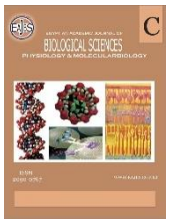
.

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 16(2) pp63-74 (2024)*
DOI: 10.21608/EAJBSC.2024.371518

Egypt. Acad. J. Biolog. Sci., 16(2):63-74 (2024)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Evaluation and Modification of Some Traditional Methods to Extract Genomic DNA in High Quality and Quantity from Different Plant Families

Mona M. Moghazee[1], Shaimaa A. Abdelaziz[1], Rehab A. Hasanien[1], Rawan S. Zaid[1], Rawan Y. Fawzy[1], Khaled T. Mohamed[2] ,Ahmad A. Suliman[3], Ghada M. Samaha[4], Hala M. Zoghly[1]

[1]Genetic Department, Faculty of Agriculture, Ain Shams University, P.O. Box 68, Hadayek Shoubra 11241, Qalyubia , Egypt.
[2]Plant Department, Genetics Division, Faculty of Agriculture, Al-Azhar University, Egypt.
[3]Horticultural Crop Technology Department, Agricultural and Biological Institute, National Research Centre, Dokki, Egypt.
[4]Field Crops Research Department, Agricultural and Biological Institute, National Research Centre, Dokki, Egypt.

***E-mail:** m_moghazee@agr.asu.edu.eg

ARTICLE INFO
Article History
Received:30/6/2024
Accepted:3/8/2024
Available:7/8/2024

Keywords:
DNA extraction protocols, Solid-phase technology, Molecular markers**,** PCR.

ABSTRACT

Background: Plants are rich in complex compounds, which hinder obtaining intact and pure DNA, especially by traditional manual methods. Extraction of high-quality and quantity genomic DNA is the most important step for all DNA-based biological applications, such as polymerase chain reaction (PCR), cloning, and sequencing. In this study, four DNA extraction modified protocols (CTAB, TNES) and (CTAB, TENS with a solid-phase column) were evaluated to produce highly purifiedand high-quantity DNA from the leaves and seeds of 11 species. They were compared with a kit (QIAGEN) which was used as a positive control. The techniques were evaluated for their capacity to produce DNA devoid of sugars, polyphenols, RNA, and other significant contaminants, as well as their apparent, viscosity, OD260 reading, and appropriateness for PCR-based assays. **Results:** The most obvious result was that using traditional methods such as CTAB and TENS with a solid-phase column increased the efficiency of the method and gave better results in spectrophotometer readings and polymerase chain reaction. **Conclusion:** To get results comparable to those from Kit procedures, it is possible to enhance the conventional DNA extraction techniques. To achieve high-quality DNA at the lowest possible cost, this is accomplished by combining several extraction buffers and utilizing a solid phase column.

INTRODUCTION

Nucleic acids are the source of life for all organisms and living systems, and all the information secrets lie in them. Therefore, it must be extracted to reveal these secrets. Because DNA extraction is the key step for many successive analytical techniques, this step must be given more attention (Sirakov, 2016). The first trial for DNA extraction was in 1869 when Swiss physician Friedrich Miescher searched for proteins in leukocytes in puss cells, he accidentally isolated a new substance from the nucleus and named it “nuclein” (Dairawan and Shetty, 2020).

Since then, DNA extraction and analysis have become important in many scientific fields. Subsequently, many studies have been conducted on DNA extraction protocols, which should include three main steps: disruption of cytoplasmic and nuclear membranes, separation, and purification of DNA from other cellular components, and subsequent concentrations and purifications of DNA for use in research applications and diagnosis (Sheershika and Ram, 2024). Doyle and Doyle (1987) and Dellaporta *et al.* (1983) detected the most used and developed methods of extracting DNA from plants. The different the plant species, the more changes these methods require as all plant species are not the same in terms of their complex components. Not only inter-species but also intra-species content of secondary metabolites should be considered. Interestingly, the type of tissue and the age at which the tissue is collected affect the extraction procedure thus, mature plant tissue is not recommended for DNA extraction due to its higher content of secondary metabolites such as sugars and polyphenols (Granier, 1988; Wu *et al.,* 2014). Researchers often modify the extraction buffer pH and composition or include nuclei isolation as a beginning in the most used methods to extract DNA from different plant speciesthese adjustments are required for standard protocols (Porebski *et al.,* 1997; Abdel-Latif and Osman, 2017; Aboul-Maaty and Oraby, 2019; Xia *et al.,* 2019). The integrity and purity of DNA are the biggest challenges for many protocols, especially those applied to plants, because of the complex compounds it contains, such as phenols, dyes, and polysaccharides, which hinder the proper extraction of nucleic acids. In other words, the absence of good-quality DNA is a major limiting factor to the success of all DNA-based techniques (Echevarría-Zomeño *et al.,* 2012; Zhang *et al.,* 2013; Aboul-Maaty and Oraby 2019; Aggarwal *et al.,* 2022). High-quality DNA is characterized by high molecular-weight fragments with an A260/280 ratio between 1.8 and 2.0 and the lack of contaminating substances, such as polysaccharides and phenols (Abdel-Latif and Osman, 2017). DNA extraction is essential for many applications in molecular biology, including polymerase chain reaction (PCR), molecular marker investigation techniques, Southern blotting, and DNA sequencing (Manen *et al.,* 2005). Finally, a good genomic DNA extraction procedure should be fast, inexpensive, and simple (not laborious and hazardous). To produce a good quantity of intact DNA of reasonable purity using insignificant amounts of tissue.

Therefore, to achieve the best results in terms of DNA quantity and quality, the current study attempted to adapt conventional procedures by utilizing columns and adding a modified extraction buffer.

MATERIALS AND METHODS

Plant Materials:

Eleven plant species representing six plant families listed in Table 1, were obtained from the Dabaa farm, Marsa Matrouh, Egypt. All collected plant leaves were frozen until working, except for the corn, which was obtained as ground seeds. These plant specimens are rich in polyphenols, proteins, and polysaccharides, which explains their choice for the current study.

Table 1: List of all collected plants for the current investigation.

No.	Samples	Genus-species	Family
1.	Broccoli	*Brassica oleracea*	*Brassicaceae*
2.	Eggplant	*Solanum melongena*	*Solanaceae*
3.	Tomato	*Solanum lycopersicum*	
4.	Paprika	*Capsicum annuum*	
5.	coloured Pepper	*Capsicum annuum*	
6.	Hot Pepper	*Capsicum annuum*	
7.	Bean	*Vicia faba*	*Fabaceae*
8.	Green Beans	*Phaseolus vulgaris*	
9.	Strawberry	*Fragaria ananassa*	*Rosaceae*
10.	Cucumber	*Cucumis sativus*	*Cucurbitaceae*
11.	Maize	*Zea mays*	*Poaceae*

Modified DNA Extraction Protocols:

-Protocol no.1 Doyle and Doyle (CTAB) modified method.

-Protocol no.2 Dellaporta (home-made extraction method or "TENS") modified method.

-Protocol no.3 (CTAB modified + Solid phase technology) or (CTAB+ Co.).

-Protocol no.4 (TENS modified + Solid phase technology) or (TENS+ Co.)

-Protocol no.5 (QIAGEN kit)

Every stage in the procedures used is taken into consideration, and Table 2, lists the most significant changes we made to the conventional laboratory procedures in contrast to the Kit technique.

Table 2: A table illustrating the primary differences between the methodologies utilized and the changes made to them.

No.	Methods	Base methods	Modification	Solid phase	References
1	CTAB modified	Doly and Doly (CTBA)	Buffer with (0.4 M glucose, 20 mM EDTA pH 8.0 and 3% (w/v) PVP-40 (MW:40,000)), altered incubation/ centrifugation	No	Doyle and Doyle 1987, Arbi et al., 2009; Spadoni et al., 2019; Qarni et al., 2022
2	TENS modified	Dellaporta (TENS)	Same modifications as protocol 1, selectively precipitations DNA while maintaining polysaccharide solubility	No	Dellaporta et al. (1983), Csaikl et al., 1998; Bergallo et al., 2006.
3	CTAB+ Co.	CTAB modified	Same modifications as protocol 1, and column introduced before DNA precipitation, combined with isopropanol and potassium acetate, washing and resuspension with spin column.	Yes	Genetix Total Nucleic Acid Extraction Kit. (Cat. #NP-BD-050)
4	TENS+ Co.	TENS modified	Same procedure as protocol 2, except DNA mixture from precipitation step with isopropanol and potassium acetate applied to column and centrifuged.	Yes	
5	QIAGEN kit	N/A	N/A	N/A	DNeasy Plant Mini Kit, Qiagen, Hilden, Germany) (Cat. No./ID: 69204)

Quantification and Qualification of Extracted DNA:

For DNA qualification, the genomic DNA was electrophoresed on 0.8% agarose gel . Electrophoresis was performed using 1×TBE buffer (0.089M Tris-HCl pH 7.5, 0.089M boric acid, and 0.002M EDTA) at a constant voltage of 100 V for 15 minutes. However, DNA quantification was investigated by a spectrophotometer (SmartSpec. Plus, UV/Vis Spectrophotometer 200–800 NM DNA RNA QUANTITATION (BIO-RAD)). The DNA yield was measured by OD 260 reading. DNA quantity per tissue

was calculated by multiplying the result of the following equation: (A_{260}* 50* DF/1000) (DF= dilution factor), by the buffer volume in which DNA was extracted from each tissue.

Appropriateness of extracted DNA for PCR:

Molecular markers such as ribulose-1,5-bisphosphate carboxylase (*rbcL*) and inter simple sequence repeats (ISSRs) were performed. The sequence of used primers, their references and PCR conditions are listed in Table 3. All PCR reactions were performed in a total volume of 25 µl in 96-well plates using a Veriti™ 96-Well Fast thermal cycler. The PCR mixture contained 12.5 µl of COSMO PCR RED Master Mix DNA Polymerase (WF10203001 Co., Ltd.), 2.0 µl of genomic DNA (20 ng/µL), 2.0 µl of each primer (10 nm), and 6.5 µl of sterilized water. PCR products were visualized on 1.5% agarose gel with a 1Kbp DNA ladder (TIANGEN, Cot. No.MD113).

Table 3: Sequence of the used primers for *rbcL* and ISSRs markers.

Marker		Sequence 5´→ 3´	PCR conditions	Ref.
rbcL	F	CGGTAGCTGCCGAATCTTCT	94°C for 5 minutes, 94°C for 15 seconds, 55°C for 30 seconds, 72°C for 30 72°C for 5 minutes	Abdelaziz et al. 2024.
	R	ACCTGTTTCAGCCTGTGCTT		
ISSR-7		GAGAGAGAGAGAGAGAT	94°C for 3 minutes, 94°C for 1 min, 48°C for 1 min, and 72°C for 2 min.,72°C for 5 minutes.	Shaban et al. 2022

RESULTS

Grinding of Plant Tissues:

This step was the most time-consuming of each method for extracting DNA from plants, therefore more effort and additional work were needed. Table 4 & Figure 1 shows the time spent by each of the applied methods. All plants took time and relative effort to grind, except for the corn that was collected as ground from the beginning.

Table 4: Characterization and qualification of used methods for DNA extraction

Protocol	CTAB	TENS	CTAB+ Co.	TENS+ Co.	Kit
Lysis pH	pH 8.0	pH7.5	pH 7.5	pH 7.5	
Lysis "incubation"	30 min	20 min	25 min	15 min	15 min
DNA purification	Phenol/ Chloroform / isoamyl	Chloroform/ isoamyl	Phenol/ Chloroform / isoamyl+ Column	Chloroform/ isoamyl + Column	Column
Prescription "incubation"	Overnight	1 hours	1 hours	1 hours	30 min
Elution	30 µl	30 µl	30 µl	30 µl	40 µl
Time-consuming*	> 1 day	3 hours	2.5 hour	2 hours	1.45 hour
Spectrophotometer**	43	27	32	29	44
PCR amplification of *rbcL****	8	7	11	11	9
PCR amplification of ISSR***	4	6	10	9	10
Number of PCR points	12	13	21	20	19
Tot. number of points	55	40	53	49	63

* =Time required to prepare DNA from 11 pulverized samples.

** =DNA score is calculated by averaging the DNA concentrations (µg/µl) generated from the A_{260} reads (not shown data).

*** = one point is given for each clear positive PCR result for each amplified sample (total is 11).

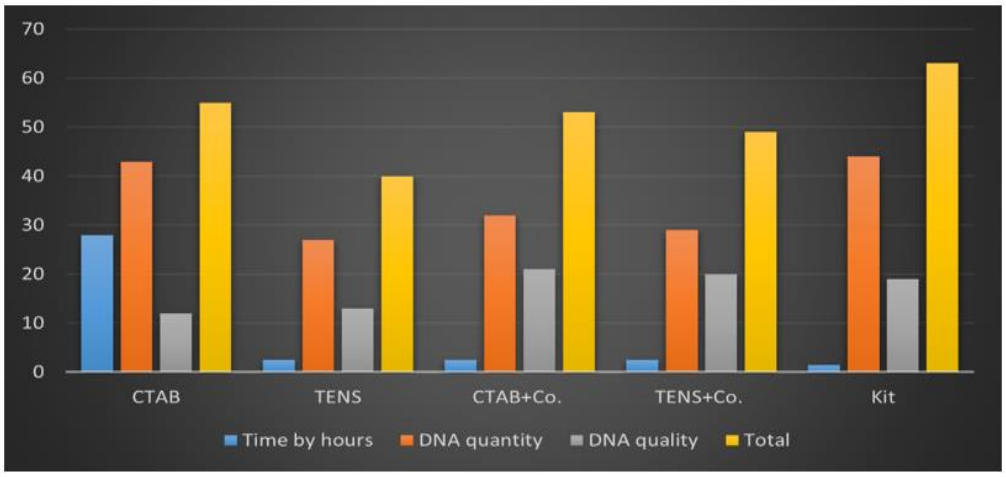


Fig.1: An illustration of the effectiveness of applied protocols for DNA extraction.

The Quantity and Quality of Extracted DNA:

Both electrophoretic and spectrophotometric techniques were used twice to evaluate the overall quality and quantity of extracted DNA, respectively. Intact and abundant DNA bands were obtained for most samples for all the applied methods (Fig. 2). In the CTAB and TENS approaches, genomic DNA bands were absent in certain plants shwon in (Fig. 2; a & b),, while both the (CTAB +Co.) and (TENS +Co.) methods showed satisfactory results with all samples, except for the corn sample, which did not appear clearly on the gel (Fig. 2; c & d), respectively). Samples extracted by the Kit method showed well-defined bands except for the cucumber plant sample (Fig. 2; e). All DNA quality and quantity characteristics resulting from the used methods were summarized in Table 5. It is noted that the results of time (average of two replicates) were identical for many samples. DNA quality was evaluated by measuring the OD 260 readings results were extremely varied, as shown in Table 5.

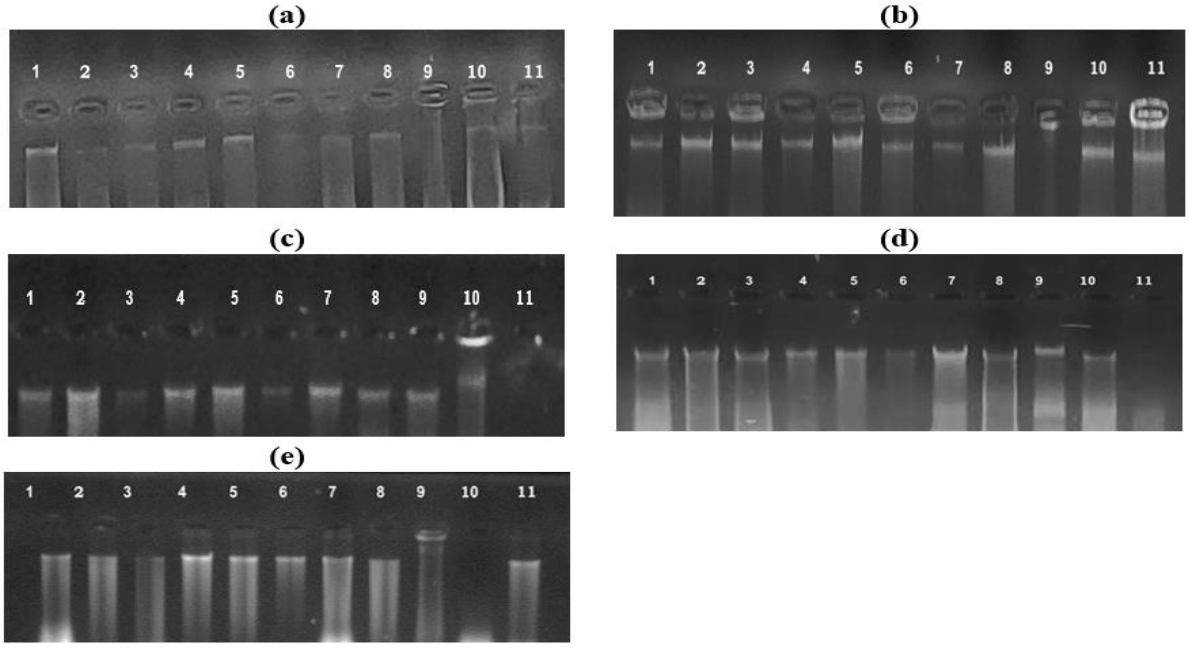


Fig.2: Evaluation of extracted DNA quality by gel electrophoresis (represented by gels figures) for used methods; (a) CTAB; (b) TENS; (c) CTAB+ Co.; (d) TENS+ Co. and (e) Kit. For samples; 1) Broccoli, 2) Eggplant, 3) Tomato, 4) Paprika, 5) Colored Pepper, 6) Hot Pepper, 7) Bean, 8) Beans, 9) Strawberry, 10) Cucumber and 11) Maize.

Table 5: Evaluation of extracted DNA quantity by spectrophotometry (represented by values of OD 260) for used methods (M); (a) CTAB; (b) TENS; (c) CTAB+ Co.; (d) TENS+ Co. and (e) Kit. For samples (S); 1) Broccoli, 2) Eggplant, 3) Tomato, 4) Paprika, 5) Colored Pepper, 6) Hot Pepper, 7) Bean, 8) Beans, 9) Strawberry, 10) Cucumber and 11) Maize.

M / S	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
a.	47	5	2	11	3	235	8	3	4	3	35
b.	1	3	1	1	2	2	3	2	1	3	3
c.	1	2	2	3	1	8	268	1	2	22	11
d.	1	1	2	3	3	1	1	2	1	3	2
e.	13	7	5	5	7	6	1	1	7	4	6

PCR Outputs:

It should be noted that the experiment was conducted twice with identical outcomes. PCR results were obtained at two levels: *rbcL* and ISSR. As for the *rbcL* reaction, the size of the amplicon obtained with this primer ranged from 540 to 580 bp (average 560) and PCR products were clarified in (Fig. 3). The number of absent bands in modified CTAB (Fig. 3a) and TENS (Fig. 3b) methods was bigger than CTAB (Fig. 3c) and TENS (Fig. 3d) with the column and kit (Fig. 3e). When CTAB compared to TENS, whether with column or without, TENS outperformed but incorporation of columns gave the best results in general.

As for the ISSRs reaction, PCR products were clarified in (Fig. 4). Generally, not all samples succeeded with this marker, and the average number of successful sample bands was 3 to 6, with an average amplicon size of 300-2200 bp. Comparing the results of our modified techniques, the technique containing column showed better results and was close to the results of DNA extracted with Kit, which is the standard for this ISSR marker.

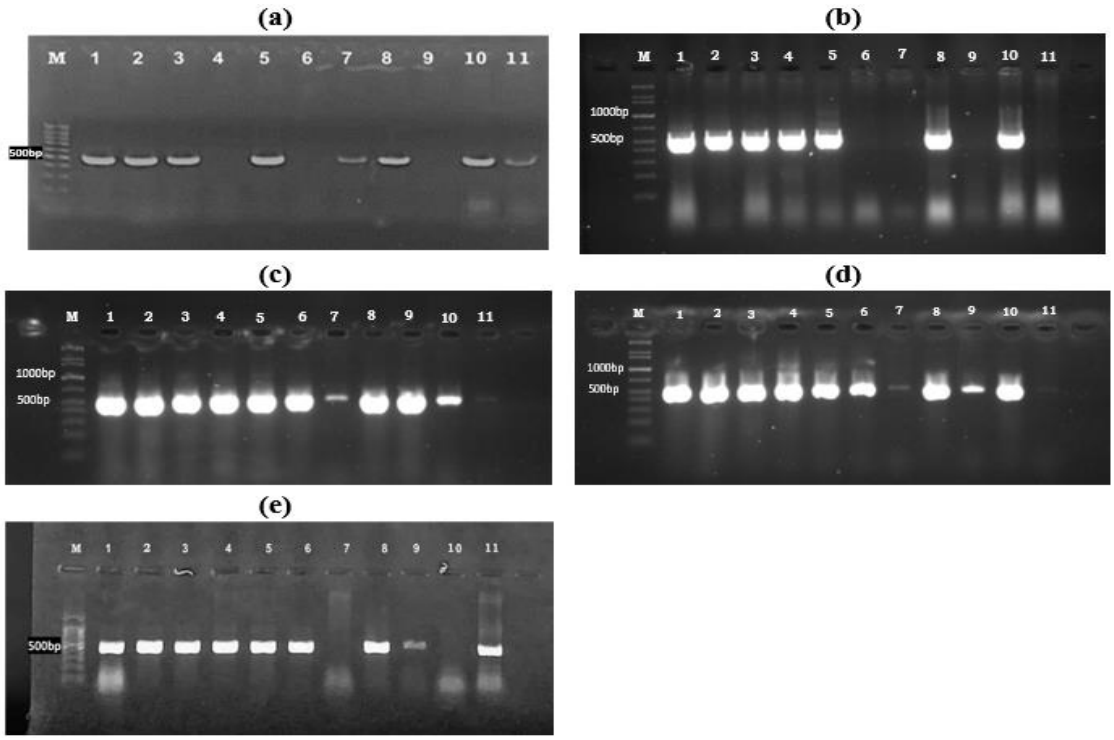


Fig.3: Agarose gel electrophoresis of *rbcL* PCR products for the DNA extracted by; (a) CTAB; (b) TENS; (c) CTAB+ Co. and (d) TENS+ Co.; and (e) Kit.

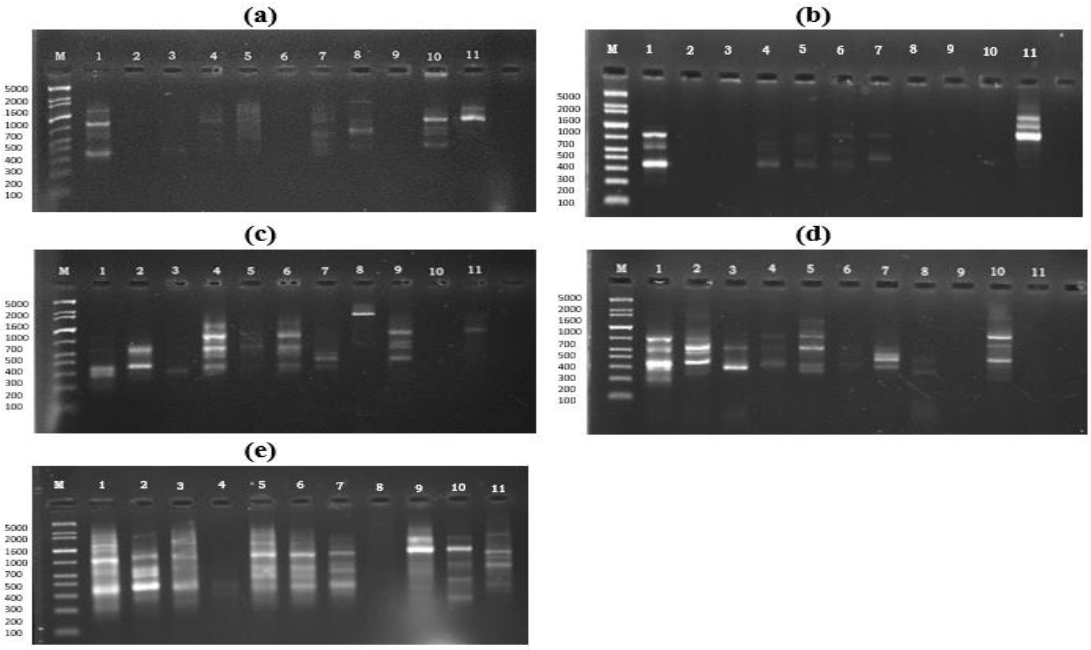


Fig. 4: Agarose gel electrophoresis of ISSR PCR products for the DNA extracted by; (a) CTAB; (b) TENS; (c) CTAB+ Co. and (d) TENS+ Co.; and (e) Kit.

DISCUSSION

Traditional techniques for extracting DNA from plants involve labour-intensive and time-consuming procedures, including solutions preparation, the time-consuming grinding process depending on the tissue type (leaf, seed, or root), purification, and sedimentation according to Júnior *et al.* (2016). In addition to secondary metabolite residues, subsequently cause an obstacle in molecular analyses. This confirms that the idea of the current study is urgent to overcome the previous problems.

The mortar and pestle were used in the plant sample grinding step, and it was easy to manipulate all types of plant tissues to produce DNA with more reliable quality. To further facilitate the grinding process, liquid nitrogen can be used. However, with corn samples, a hand blender was used to obtain the best results, according to Abdel Latif and Othman (2017) and Aboul-Maaty and Oraby (2019). Regarding time consumption, the TENS method was the best, whether with or without a column. Although it is known that the tissue and the preservation method types play a fundamental role, therefore, appropriate tissue type and sample collection methods are recommended.

Regarding the time, The grinding process - took an average of 30 to 45 seconds per sample, with an average of 7 to 8.5 minutes for all samples in the CTAB and CTAB column methods, respectively, while the average grinding process per sample was 25 to 35 seconds at a rate of 5.5 to 6 minutes for all samples using the TENS and TNES method with the column, respectively. The Kit method took an average of 25 to 30 seconds per sample, and an average of 2.5 and 3 minutes for all samples. As for completing the rest of the steps, the real differences appeared; a full day to complete the extraction stages with the CTAB method, (because the best result is when the samples are incubated at -20 for as long as possible) while CTAB with the column, TENS and TNES with the column both took 2.5 hours as average time of extraction. While the Kit method was the least time-consuming to obtain the result, with an average of 1.4 hours**.**

Regarding DNA quality, the current study resulted in an effect of the duration of sample preservation on the extracted DNA, in addition to other known factors such as age, tissue type, and extraction method. It turned out that a long preservation period negatively affects the DNA extraction process.

Therefore, the extraction process must be rapid immediately after sample collection.

As for contamination of secondary metabolite residues, our modifications proved effective results. To improve the efficiency of traditional procedures such as Doyle and Doyle and Dellaporta**,** we have made certain adjustments by adding a solution containing specific compounds (glucose, EDTA, and PVP) that help to raise the purity of DNA. Glucose prevents contamination and browning by polyphenols, which improves DNA quality (Uddin *et al.,* 2014). High-concentrated PVP binds to phenolic compounds, through hydrogen bonds, allowing them to be separated from DNA and reducing the levels of polyphenols in the product (Porebski *et al.,* 1997). During the DNA extraction process, the DNA should be protected from endogenous nucleases, and this is achieved using ethylenediaminetetraacetic acid (EDTA) which is included in DNA extraction buffer to chelate magnesium ions, a necessary co-factor for most nucleases (Vinod, 2004).

Polysaccharides inhibit PCR amplifications and can lead to erroneous interpretations (Kotchoni *et al.,* 2003). The co-precipitation of polysaccharides was avoided by adding higher concentrations of selective precipitants of nucleic acid, CTAB (0.04 g/mL), and NaCl (3 M) (Dellaporta *et al.,* 1983); the latter was used at 1.4 M in our modified method. Long-tail surfactants such as CTAB produce a conformational change in the DNA from a "random coil" to a "compact globule" making DNA precipitation more effective (Azmat *et al.,* 2012). Phenolic compounds are powerful oxidizing agents and bind covalently to the extracted DNA, making them useless for most molecular manipulations (Porebski *et al.,* 1997; Padmalatha and Prasad 2006). A high concentration (0.02 g/ml) of PVP mixed in the extraction buffer (Fang *et al.,* 1992; Moller *et al.,* 1992; Lodhi *et al.,* 1995) binds to phenolic compounds and helps to remove them.

As for DNA quantity, the spectrophotometer readings were very variable, which related to the variation of the samples in terms of integrity and purity (Shokere *et al.,* 2009). These results confirm that choosing the appropriate protocol is an important factor in determining the level of results desired to be achieved. However, the fluctuation of readings may also be due to the lack of spectrophotometer efficiency, as it lacks accuracy in the case of low-concentrated or contaminated samples (Masago *et al.,* 2021). Therefore, DNA quantification is prescribed to be measured by other accurate methods such as Nanodrop DNA quantification (Simbolo *et al.,* 2013) in case accessible.

As for the appropriateness of PCR, each method's effectiveness is assessed by assigning a score**.** The results were as expected, as the modifications were useful in obtaining DNA suitable for PCR applications, but to varying degrees. The column inclusion was remarkably effective to the point of approaching the Kit results. The results of *rbcL* agreed with Urumarudappa *et al.* (2022), and the results of ISSR agreed with Pradeep Reddy *et al.* (2002), Wang *et al.* (2012), and Abdulhamed *et al.* (2021). However, one of the strong observations is that some samples, even those extracted with the kit, dropped, which explains the possibility of problems controlling the conditions of the PCR itself and not the sample.

CONCLUSION

Finally, we can conclude from the current study that if we want to obtain intact, high-quality DNA at the cheapest cost and time, and suitable for many plant species, we must identify the appropriate tissue and extract DNA from it as quickly as possible without resorting to a preservation period. The current study confirmed the high quality of the conventional techniques by simply inserting the precipitation columns. Therefore, we must buy precipitation columns only instead of buying the kit totally and including these columns among the extraction steps. If the purification columns are also introduced we expect better results. We also recommend having a standard sample as a positive control when applying PCR because the poor results are not necessarily due to the

sample conditions alone.

Abbreviation:

CTAB, Cetyl Trimethyl Ammonium Bromide or Hexadecyl Trimethyl Ammonium bromide; EtOH, Ethanol; TBE (Tris-Borate-EDTA); TENS (Tris, EDTA, NaCl & SDS), *rbcL* (ribulose bisphosphate carboxylase) DNA barcode marker**.**

Declarations:

Ethical Approval: Not applicable

Conflict of interest: The authors declare no conflict of interest.

Authors Contributions: Mona M. Moghazee and Ghada M. Samaha, with Ahmad A. Suliman contributing conceptualization, Rehab A. Hasanien, Rawan S. Zaid, Rawan Y. Fawzy, Khaled T. Mohamed did manual scientific experiments while Shaimaa A. Abdelaziz did Kit scientific experiments and follow-up polymerase chain reaction. Mona M. Moghazee collected, prepared, analyzed, and drafted data. Mona M. Moghazee and Hala M. Zoghly contributed writing and approval of the contents. Ghada M. Samaha and Hala M. Zoghly did the paraphrasing and measuring the plagiarism ratio. Ahmad A. Suliman supplied the research with plant materials. Ghada M. Samaha and Hala M. Zoghly generated the numbers and did the final revision. Mona M. Moghazee, Hala M. Zoghly and Ghada M. Samaha supervised all stages of manuscript preparation. Mona M. Moghazee, Ghada M. Samaha and Hala M. Zoghly have read, reviewed and approved the content of the final version of this review.

Funding: No funding was received.

Availability of Data and Materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: My thanks and gratitude to Professor Dr. Fatma Mohamad Ibrahim Badawy (may she rest in peace), Professor of Microbial Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University, who taught me the fundamentals of scientific life. The authors would like to express their thanks to the Cyto-Molecular Genetics Laboratory in the Department of Genetics, Faculty of Agriculture, Ain Shams University, for sponsoring the work of the experiment. Thank you to Professor Dr. Eman M. Fahmy, Professor of Genetics, for her help in the linguistic review of the research.

REFERENCES

Abdelaziz, S. A., Khaled, K. A., Younis, R. A., Al-Kordy, M. A., El-Domyati, F. M. and Moghazee, M. M. (2024). Comparison of four DNA barcoding loci to distinguish between some *Apiaceae* family species. *Beni-Suef University Journal of Basic and Applied Sciences*, 13(1), 12. doi.org/ 10.1186/s43088-023-00457-7

Abdel-Latif, A. and Osman, G. (2017). Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. *Plant Methods*, 13, 1-9. https://doi.org/10. 1186/s13007-016-0152-4

Abdulhamed, Z. A., Abdulkareem, B. M. and Noaman, A. H. (2021). Efficiency of ISSR markers detect genetic and molecular variation between barley genotypes. *International Journal of Agricultural and Statistical Sciences*, 17, p1503.

Aboul-Maaty, N. A. F. and Oraby, H. A. S. (2019). Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. *Bulletin of the National Research Centre*, *43*(1), 1-10. https://doi.org/10.1186/s42269-019-0066-1

Aggarwal, G., Edhigalla, P. and Walia, P. (2022). A comprehensive review of high-quality plant DNA isolation. *The Pharma Innovation Journal*. SP-11 (6), 171, 176. https://www.thepharmajournal.com/

Arbi, G., Naceur, B., Chokri, M., Mohamed, B. and Mohamed, N. (2009). A simple, rapid, and efficient method for the extraction of genomic DNA from *Allium roseum* L. (Alliaceae). *African Journal of Biotechnology*, 8(17). www.academicjournals. org/ AJB

Azmat, M. A., Khan, I. A., Cheema, H. M. N., Rajwana, I. A., Khan, A. S. and Khan, A. A. (2012). Extraction of DNA suitable for PCR applications from mature leaves of *Mangifera indica* L. *Journal of Zhejiang University Science B, 13*, 239-243. https://doi.org/10.1631/jzus.B1100194

Bergallo, M., Costa, C., Gribaudo, G., Tarallo, S., Baro, S., Ponzi, A. N. and Cavallo, R. (2006). Evaluation of six methods for extraction and purification of viral DNA from urine and serum samples. *New Microbiologica*, *29*(2), 111-119.

Csaikl, U. M., Bastian, H., Brettschneider, R., Gauch, S., Meir, A., Schauerte, M. ...and Ziegenhagen, B. (1998). Comparative analysis of different DNA extraction protocols: a fast, universal maxi-preparation of high-quality plant DNA for genetic evaluation and phylogenetic studies. *Plant Molecular Biology Reporter*, *16*, 69-86. https://doi.org/10.1023/A:1007428009556.

Dairawan, M., & Shetty, P. J. (2020). The evolution of DNA extraction methods. *American Journal of Biomedical Science and Research,* 8(1), 39–45. http://dx.doi.org/10.34297/AJBSR.2020.08.001234

Dellaporta, S. L., Wood, J. and Hicks, J. B. (1983). A plant DNA minipreparation: version II. *Plant Molecular Biology Reporter*, *1*, 19-21.

Doyle JJ, Doyle JL.(1987). A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochem Bull. 1987;19:11–5.

Echevarría-Zomeño, S., Abril, N., Ruiz-Laguna, J., Jorrín-Novo, J. and Maldonado-Alconada, A. M. (2012). Simple, rapid, and reliable methods to obtain high quality RNA and genomic DNA from Quercus ilex L. leaves suitable for molecular biology studies. *Acta Physiologiae Plantarum*, 34, 793-805. https://doi.org/10.1007/s11738-011-0880-z.

Fang, G., Hammar, S. and Grumet, R. (1992). A quick and inexpensive method for removing polysaccharides from plant genomic DNA. *Biotechniques*, *13*(1), 52-4. PMID: 1503775.

Granier, F. (1988). Extraction of plant proteins for two-dimensional electrophoresis. *Electrophoresis*, 9(11), 712-718. https://doi.org/10.1002/elps. 1150091106.

Júnior, C. D. S., Teles, N. M. M., Luiz, D. P. and Isabel, T. F. (2016). DNA extraction from seeds. *Sample Preparation Techniques for Soil, Plant, and Animal Samples*, 265-276. https://doi.org/10.1007/978–1-4939-3185-9_18

Kotchoni, S. O., Gachomo, E. W., Betiku, E. and Shonukan, O. O. (2003). A homemade kit for plasmid DNA mini preparation. *African Journal of Biotechnology*, *2*(4). http://www.academicjournals.org/AJB

Lodhi, M. A., Ye, G. N., Weeden, N. F., Reisch, B. I. and Daly, M. J. (1995). A molecular marker-based linkage map of Vitis. *Genome*, *38*(4), 786-794. https://doi.org/10.1139/g95-100

Manen, J. F., Sinitsyna, O., Aeschbach, L., Markov, A. V. and Sinitsyn, A. (2005). A fully automatable enzymatic method for DNA extraction from plant tissues. *BMC Plant Biology*, 5, 1-9. https://doi.org/10.1186/1471-2229-5-23.

Masago, K., Fujita, S., Oya, Y., Takahashi, Y., Matsushita, H., Sasaki, E. and Kuroda, H. (2021). Comparison between fluorimetry (Qubit) and spectrophotometry (NanoDrop) in the quantification of DNA and RNA extracted from frozen and FFPE tissues from lung cancer patients: A real-world use of genomic tests. *Medicina*, 57(12), 1375. doi.org/10.3390/medicina57121375.

Moller, E. M., Bahnweg, G., Sandermann, H. and Geiger, H. H. (1992). A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic acids research*, *20* (22), 6115. doi.org/10.1093%2Fnar%2F20.22.6115

Padmalatha, K. and Prasad, M. N. V. (2006). Optimization of DNA isolation and PCR protocol for RAPD analysis of selected medicinal and aromatic plants of conservation concern from *Peninsular India*. *African journal of Biotechnology*, *5*(3), 230-234. DOI: 10.5897/AJB05.188

Porebski, S., Bailey, L. G. and Baum, B. R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter*, 15, 8-15. doi.org/10.1007/BF02772108.

Pradeep Reddy, M., Sarla, N. and Siddiq, E. A. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, 128, 9-17. https://doi.org/10.1023/A:1020691618797.

Qarni, A., Muhammad, K., Wahab, A., Ali, A., Khizar, C., Ullah, I., ... and Rahimi, M. (2022). Molecular characterization of wild and cultivated Strawberry (Fragaria× ananassa) through DNA barcode markers. *Genetics Research*, 2022. https://doi.org/10.1155/2022/9249561

Shaban, A. S., Arab, S. A., Basuoni, M. M., Abozahra, M. S., Abdelkawy, A. M. and Mohamed, M. M. (2022). SCoT, ISSR, and SDS-PAGE investigation of genetic diversity in several Egyptian wheat genotypes under normal and drought conditions. *International Journal of Agronomy*, 2022, 1-14. https://doi.org/10.1155/2022/7024028

Sheershika, S. and Ram, M. (2024). Advances in DNA Extraction Techniques: A Comprehensive Review of Methods and Applications. *Plant Cell Biotechnology And Molecular Biology*, 25(5-6), 30-42. https://doi.org/10.56557/PCBMB/2024/v25i5-68683.

Shokere, L. A., Holden, M. J. and Jenkins, G. R. (2009). Comparison of fluorometric and spectrophotometric DNA quantification for real-time quantitative PCR of degraded DNA. *Food Control*, 20(4), 391-401. https://doi.org/10.1021/ jf 901165h.

Simbolo, M., Gottardi, M., Corbo, V., Fassan, M., Mafficini, A., Malpeli, G., ... & Scarpa, A. (2013). DNA qualification workflow for next generation sequencing of histopathological samples. *PloS one*, 8(6), e62692. https://doi.org/10.1371/journal.pone.0062692.

Sirakov, I. N. (2016). Nucleic acid isolation and downstream applications. *Nucleic Acids-From Basic Aspects to Laboratory Tools*, 10, 1-26. https://doi.org/10.5772/61833.

Spadoni, A., Sion, S., Gadaleta, S., Savoia, M. A., Piarulli, L., Fanelli, V., ... and Sabetta, W. (2019). A simple and rapid method for genomic DNA extraction and microsatellite analysis in tree plants. *Journal of Agricultural Science and Technology*, 21(5), 1215-1226. doi.org/10.1155/2022/9249561

Uddin, M., Sun, W., He, X., Teixeira da Silva, J. and Cheng, Q. (2014). An improved method to extract DNA from *Mango mangifera* indica. *Biologia*, *69*(2), 133-138. https://doi.org/10.2478/s11756-013-0311-2

Urumarudappa, S. K. J., Tungphatthong, C., Jaipaew, J., Pornputtapong, N., Pakdeesattayapong, D., Vimolmangkang, S. and Sukrong, S. (2022). Development of a DNA barcode library of plants in the Thai Herbal Pharmacopoeia and Monographs for authentication of

herbal products. *Scientific Reports*, 12(1), 9624. https://doi.org/10.1038/s41598-022-13287-x

Vinod, K. K. (2004). Total genomic DNA extraction, quality check and quantitation. *Tamil Nadu Agricultural University, Coimbatore* ,109-121. https://www.researchgate.net/publication/265490368

Wang, H. F., Zong, X. X., Guan, J. P., Yang, T., Sun, X. L., Ma, Y. and Redden, R. (2012). Genetic diversity and relationship of global faba bean (Vicia faba L.) germplasm revealed by ISSR markers. *Theoretical and applied genetics*, 124, 789-797. https://doi.org/10.1007/s00122-011-1750-1.

Wu, X., Gong, F. and Wang, W. (2014). Protein extraction from plant tissues for 2DE and its application in proteomic analysis. *Proteomics*, 14(6), 645-658. https://doi.org/10.1002/pmic.201300239

Xia, Y., Chen, F., Du, Y., Liu, C., Bu, G., Xin, Y. and Liu, B. (2019). A modified SDS-based DNA extraction method from raw soybean. *Bioscience Reports*, *39*(2), BSR20182271. https://doi.org/10.1042/BSR20182271

Zhang, L., Wang, B., Pan, L. and Peng, J. (2013). Recycling isolation of plant DNA, a novel method. *Journal of Genetics and Genomics*, 40(1), 45-54. https://doi.org/10.1016/j. jgg. 2012.10.001

ARABIC SUMMARY

تقييم وتعديل بعض الطرق التقليدية لإستخلاص الحمض النووي الجينومي بجودة وكمية عالية من العائلات النباتية المختلفة

منى محمد مغازي[1]، شيماء عبد اللطيف عبد العزيز[1]، رحاب احمد حسنين[1]، روان صبري زيد[1]، روان ياسر فوزي[1]، خالد طاهر محمد[2]، أحمد علي سليمان[3]، غادة منصور سماحة[4]، هالة محمد زغلي[1]

1 قسم الوراثة، كلية الزراعة، جامعة عين شمس، ص.ب. ص.ب 68، حدائق شبرا 11241، القليوبية، مصر.

2 قسم النبات، شعبة الوراثة، كلية الزراعة، جامعة الأزهر، مصر.

3 قسم تكنولوجيا الحاصلات البستانية، معهد البحوث الزراعية والبيولوجية، المركز القومي للبحوث، الدقي، مصر.

4 قسم بحوث المحاصيل الحقلية، معهد البحوث الزراعية والبيولوجية، المركز القومي للبحوث، الدقي، مصر.

النباتات غنية بالمركبات المعقدة، التي تعيق الحصـول على الحمض النووي السـليم والنقي، وخاصـة بالطرق اليدوية التقليدية. يعد إسـتخراج الحمض النووي الجينومي عالي الجودة والكمية أهم خطوة لجميع التطبيقات البيولوجية المعتمدة على الحمض النووي، مثل تفاعل البوليميراز المتسلسل (PCR)، والاستنساخ، والتسلسل. في هذه الدراسة، تم تقييم أربعة بروتوكولات معدلة لإسـتخراج الحمض النووي (CTAB، TNES) و(CTAB، TENS مع عمود المرحلة الصـلبة) لإنتاج DNA عالي النقاء والكمية من أوراق وبذور 11 نوعاً نباتي. وتمت مقارنتهم مع مجموعة (QIAGEN) والتي تم إسـتخدامها كعنصـر تحكم إيجابي. تم تقييم التقنيات لقدرتها على إنتاج الحمض النووي المعزول خالياً من السكريات والبوليفينول والحمض النووي الريبي (RNA) وغيرها من الملوثات المهمة، بالإضافة إلى اللزوجة الواضحة ونسبة قراءات OD260 ومدى ملاءمتها لفحوصات تعتمد على تفاعل البوليميراز المتسلسل. أدى استخدام الطرق التقليدية مثل CTAB وTENS مع عمود المرحلة الصـلبة إلى زيادة كفاءة الطريقة وإعطاء نتائج أفضـل في قراءات مقياس الطيف الضـوئي وتفاعل البوليميراز المتسـلسـل. للحصـول على نتائج مماثلة لتلك التي يتم الحصـول عليها من إجراءات المجموعة، من الممكن تحسين تقنيات إستخراج الحمض النووي التقليدية. لتحقيق الحمض النووي عالي الجودة بأقل تكلفة ممكنة، يتم تحقيق ذلك من خلال الجمع بين العديد من محاليل الإستخلاص المنظمة وإستخدام عمود المرحلة الصلبة.