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A rapid and efficient DNA extraction method from high oily content seeds: *Ricinus communis* L.- apt for PCR based assay

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Abstract: *Ricinus communis* seeds harbor high oil and polyphenolics contents that hinder DNA extraction. Here, a rapid and efficient protocol for isolating total DNA from *R. communis* seeds was developed. The current method implies the use of repeated cycles of freeze/heat shock for the seed tissue to lyse the cells. DNA isolated with this protocol was successfully used as a template for PCR amplification of the internal transcribed spacer (ITS) region of the rRNA encoding gene that widely used for molecular identification of different plant species. As far to our knowledge, this study is the first one that report the efficient use of freeze/heat shock repeated cycles for isolation of a high-quality DNA from plant cells. The current protocol would support the subsequent analysis for seed lot purity analysis.

Key words: castor bean seed, cell lysis, heat shock, ITS, PCR, sequencing

Hitra in učinkovita metoda DNK ekstrakcije iz semen kloščevca (*Ricinus communis* L.), bogatih na oljih, primerna za PCR analizo

Izvleček: Semena kloščevca so bogata na oljih in polifenolih kar ovira ekstrakcijo DNK. V tej raziskavi je bil razvit hiter in učinkovit protokol za izolacijo celokupne DNK iz semen kloščevca. Metoda uporablja ponavljajoče se cikle zmrzovanja in segrevanja, kar povzroči lizijo celic v tkivu semena. DNK izolirana po tem protokolu je bila uspešno uporabljena kot osnova za PCR namnoževanje ITS regij v rRNK, ki kodirajo gene, na širokouporabljene pri molekularni identifikaciji različnih rastlinskih vrst. Kolikor nam je znano, je to prva raziskava, ki poroča o učinkoviti rabi ponavljajočih se cikličnih šokov zmrzovanja in segrevanja za izolacijo visoko kakovostne DNK iz rastlinskih celic. Ta protokol bo pripomogel k hitri analizi čistosti semen.

Ključne besede: kloščevec, lizija celic, vročinski šok, ITS, PCR, sekvenciranje

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1 INTRODUCTION

Castor bean (*Ricinus communis* L.) seed is one of the most common oil seed plants that is widely used economically in biodiesel production, cosmetics industry, lubricants, biomedical applications and as a rich animal fodder (Patel et al., 2016; Sturtevant et al., 2019; Sánchez et al., 2019; Awais et al., 2020). The seeds are externally protected by hard, brittle, mottled brown and shinning testa. The outermost layer of the seeds coat is the waxy cuticle, which represents the first barrier to water imbibition. The mature desiccated seed coat is rich in the oxidative products of polyphenolic compounds including phenolic acids, tannins, and flavonoids which are the source of the brown color of the mature seed coat, and play a significant role in plant disease resistance. Also, peroxidases and other antioxidant scavenging enzymes are commonly found in seeds coats (Moïse et al., 2005).

Employing genomics and molecular technologies promise to accelerate our knowledge of seeds and thus open new potentials for uses and conservation. The low concentration of DNA present in seed tissues makes it difficult to isolate intact DNA. Additionally, the DNA in seeds can be tightly bound to various proteins, polyphenols, and sugars. These biochemical interactions further complicate the process of extracting high quality DNA from seeds (Roy Davies, 1977; Sliwinska, 2006; Lee et al., 2020). The isolation of a good quality DNA is a prerequisite for any molecular biology work because proteins, polyphenols and polysaccharides impurities that co-precipitate with the DNA may hinder any enzymatic action, such Taq DNA polymerase in Polymerase Chain Reaction (PCR) and subsequent sequencing (Lakay et al., 2007; Wnuk et al., 2020) and endonucleases in genotyping and blotting techniques (Brown, 2001; Harju et al., 2004; Shiraishi and Iwai, 2020).

PCR based technique is successfully used to detect *R. communis* candidate genomic loci that are associated with important agronomic traits (Fan et al., 2019). PCR is essential for specific gene detection that prevailed in molecular identification and characterization (Manjunath and Sannappa, 2014). Enhancing the current seed traits to add value or to overcome an existing problem may be achieved by the generation of genetically modified plants. This wouldn't be achieved without isolating a good quality DNA that would serve as a template for a PCR reaction. The production of transgenic *R. communis* plants expressing the *Bacillus thuringiensis cry1Aa* gene help in lepidopteran insect pest management that were responsible for 30–50 % of yield losses (Muddanuru et al., 2019).

CTAB (Cetyl trimethylammonium bromide) DNA based extraction protocol and tailored modification versions of it, is widely used for isolating DNA from different plant tissues. However efficient, the CTAB is harsh and toxic in nature for human health and should be followed by phenol purification to ensure sufficient DNA purity. The phenol is volatile and can burn the skin (Doyle and Doyle, 1990; Shukla et al., 2018; Aboul-Maaty and Oraby, 2019). The used CTAB based protocol is time consuming as it requires long incubation time reached up to 60 min at 65 ^{0}C (Novaes et al., 2009). Alternatively, the commercial DNA extraction kits is an efficient substitute, despite the associated cost burden. So, a rapid efficient isolation method is of demand to facilitate any genomics and molecular biology work.

Therefore, the current study was designed for developing a rapid and efficient seed DNA isolation method that implies repeated cycles of freeze/heat shock to lyse the seed cells. Subsequent removal of the coagulated proteins and lipids by chloroform extraction was made and finally, high quality DNA was precipitated by ethanol.

2 MATERIALS AND METHODS

2.1 PLANT MATERIALS

Ten different samples of *R. communis* seeds were collected from their wild habitat in Egypt. Their DNA was extracted according to the newly developed protocol and used as a template for PCR amplification to amplify the ITS region of the corresponding rRNA encoding gene (Cheng et al., 2016).

2.2 MATERIALS AND EQUIPMENTS

- 1.5 ml Eppendorf tubes
- Mortar and pestle
- Tips
- Cooling centrifuge (Hettich MIKRO 22)
- Crushed ice
- UV-transilluminator (Vilber Lourmat-Germany)
- Digital balance (RADWAG Wagi Elektroniczne, AS 220/C/2)
- Micropipettes
- Vortex (VELP SCIENTIFICA)
- Water bath (MLW W21)
- Gel electrophoresis unit
- Thermal cycler (Biometra, Germany)

2.3 REAGENTS

- Lysis buffer: 2 % Triton X-100 (ADVENT), 100

mM NaCl (POWER CHEMAL), 1 % SDS (Sodium Dodecyl Sulfate), 10 mM Tris-HCl (pH 8.0) (OX FORD) and 1 mM EDTA (pH 8.0) (Hoffman and Winston, 1987, Harju et al., 2004)
- Ice cold 99 % ethanol (POWER CHEMICAL) or isopropyl alcohol
- 70 % ethanol
- 1 × TAE buffer
- Ethidium bromide (ALPHA CHEMIKA)
- Chloroform (SIGMA-ALDRICH)
- 3 M sodium acetate pH 5.2
- TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0, autoclaved)
- Agarose (molecular grade, Cleaver Scientific Ltdm CAS 9012-36-6)

2.4 THE DETAILED PROTOCOL

1. In 1.5 ml Eppendorf tubes, 0.1 g crushed decoated *R. communis* seeds were combined with 400 µl of the DNA lysis buffer. And then vortexed vigorously in a bench top vortex for up to one minute, followed by incubation on ice.

a. TIP: Avoid using a large amount of seed sample because the polysaccharides, polyphenols, and their derivatives in the seed would increase concurrently. These substances could interfere negatively with proper DNA isolation, reducing the isolated amount.

b. TIP: Rather than using a mortar and pestle, the sample could be crushed inside a round end 2 ml Eppendorf tube using a glass rod.

c. TIP: Grinding in liquid nitrogen would facilitiate the grinding process and increase DNA integrity.

2. After two minutes on crushed ice, the tubes were immersed abruptly in a 95 °C water bath for 5 minutes with interval vortexing.

3. After repeating the freeze/heat shock procedure exactly as described above, the tubes were vigorously vortexed for a continuous 30 seconds.

TIP: The solution takes on the appearance of a milky emulsion.

4. 400 µl of chloroform was added and vigorously vortexed for one minute, followed by centrifugation at 4 °C, 5 min, 10,000 rpm (RCF: 17,507).

TIP: Chloroform and isoamyl-alcohol can be mixed in a ratio of (24:1 v/v).

5. The aqueous layer was transferred to a new tube and the chloroform purification was repeated twice.

TIP: The chloroform purification should be repeated whenever necessary till the aqueous fraction is clear.

6. To allow DNA precipitation, the clear aqueous layer was transferred to a new tube containing 1 ml of ice-cold 99 % absolute ethanol and 40 µl of 3 M sodium acetate pH 5.2. After 15 minutes on ice, the tubes were centrifuged for 10 minutes at 4 °C and 10,000 rpm.

TIP: Instead of absolute ethanol, isopropyl alcohol may be used.

7. Following the removal of supernatants, DNA pellets were washed with 0.5 ml of 70 % ethanol and quickly centrifuged at 4 °C for 2 minutes at 10,000 rpm. The pellet was then air dried for 5 minutes.

TIP: To expedite the drying of the DNA pellet, invert the Eppendorf on a piece of clean. tissue.

8. Resuspend the DNA in 30 µl TE buffer [10 mM Tris, 1 mM EDTA (pH 8.0)]. The samples were stored at -20 °C until they were used.

TIP: It is recommended to dissolve the DNA pellet in a small volume of TE buffer to ensure that the DNA is adequately concentrated. If the sample was dissolved in a larger volume, it resulted in a low concentration of DNA. Reprecipitate your DNA and resuspend it in a reduced volume of TE buffer.

2.5 QUANTIFICATION AND VISUALIZATION OF DNA

The concentration of isolated DNA was determined using a NanoDrop® ND-1000 UV-Vis Spectrophotometer (Thermo Scientific-USA). The optical density (OD) at A260 and A280 was used to determine the purity. Electrophoresis of samples was performed on a 1 % agarose gel in 1× TAE (Tris-Acetate- Ethylenediaminetetraacetic acid) buffer containing 0.5 µg ml^{-1} ethidium bromide. The DNA was visualized and photographed with the aid of a UV-transilluminator embedded in Gel Documentation system (Virballurmate-Germany).

2.6 PCR AMPLIFICATION AND SEQUENCING

The PCR was carried out according to the manufacturer's instructions in a 50 µl reaction volume using (BIOLINE cat # BIO-21108) Mytaq Red DNA polymerase master mix. ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') primers were used. The reaction mixture contains 1 × mytaq Red DNA polymerase master mix, 2.0 µl of each primer at a concentration of 10 pm l^{-1}, 1.0 µl of DNA template, and 0.25 µl of MyTaq™ DNA polymerase (5U $µl^{-1}$). The following PCR cycles were run in a Thermal Cycler (Biometra, Germany): Initial denaturation was carried out for 3 minutes at 95 °C, followed by 35 cycles of 20 seconds at 95 °C (denaturation), 20 seconds at 55 °C (annealing), 30 seconds at 72 °C (extension), and then a final extension

for 10 minutes at 72 °C. On a 1 % agarose gel, the PCR products were separated.

The amplified fragments were excised and gel purified according to the manufacturer's protocol using the PCR-M clean up system (VIOGENE cat# PF1001). Following that, their sequences were determined at GATC Company using an ABI 3730xl DNA sequencer and an ITS4 primer. The obtained nucleotide sequences were validated against the NCBI database using the MEGA blast tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Geneious 11.1.5 software was used to align all sequences. The following sequences were deposited in the NCBI GenBank: MN880879, MN880880, MN880881, MN880882, MN880885, MN880886, MN880887, MN880888, MN880889, and MN880890.

3 RESULTS

The following is a description of a rapid DNA isolation protocol called the "ES DNA isolation method" (Fig. 1). The method is based on the use of repeated freeze-and-heat shock cycles to disrupt the cell wall and release genomic DNA from crushed de-coated *R. communis* seeds in lysis buffer. Following that, two rounds of chloroform purification were performed to remove co-bound proteins and lipids to the DNA following cell lysis. After transferring the aqueous phase to a clean tube, the DNA is easily precipitated using absolute (Abs.) ethanol and 3 M sodium acetate (pH 5.2). Following a 70 % ethanol wash, the air-dried pellet was dissolved in TE buffer to ensure the extracted DNA was preserved for the long term.

The isolated DNA's purity and lack of degradation are demonstrated by gel electrophoresis (Fig. 2).

Table 1: Quantitative estimates of DNA purity and concentration revealed by Nanodrop Spectrophotometer

Sample ID	Concentration ng µl^{-1}	A260/A280 readings (DNA Purity)
Rc1	95.1	2
Rc 2	81.4	2.57
Rc 3	49.5	2.3
Rc 4	55.6	2.28
Rc 5	79.7	1.99
Rc 6	52.8	2.11
Rc 7	56.9	2.3
Rc 8	60.1	2.5
Rc 9	66.3	1.99
Rc 10	55.7	2.44

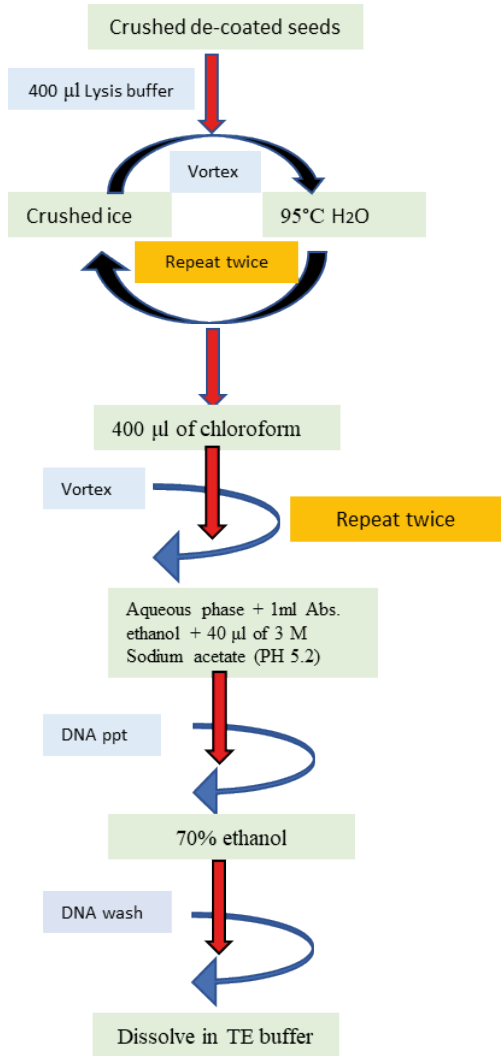

Figure 1: Flow chart representation of the current *R. communis* seed DNA isolation protocol termed "ES DNA isolation method"

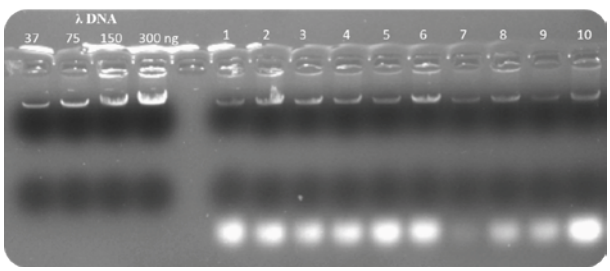

Figure 2: Gel electrophoresis of DNA isolated from the de-coated *R. communis* seeds used in the current protocol; "ES DNA isolation method". Lanes 1 to 10 refers to the ten samples used. λ DNA refers to serial dilution of undigested λ DNA for quality control and quantification of isolated DNA. The flourecent bands towards the bottom of the gel likely represent degraded RNA, which commonly co-purifies during DNA extraction protocols

The A260/280 ratio, which ranges between 1.99 and 2.57 for all *R. communis* seed DNA samples examined, indicates the quality of the isolated DNA (Table 1). The isolated DNA concentration ranges between 49.5 and 95.1 ng μl^{-1}. The yield and purity of the isolated DNA were sufficient for performing polymerase chain reaction amplification (Fig. 3). All samples were amplified to the expected product size of 700 bp.

All PCR products were sequenced and the nucleotide sequences verified using the National Center for Biotechnology Information's (NCBI) website. Alignment of the ITS sequences was performed to demonstrate their homology (Fig.4). The matched sequences revealed that all samples shared a significant degree of homology. The results demonstrates that the sequenceable PCR products obtained are of a high grade.

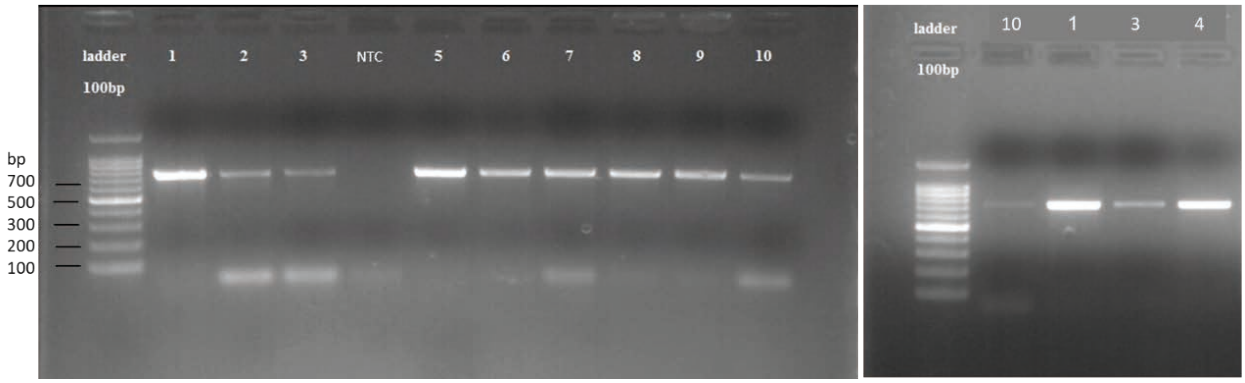

Figure 3: PCR-amplification of the ITS-rRNA encoding gene of the different *R. communis* seeds using the DNA extracted by the current method "ES DNA isolation method" as a template. Lanes 1 to 10 represent the ten *R. communis* samples. 100 bp ladder refers to the DNA ladder. NTC refers to non-DNA template of the PCR, no amplification means no PCR contamination

```
          1         10        20        30        40        50        60
          |         |         |         |         |         |         |
Rc 6      AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
Rc 7      AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
Rc 2      AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
Rc 9      AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
Rc 3      AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
Rc 1      AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
Rc 4      AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
Rc 8      AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
Rc 5      AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
Rc 10     AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT

Rc 6      CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
Rc 7      CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
Rc 2      CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
Rc 9      CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
Rc 3      CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
Rc 1      CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
Rc 4      CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
Rc 8      CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
Rc 5      CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
Rc 10     CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC

Rc 6      AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
Rc 7      AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
Rc 2      AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
Rc 9      AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
Rc 3      AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
Rc 1      AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
Rc 4      AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
Rc 8      AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
Rc 5      AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
Rc 10     AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA

Rc 6      ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
Rc 7      ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
Rc 2      ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
Rc 9      ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
Rc 3      ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
Rc 1      ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
Rc 4      ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
Rc 8      ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
Rc 5      ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
Rc 10     ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG

Rc 6      CCGAGGGCACGCCTGCCTGGGTGTCACGCAATTGTCGCCCCCAACCCTTTCGATACATCG
Rc 7      CCGAGGGCACGCCTGCCTGGGTGTCACGCAATTGTCGCCCCCAACCCTTTCGATACATCG
Rc 2      CCGAGGGCACGCCTGCCTGGGTGTCACGCAATTGTCGCCCCCCAACCCTTTCGATACATCG
Rc 9      CCGAGGGCACGCCTGCCTGGGTGTCACGCAATTGTCGCCCCCCAACCCTTTCGATACATCG
Rc 3      CCGAGGGCACGCCTGCCTGGGTGTCACGCAATTGTCGCCCCCCAACCCTTTCGATACATCG
Rc 1      CCGAGGGCACGCCTGCCTGGGTGTCACGCAATTGTCGCCCCCCAACCCTTTCGATACATCG
Rc 4      CCGAGGGCACGCCTGCCTGGGTGTCACGCAATCGTCGCCCCCCAACCCTTTCGATACATCG
Rc 8      CCGAGGGCACGCCTGCCTGGGTGTCACGCAATCGTCGCCCCCCAACCCTTTCGATACATCG
Rc 5      CCGAGGGCACGCCTGCCTGGGTGTCACGCAATCGTCGCCCCCCAACCCTTTCGATACATCG
Rc 10     CCGAGGGCACGCCTGCCTGGGTGTCACGCAATCGTCGCCCCCCAACCCTTTCGATACATCG
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Figure 4: Partial sequence alignments of the ITS-rRNA encoding gene sequences obtained from the 10 *R. communis* seeds using Geneious 11.1.5 software. The data confirms the quality of the obtained PCR products that could be sequenced

4 DISCUSSION

While a PCR-based assay requires adequate quality genomic DNA, a rapid isolation methodology is required to permit the processing of large numbers of samples. Because the majority of polyphenolic chemicals in seeds are localized in their seed coats, de-coating the seeds reduces their interference with DNA isolation (Moïse et al., 2005). Repeated freeze-heat shock cycles are employed to shatter the cell wall and release genomic DNA from crushed de-coated *R. communis* seeds in lysis buffer, obviating the necessity for enzymatic or mechanical degradation (Harju et al., 2004). The lysis buffer contains mild detergents such as Triton X-100 and SDS and is frequently used to lyse cells, extract proteins, extract oils, and permeabilize live cell membranes by dissolving protein-lipid and lipid-lipid interactions without denaturing proteins (Johnson, 2013). However, freezing and heat shock may affect cell wall permeability and denature proteins, thereby expediting their removal, without the need for hazardous polyvinyl pyrrolidone (PVP), phenol, or β-mercaptoethanol (John, 1992; Shukla et al., 2018).

Seed polysaccharides, polyphenols, and their derivatives may impair the integrity of isolated DNA from *R. communis* seed (Porebski et al., 1997). When cells are lysed, these compounds covalently link to DNA, impairing DNA integrity and impeding PCR amplification (Wnuk et al., 2020). Therefore, the 60 °C CTAB incubation may be particularly important to dissociate polysaccharides from DNA, allows efficient and thorough lysis of cells and breakdown of proteolytic enzymes that could otherwise degrade DNA. This contributes to high DNA yields with CTAB and improve purity of the extracted DNA. The current lysis protocol using repeated freeze-heat shock cycles may achieve comparable degree of enzymatic degradation and release of DNA from cellular components without prolonged optimum heated incubation (Carey et al., 2023). Chloroform can be used to remove these compounds during DNA extraction. Additional to removing denatured proteins, it aids in the removal of other colouring chemicals such as pigments and dyes. Chloroform facilitates the separation of lipids, proteins, and cellular detritus into the organic phase, while the recoverable DNA is dissolved in the aqueous phase and then easily precipitated with absolute (Abs.) ethanol. Because the low seed DNA content necessitates efficient DNA precipitation, 3 M sodium acetate (pH 5.2) was added to the absolute ethanol during the precipitation process (Júnior et al., 2016; Heikrujam et al., 2020).

Gel electrophoresis was used to determine the feasibility of the current DNA isolation approach. No degradation was seen on the gel. Additionally, this was also observed with the nanodrop measurements. The A260/280 ratio of isolated DNA varied between 1.99 to 2.57 for all *R. communis* seed DNA samples examined, indicating that the DNA was of acceptable quality (Nzilibili et al., 2018). The isolated DNA concentration ranged between 49.5 and 95.1 ng μl^{-1}, which was sufficient for downstream applications such as PCR. Although the A260/280 ratio for high-quality DNA should be between 1.8 and 2, values greater than 2 have been observed previously for DNA samples isolated from various plant tissues using a modified CTAB-based approach (Aboul-Maaty and Oraby, 2019). This could be attributed to ionic strength and altered pH of the solutions used in the extraction process (Wilfinger et al., 1997; Boesenberg-Smith et al., 2012). Despite the fact that the DNA isolated using the current approach had a slightly higher A260/280 ratio, the DNA isolated was effectively employed for the downstream application, PCR. The extracted DNA was used as a template for PCR amplification of the ITS region of the rRNA encoding gene, which is commonly used for molecular identification and assessment of the molecular diversity of eukaryotic cells (Cheng et al., 2016; Yang et al., 2018; Ghareb et al., 2020; Soliman and A., 2021). PCR amplification of the extracted DNA samples yielded the expected product size of approximately 700 bp, as shown in Figure 3. The amplified PCR products were successfully sequenced, and the obtained sequences were deposited in the NCBI database (Fig. 4) (Soliman and A., 2021). These results demonstrate that the isolated DNA was of suitable quality for PCR amplification and downstream sequencing applications.

5 CONCLUSION

This rapid DNA extraction protocol demonstrates the ability to isolate DNA from seed tissues even without relying on specific chemical reagents typically used for extraction, such as CTAB. By utilizing simple, readily accessible lysis buffer components and efficient physical disruption of cells through freeze-heat cycles. This method provides an alternative approach to extract PCR-suitable DNA without requiring specialized chemicals. While CTAB is considered a staple reagent for high-quality DNA purification, this work shows that effective extraction is still achievable using very basic buffers and lysing techniques. The simplicity and accessibility of the reagents needed could make this rapid protocol easily adoptable, especially in resource-limited settings where procuring chemicals like CTAB may be difficult or cost-prohibitive. The proposed approach would facilitate the molecular investigation of seed lot quality and the characterization of germplasm.

5.1 DECLARATIONS

5.1.1 List of abbreviations

PVP, polyvinyl pyrrolidone. ITS-rRNA, Internal transcribed spacer of ribosomal RNA encoding gene.

5.1.2 Ethics approval and consent to participate

"Not applicable"

5.1.3 Consent for publication

"Not applicable"

5.1.4 Availability of data and material

All data generated or analyzed during this study are included in this article.

5.1.5 Competing interests

"The author declare that she has no competing interests"

5.1.6 Funding

This study received no funding grant

5.1.7 Authors' contributions

ERSS: generates the idea, performs all experimental work, analyze the data, submit the sequences to the database, wrote the manuscript, revised it, and corresponding the publication.

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6 REFERENCES

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*(25), 1–10. https://doi.org/10.1186/S42269-019-0066-1

Awais, M., Musmar, S.A., Kabir, F., Batool, I., Rasheed, M.A., Jamil, F., Khan, S.U. and Tlili, I. (2020). Biodiesel production from *Melia azedarach* and *Ricinus communis* oil by transesterification process. *Catalysts*, *10*, 427. https://doi.org/10.3390/catal10040427

Boesenberg-Smith, K.A., Pessarakli, M.M. and Wolk, D.M. (2012). Assessment of DNA yield and purity: an overlooked detail of PCR troubleshooting. *Clinical Microbiology Newsletter*, *34*, 1–6. https://doi.org/10.1016/J.CLINMICNEWS.2011.12.002

Brown, T.A. (2001). Southern blotting and related DNA detection techniques. *Encyclopedia of Life Sciences*, John Wiley & Sons, Ltd, Chichester, UK. https://doi.org/10.1038/npg.els.0000996

Carey, S.J., Becklund, L.E., Fabre, P.P. and Schenk, J.J. (2023). Optimizing the lysis step in CTAB DNA extractions of silica-dried and herbarium leaf tissues. *Applications in Plant Sciences*, *2*711(3):e11522. https://doi.org/10.1002/aps3.11522. PMID: 37342163; PMCID: PMC10278933.

Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y. and Zhou, S. (2016). Barcoding the kingdom Plantae: New PCR primers for ITS regions of plants with improved universality and specificity. *Molecular Ecology Resources*, 16, 138–149. https://doi.org/10.1111/1755-0998.12438

Doyle, J. and Doyle, J. (1990). Isolation of plant DNA from fresh tissue. *Focus,* 12, 13-15. https://doi.org/10.2307/2419362

Fan, W., Lu, J., Pan, C., Tan, M., Lin, Q., Liu, W., Li, D., Wang, L., Hu, L., Wang, L., Chen, C., Wu, A., Yu, X., Ruan, J., Yu, J., Hu, S., Yan, X., Lü, S. and Cui, P. (2019). Sequencing of Chinese castor lines reveals genetic signatures of selection and yield-associated loci. *Nature Communications*, *10*, 1–11. https://doi.org/10.1038/s41467-019-11228-3

Ghareb, H.E.S., Ibrahim, S.D. and Hegazi, G.A.E.M. (2020). In vitro propagation and DNA barcode analysis of the endangered Silene schimperiana in Saint Katherine protectorate. *Journal of Genetic Engineering and Biotechnology*, 18. https://doi.org/10.1186/s43141-020-00052-8

Harju, S., Fedosyuk, H. and Peterson, K.R. (2004). Rapid isolation of yeast genomic DNA: Bust n' Grab. *BMC Biotechnology*, *4*. https://doi.org/10.1186/1472-6750-4-8

Heikrujam, J., Kishor, R. and Behari Mazumder, P. (2020). The Chemistry Behind Plant DNA Isolation Protocols. *Biochemical Analysis Tools - Methods for Bio-Molecules Studies*, IntechOpen, DOI: 10.5772/intechopen.92206

Hoffman, C.S. and Winston, F. (1987). A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli. Gene*, *57,* 267-272. https://doi.org/10.1016/0378-1119(87)90131-4

John, M.E. (1992). An efficient method for isolation of RNA and DNA from plants containing polyphenolics. *Nucleic Acids Research*, *20*, 2381. https://doi.org/10.1093/nar/20.9.2381

Johnson, M. (2013). Detergents: Triton X-100, Tween-20, and More. *Materials and Methods*, 3. https://doi.org/10.13070/mm.en.3.163

Júnior, C.D.S., Teles, N.M.M., Luiz, D.P. and Isabel, T.F. (2016). DNA Extraction from seeds. pp. 265–276, Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-3185-9_18

Lakay, F.M., Botha, A. and Prior, B.A. (2007). Comparative analysis of environmental DNA extraction and purification methods from different humic acid-rich soils. *Jour-*

nal of Applied Microbiology, *102*, 265–273. https://doi.org/10.1111/j.1365-2672.2006.03052.x

Lee, C.-L., Huang, Y.-H., Hsu, I.C. and Lee, H.C. (2020). Evaluation of plant seed DNA and botanical evidence for potential forensic applications. *Forensic Sciences Research*, *5*, 55–63. https://doi.org/10.1080/20961790.2019.1594599

Manjunath, K.G. and Sannappa, B. (2014). Identification of castor bean (*Ricinus Communis* L.) ecotypes through molecular characterization in the selected regions of the western ghats of Karnataka, India. *International Jornal of Bioassays*, *3*, 3492–3498.

Moïse, J.A., Han, S., Gudynait-Savitch, L., Johnson, D.A. and Miki, B.L.A. (2005). Seed coats: Structure, development, composition, and biotechnology. *In Vitro Cellular and Developmental Biology- Plant*, *41,* 620–644. https://doi.org/10.1079/IVP2005686

Muddanuru, T., Polumetla, A.K., Maddukuri, L. and Mulpuri, S. (2019). Development and evaluation of transgenic castor (*Ricinus communis* L.) expressing the insecticidal protein Cry1Aa of *Bacillus thuringiensis* against lepidopteran insect pests. *Crop Protection*, *119*, 113–125. https://doi.org/10.1016/j.cropro.2019.01.016

Novaes, R., Rodrigues, J. and Lovato, M. (2009). An efficient protocol for tissue sampling and DNA isolation from the stem bark of leguminosae trees. *Genetics and Molecular Research, 8*(1), 86-96. https://doi.org/10.4238/vol8-1gmr542

Nzilibili, S.M.M., Ekodiyanto, M.K.H., Hardjanto, P. and Yudianto, A. (2018). Concentration and purity DNA spectrophotometer: Sodium monofluorophosphate forensic impended effect. *Egyptian Journal of Forensic Sciences*, *8*, 34. https://doi.org/10.1186/s41935-018-0065-7.

Patel, V.R., Dumancas, G.G., Viswanath, L.C.K., Maples, R. and Subong, B.J.J. (2016). Castor bean oil: properties, uses, and optimization of processing parameters in commercial production. *Lipid Insights*, *9*, 1-12. https://doi.org/10.4137/LPI.S40233. PMID: 27656091.

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. https://doi.org/10.1007/BF02772108

Roy Davies, D. (1977). DNA contents and cell number in relation to seed size in the genus vicia. *Heredity*, *39*, 153–163. https://doi.org/10.1038/hdy.1977.52

Sánchez, N., Encinar, J.M., Nogales, S., and González, J.F. (2019). Biodiesel production from castor oil by two-step catalytic transesterification: optimization of the process and economic ssessment. *Catalysts*, *9*, 864. https://doi.org/10.3390/catal9100864

Shiraishi, M. and Iwai, S. (2020). Molecular basis of substrate recognition of endonuclease Q from the euryarchaeon pyrococcus furiosus. *Journal of Bacteriology*, *202*. https://doi.org/10.1128/JB.00542-19

Shukla, R., Sharma, D.C., Pathak, N. and Bajpai, P. (2018). Genomic DNA isolation from high polyphenolic content *Grewia asiatica* L. leaf without using liquid nitrogen. *Iranian Journal of Science and Technology, Transaction A: Science*, *42*, 347–351. https://doi.org/10.1007/s40995-016-0081-0

Sliwinska, E. (2006) Nuclear DNA content analysis of plant seeds by flow cytometry. *Current protocols in cytometry,* J. Paul Robinson, managing editor, Chapter 7. https://doi.org/10.1002/0471142956.cy0729s35

Soliman, E.R.S. and A., S.M.S. (2021). Diversity assessment by molecular barcoding and seed morphology in *Ricinus communis* L. *Baghdad Science Journal*, *18*(1Suppl.), 708–715. https://doi.org/10.21123/bsj.2021.18.1(Suppl.).0708

Sturtevant, D., Romsdahl, T.B., Yu, X.-H., David, Burks, J., Azad, R.K., Shanklin, J., Kent, and Chapman, D. (2019). Tissue-specific differences in metabolites and transcripts contribute to the heterogeneity of ricinoleic acid accumulation in *Ricinus communis* L. (castor) seeds. *Metabolomics*, *15*, 6. https://doi.org/10.1007/s11306-018-1464-3

Wilfinger, W.W., Mackey, K. and Chomczynski, P. (1997). Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *BioTechniques*, *22*, 474–481. https://doi.org/10.2144/97223ST01

Wnuk, E., Waśko, A., Walkiewicz, A., Bartmiński, P., Bejger, R., Mielnik, L. and Bieganowski, A. (2020) The effects of humic substances on DNA isolation from soils. *PeerJ*, *8*. https://doi.org/10.7717/peerj.9378

Yang, R.-H., Su, J.-H., Shang, J.-J., Wu, Y.-Y., Li, Y., Bao, D.-P. and Yao, Y.-J. (2018) Evaluation of the ribosomal DNA internal transcribed spacer (ITS), specifically ITS1 and ITS2, for the analysis of fungal diversity by deep sequencing.(ed D Cullen). *PLOS ONE*, *13*, e0206428. https://doi.org/10.1371/journal.pone.0206428