Biotechnology*, 12(15).

Singh, B. D. & Singh, A. K. (2015). Marker-assisted plant breeding: principles and practices. *Springer*.Book Edition (1). *P*p. 1-514.

Wang, X., Liu, X., Shi, N., Zhang, Z., Chen, Y., Yan, M., & Li, Y. (2023). Response surface methodology optimization and HPLC-ESI-QTOF-MS/MS analysis on ultrasonic-assisted extraction of phenolic compounds from okra *(Abelmoschus esculentus*) and their antioxidant activity. *Food chemistry*. Pp. 134-142.

Xie, J., Del Tredici, P., LaPorte, M., Bekmetjev, A., Lemmon, A. R., Lemmon, E. M., Gong, W., Tang.Y., & Li, J. (2023). Phylogenetic relationships of Tilia (Malvaceae) inferred from multiple nuclear loci and plastid genomes. *International Journal of Plant Sciences*, 184(1), 56-67.

Zhan, Y., Wu, Q., Chen, Y., Tang, M., Sun, C., Sun, J., & Yu, C. (2019). Comparative proteomic analysis of okra (*Abelmoschus esculentus* L.) seedlings under salt stress. *BMC genomics*, 20, 1-12.

Zhang, X., Li, J., Shao, L., Qin, F., Yang, J., Gu, H., Zhai, P., & Pan, X. (2023). Effects of organic fertilizers on yield, soil physico-chemical property, soil microbial community diversity, and structure of *Brassica rapa* var. *Chinensis. Frontiers in Microbiology*. Pp.123-137.

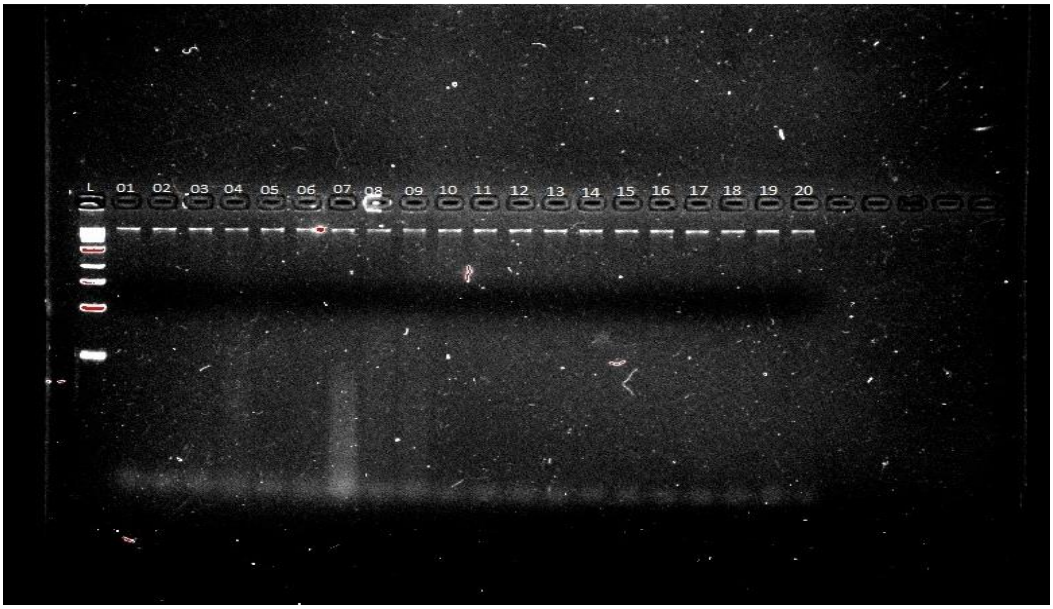
Table 1. List of total number of genotypes and germination percentage

S. No.	Genotypes	Germination Percentage	Geographical Location
1	AB01	65 per cent	28.6923° N, 77.0916° E
2	AB02	65 per cent	28.6850° N, 77.0982° E
3	AB03	65 per cent	23.1765° N, 75.7885° E
4	AB04	65 per cent	31.3260° N, 75.5762° E
5	AB05	65 per cent	28.6377° N, 77.1571° E
6	AB06	60 per cent	18.1124° N, 79.0193° E
7	AB07	65 per cent	28.7147° N, 77.1771° E
8.	AB08	65 per cent	18.4751° N, 73.9163° E
9.	AB09	65 per cent	19.0760° N, 72.8777° E
10.	AB10	65 per cent	18.5204° N, 73.8567° E
11.	AB11	65 per cent	19.8347° N, 75.8816° E
12.	AB12	65 per cent	28.7147° N, 77.1770° E
13.	AB13	65 per cent	17.4065° N, 78.4772° E
14.	AB14	65 per cent	12.9716° N, 77.5946° E
15.	AB15	65 per cent	31.3260° N, 75.5762° E
16.	AB16	70 per cent	30.9010° N, 75.8573° E
17.	AB17	65 per cent	29.0588ºN, 76.0856° E
18.	AB18	60 per cent	19.8762° N, 75.3433° E
19.	AB19	65 per cent	12.9716° N, 77.5946° E
20.	AB20	65 per cent	28.6975° N, 77.1604° E

Table 2. Quantification data of Isolated DNA for pcr amplification.

Genotypes	Ab 260	Ab 280	260/280	µg/ml
1	0.065	0.04	1.63	1300
2	0.067	0.039	1.72	1340
3	0.068	0.041	1.66	1360
4	0.069	0.043	1.6	1380
5	0.071	0.044	1.61	1420
6	0.074	0.046	1.61	1480
7	0.067	0.041	1.63	1340
8	0.071	0.043	1.65	1420
9	0.091	0.056	1.63	1820
10	0.077	0.047	1.64	1540
11	0.056	0.034	1.65	1120
12	0.062	0.036	1.72	1240
13	0.076	0.045	1.69	1520
14	0.069	0.043	1.6	1380
15	0.057	0.032	1.78	1140
16	0.069	0.042	1.64	1380
17	0.063	0.038	1.66	1260
18	0.072	0.043	1.67	1440
19	0.068	0.038	1.79	1360
20	0.063	0.038	1.66	1260
Mean	0.07	0.04	1.66	1375
Range	0.056-0.091	0.032-0.056	1.60-1.79	1120-1820

Fig. 3. Electrophoresis gel image for different okra germplasm



Germplasm Code: -1-AB01, 2-AB02, 3-AB03, 4-AB04, 5-AB05, 6-AB06, 7-AB07, 8-AB08, 9-AB09, 10-AB10, 11-AB11, 12-AB12, 13-AB13, 14-AB14, 15-AB15, 16-AB16, 17-AB17, 18-AB18, 19-AB19, 20-AB20**.**