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A Simple and Efficient Method for DNA Extraction from Algerian *Vitis vinifera* L. Cultivars

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Abstract
The existence of numerous grapevine varieties has led to the emergence of many cultivars that are often poorly identified or known by different names in various regions. Currently, molecular biology methods that require high-quality genomic DNA allow for the genetic identification of these different varieties. Our study is considered a first step toward the genetic identification of grapevines in Algeria and the optimization of a protocol for better nucleic acid extraction. This development was carried out on 20 grapevine cultivars sampled from the El-Kantara commune, in the province of Biskra, Algeria. We optimized a cost-effective CTAB-based protocol and developed a simple method for preparing leaf samples as a preserved powder, which can be used at any time to obtain intact genomic DNA. The average concentration, yield (2385 µg/g), and purity of the genomic DNA extracted using the optimized protocol are very satisfactory compared to the reference protocol.
Keyword: Local vines, DNA extraction, CTAB protocol, optimization, quality of nucleic acids.

1. Introduction

In the Maghreb countries, particularly Algeria, viticulture has historically been characterized by a rich diversity of local grapevine varieties (Levadoux et al., 1971). Since its expansion during the Phoenician, Carthaginian, and Roman periods, this culture has become a source of income alongside the cultivation of olives and figs (Hamama et al., 2014). In Algeria, the

diversity of grape varieties is impressive (Laiadi et al., 2009; Laiadi et al., 2013; Rahali et al., 2019; Khouni et al., 2023). Notably, the Algerian gene pool is relatively unexplored, and misidentification problems are common (Khouni et al., 2023). Numerous cultivars are misidentified or called by different names in different regions (Isnard, 1951). With the advent of molecular biology, it is now possible to genetically identify different grapevine varieties (This et al., 2004). However, for these techniques to be effective, high-quality genomic DNA is essential (Migliaro et al., 2013). The success of molecular analyses largely depends on the quality of the extracted DNA, which underlines the importance of robust and reliable extraction protocols (Marsal et al., 2013, Rathnayake et al., 2014). Ensuring optimal extraction not only leads to more accurate results, but also facilitates the classification and study of grape cultivars, often characterized by their great diversity and complexity (Öner et al., 2022). The species *Vitis vinifera* L. is known for its richness in secondary metabolites such as polysaccharides, polyphenols, and proteins (Lodhi et al., 1994). These compounds are considered contaminants that can interfere with DNA and inhibit the application of PCR and other subsequent applications (Khanuja et al., 1999; Dani et al., 2010; Anđelković et al., 2015; Aboul-Maaty and Oraby, 2019). Polyphenols precipitate with DNA and prevent the isolation of high-quality DNA (Porebski et al., 1997). Polysaccharides can also inhibit the enzymatic activity of Taq polymerase (Fang et al., 1992) and the activity of restriction des enzymes (Pandey et al., 1996). A viscous solution of isolated DNA was due to the presence of polysaccharides (Do and Adams, 1991) which covalently binds to DNA and giving a brown color (Katterman and Shattuck, 1983). The yield, purity, and integrity of nucleic acids are crucial elements for ensuring the efficiency and reliability of molecular biology analyses (Shukla et al., 2018). This is particularly challenging for plant samples, where achieving optimal yield and/or satisfactory purity is often more difficult (Friar, 2005). Numerous extraction protocols are well-known, including those proposed by Dellaporta et al., (1983), Doyle and Doyle, (1987), Bowers et al., (1993), Piccolo et al., (2012). However, their application is often limited by the availability of appropriate resources and equipment (Scobeyeva et al., 2018). In addition to manual extraction techniques, commercial kits for genomic DNA extraction have also been developed and used especially in grapevine characterization studies (This et al., 2004). These kits are often either expensive, yield a small amount of DNA (Öner et al., 2022), not readily available, especially for researchers in developing countries (Wang et al., 2011; Aubakirova et al., 2014). It is important to develop a simple, rapid, and cost-effective DNA extraction protocol, which is not only essential for molecular studies but also highly desirable when processing a large number of

samples (Wang et al., 2011; Aubakirova et al., 2014; Öner et al., 2022). The aim of this study was to develop a cost-effective method based on CTAB, with modifications, for extracting high-quality genomic DNA. To achieve this, we will attempt to extract genomic DNA from dehydrated leaves without using liquid nitrogen, and optimize the extraction method to establish an efficient, low-cost protocol that can be implemented in our minimally equipped laboratory, while ensuring high-quality genomic DNA and improved yield.

2. Material and methods

The plant material used in this study consists of various grapevine varieties from the El-Kantara commune (province of Biskra, Algeria). Nine varieties were chosen (El-Tamri, and the others are unknown).

We selected young leaves for genomic DNA extraction because they are often used in genetic studies (Lucas et al., 2019). According to Varma et al., (2007), genomic DNA extraction can be influenced by factors such as the age and type of tissue used, including leaves, stems, roots, and fruits, as well as the sample preparation method. Contrarily, adult leaves are rich in secondary metabolites, and the DNA extracted would be of poor quality (Hanania et al., 2004; Varma et al., 2007; Sahu et al., 2012; Marsal et al., 2013; Cantu et al., 2019). In other tissues, stems and roots are more lignified and contain high concentrations of secondary metabolites (Lewinsohn et al., 1994), that can inhibit the polymerase chain reaction (PCR) to amplify the locus of interest (Schori et al., 2013). However, young leaves are poor in secondary metabolites such as polysaccharides, polyphenols, tannins, etc. They are, therefore an ideal source for obtaining pure DNA.

After collecting the leaves, they were dried using the procedure described by Benbouza et al., (2006). They were then dried in a ventilated oven for four days at 27°C. The dehydration process preserves the leaves by reducing their water content, which in turn slows down the catabolic processes within the cells, thereby decreasing the catalytic activity of nucleases and proteases (Nunes et al., 2011).

The dry leaves were ground in a mortar without the use of liquid nitrogen. The samples were then stored in sterile boxes with silica gel at room temperature. The silica gel prevents the samples from rehydrating due to humidity during storage (Chase and Hills, 1991).

In this study, we adopted the protocol described by Piccolo et al., (2012) as a reference for extracting genomic DNA from grapevine leaves. This protocol is based on the method by Doyle and Doyle, (1990) and employs the CTAB/chloroform-isoamyl alcohol method for

liquid-liquid extraction. We optimized several steps of the protocol by Piccolo et al., (2012) to propose a relatively simple, cost-effective, and high-quality DNA extraction method suitable for our laboratory. The parameters tested to optimize this protocol compared to that of Piccolo et al., (2012) are listed in the table below (Table 1).

Table 1. Optimized Parameters for the Protocol proposed by Piccolo et al., (2012).

Step	Original protocol (Piccolo et al., 2012).	Modifications
Sample Preparation	200-300 mg of leaf powder frozen at (-80°C).	100 mg of dried leaf powder stored at room temperature.
Washing Step	No washing applied.	Washing with a sorbitol-based solution (100 mM Tris-HCl (pH 8.0), 0.35 M Sorbitol, 5 mM EDTA (pH 8.0), 1% PVP, 1% β-ME), washed 1 to 3 times (Inglis et al., 2018; Lucas et al., 2019).
Lysis buffer	700 µl of lysis buffer.	1 ml of lysis buffer.
	2% CTAB.	3% CTAB with the addition of 0.5% Tween 20 (Xin et al., 2003).
	25 mM EDTA (pH 8,0).	50 mM EDTA (pH 8,0).
	2,5 M NaCl.	2,7M NaCl.
	/	Addition of 3 µl Proteinase K (20 mg/ml) (Varma et al., 2007) and 2 M LiCl (Pirttilä et al., 2001).
Lysis	Incubation at 65°C for 60 minutes in a water bath, with agitation 3-4 times during incubation.	Incubation at 58°C for 60 minutes in a water bath, with continuous agitation.

Purification	Single wash with chloroform: isoamyl alcohol (24:1).	Wash twice with chloroform: isoamyl alcohol (24:1) (Demeke et Jenkins, 2010).
DNA Precipitation	The samples were mixed and incubated at -20°C for 30 minutes.	Incubation at -20°C overnight (Scaldaferri et al., 2013).
DNA Resuspension	200 µl of TE buffer.	60 µl of double-distilled water.

The optimized protocol:

1. Place 100 mg of dried leaf powder into a 1.5 ml Eppendorf tube containing 1 ml of washing buffer composed of 100 mM Tris-HCl (pH 8.0), 0.35 M sorbitol, 5 mM EDTA (pH 8.0), 1% PVP, and 1% β-mercaptoethanol.
2. Vortex the tubes for 5 seconds.
3. Centrifuge the tubes at 8000 rpm for 5 minutes at room temperature, and discard the supernatant.
4. If necessary, repeat the washing until the supernatant is clear.
5. Add 1 ml of preheated lysis buffer (60-65°C) consisting of 3% CTAB, 2% PVP, 100 mM Tris-HCl (pH 8.0), 50 mM EDTA (pH 8.0), 2.7 M NaCl, 2 M LiCl, 3 µl Proteinase K (20 mg/ml), 1% β-mercaptoethanol, and 0.5% Tween 20.
6. Mix the tubes by inversion to obtain a homogeneous preparation, then incubate at 58°C for 60 minutes in a water bath with continuous horizontal agitation.
7. Centrifuge the tubes at 10,000 rpm for 10 minutes at 4°C, and gently transfer the aqueous phase to a new tube.
8. Add an equal volume of chloroform: isoamyl alcohol (24:1) and mix by inversion for 5 minutes.
9. Centrifuge the tubes at 10,000 rpm for 10 minutes at 4°C, and transfer the aqueous phase to a new tube.
10. Perform a second wash with an equal volume of chloroform: isoamyl alcohol (24:1). Mix by inversion for 5 minutes.
11. Centrifuge the tubes at 10,000 rpm for 10 minutes at 4°C, and transfer the aqueous phase to a new tube.

12. Add an equal volume of cold isopropanol (-20°C) to precipitate the DNA, mix the samples gently for a few minutes, and incubate at -20°C overnight.
13. Centrifuge the precipitated DNA at 14,000 rpm for 10 minutes at 4°C, and discard the supernatant.
14. Wash the DNA pellet with 500 µl of cold 70% ethanol (-20°C), and centrifuge the tubes at 14,000 rpm for 5 minutes at 4°C.
15. Air-dry the resulting DNA pellet for 15 minutes, and dissolve it in 60 µl of double-distilled water.
16. Store the extracted DNA at -20°C.

The integrity and presence of the extracted DNA were verified by electrophoresis on a 1% agarose gel. The concentration and purity were then determined by spectrophotometry.

The yield was calculated as the ratio between the quantity of DNA obtained and the initial mass used (Bienvenu et al., 1999).

Cost of extraction methods

The total cost of extraction for 100 mg of sample was estimated by calculating the sum of the prices of the quantity of each product used for a 100 mg sample. The cost per extraction was compared to that of a GenElute™-E Single Spin Plant DNA kit (SIGMA)

3. Results

DNA obtained before optimization

The application of the protocol proposed by Piccolo et al., (2012) to our samples resulted in no detectable DNA, as confirmed by electrophoresis on a 1% agarose gel (Fig. 1). The challenges encountered with this protocol included the formation of a viscous paste after adding the lysis buffer and the complete absence of an aqueous phase after centrifugation in some tubes, or a minimal amount of aqueous phase in others (step 7).

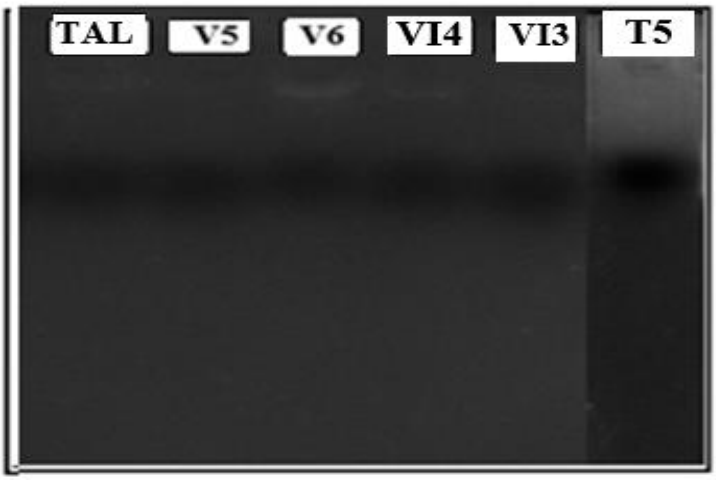

Fig.1: Electrophoretic profile of genomic DNA obtained after applying the protocol of Piccolo et al. (2012). A 1% agarose gel electrophoresis was performed, followed by BET staining and visualization under UV light. The wells TAL, V5, V6, VI4, VI 3 and T5 represent the samples used.

DNA obtained after optimization

Based on the electrophoretic profile (Fig. 2), a stronger signal was observed with samples V5, T5 and V6. However, weaker signal was detected for sample VI4, which can be explained by an error during the loading of this well.

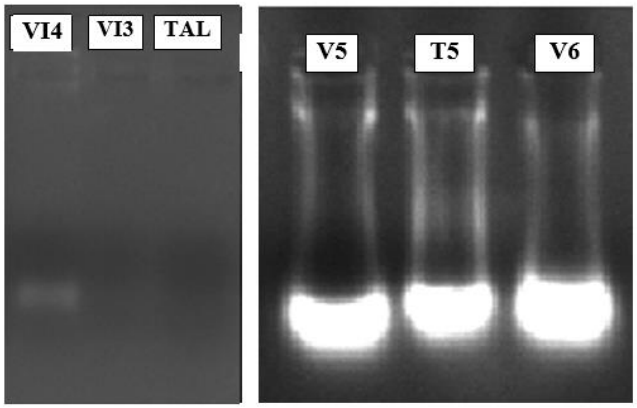

Fig.2: Electrophoretic profile of DNA obtained after applying the optimized protocol. A 1% agarose gel electrophoresis was performed, followed by BET staining and visualization under UV light. The wells VI4, VI3, TAL, V5, T5 and V6 represent the samples stored at room temperature.

According to Table (1), this protocol yields an average DNA concentration of 3975 µg/ml, a total average mass of 238.50 µg and a good extraction efficiency of 2385 µg/g.

Table 1. Average concentration, mass, and yield of DNA extracted using the optimized protocol.

Sample	DNA concentration (µg/ml)	Total DNA mass (µg)	Extraction yield (µg of DNA per g of leaf)
T5	3835	230,1	2301
V5	2520	151,2	1512
V6	5570	334,2	3342
Mean	**3975**	**238,5**	**2385**

According to the results in Table (2), the extracted DNA is of good purity for all samples (T5, V5, and V6).

Table 2. Determination of DNA purity.

Sample	A_{260nm}/A_{280nm}	$A_{260nm}/A_{230\ nm}$	Purity indication
T5	1,93	2,1	Good purity of DNA
V5	1,89	2,00	
V6	1,89	1,95	

4. Discussion

DNA extraction from plants presents particular challenges. Plants contain secondary metabolites that promote DNA degradation, making the samples viscous and the DNA non-amplifiable. Additionally, plants have cell walls that require specific techniques to be disrupted (Hanania et al., 2004). Various methods exist for grinding plant tissues, but to achieve a good particle size without altering the tissues, grinding in a mortar with liquid nitrogen is the most reliable method. However, in our laboratory, liquid nitrogen is unavailable, and its transport is difficult. Therefore, we optimized a simpler and more cost-effective grinding method that produces a fine powder without subjecting the sample to freeze/thaw cycles. To prevent contamination and DNA degradation due to humidity, silica gel is used to preserve the samples for several months at room temperature.

Based on the results obtained, it appears that the leaf drying method in the oven is very effective and allowed for long-term preservation of the samples. Before starting the extraction, we added a washing step with a sorbitol buffer. According to studies by Henry, (2008); Russell

et al., (2010); Inglis et al., (2018); Lucas et al., (2019), the sorbitol-based washing buffer helped remove mucilaginous polysaccharides present in plant tissues and also inhibited polyphenol oxidases. Polyphenols and polysaccharides are major contaminants in DNA isolation procedures. Polysaccharides are difficult to remove, and their presence in large quantities makes the tissue homogenate very viscous, giving a false indication of high DNA quantities. They often co-precipitate with DNA, resulting in a sticky and viscous consistency. In this form, they also interfere with the activity of several biological enzymes such as polymerases, ligases, and restriction endonucleases (Varma et al., 2007; Japelaghi et al., 2011).

Polyphenols can cause degradation of genomic DNA and are highly variable in their occurrence and type. During cell lysis, they escape from vacuoles and are easily oxidized by cellular oxidases. These oxidized polyphenols interact irreversibly with nucleic acids (Khanuja et al., 1999; Japelaghi et al., 2011).

In this study, we used the cationic detergent CTAB, which is an excellent detergent. It enhances the solubility of the cell membrane and disrupts the lipid bilayer proteins, thereby releasing nucleic acids (Surzycki, 2000; Palomera-Avalos et al., 2008). Additionally, increasing its concentration to 3% along with a high concentration of sodium chloride (2.7 M) leads to a significant improvement in DNA purity. The use of CTAB with a high salt concentration helps to reduce the co-precipitation of polysaccharides (Hanania et al., 2004; Hasan et al., 2008; Henry, 2008; Marsal et al., 2011; Marsal et al., 2013).

The addition of another detergent, Tween 20 (0.5%), a nonionic detergent, will aid in membrane lysis and the removal of membrane-associated proteins (Xin et al., 2003). As a third modification to enhance cell lysis, we added 3 µl of proteinase K, a nonspecific endoprotease used to denature proteins through proteolytic cleavage, which breaks peptide bonds (Varma et al., 2007).

To eliminate RNA, we added 2M lithium chloride (LiCl). According to studies by Lefort and Douglas, (1999) and Pirttilä et al., (2001), the presence of LiCl in the extraction buffer effectively removes RNA, which is considered a contaminant in genomic DNA isolation. RNA can suppress PCR amplification and inhibit downstream applications (Jobes et al., 1995). This selective precipitation by LiCl offers advantages over RNase treatment used in reference protocols, as it eliminates RNA rather than simply degrading it into smaller units (Ribeiro and Lovato, 2007) and is also more cost-effective (Jobes et al., 1995).

Time and temperature of incubation are crucial for obtaining a large amount of high-quality DNA (Thomson, 1995). Therefore, we performed this step for one and a half hours. The study by Bokszczanin and Przybyla, (2006) suggests that increasing the incubation time from one hour to one and a half hours promotes optimal action of the lysis buffer on cell membranes.

Proteins are removed by a double wash with chloroform, which denatures protein surfaces, and β-mercaptoethanol used in the lysis buffer also helps denature proteins by breaking disulfide bonds between cysteine residues. Additionally, protease K is used for protein denaturation (Sharma et al., 2002). Isoamyl alcohol maintains phase stability after centrifugation of the deproteinized solution, aiding in obtaining a well-separated aqueous phase from the organic phase (Sharma et al., 2002).

The reference protocol (Lo Piccolo et al., 2012) used TE buffer for DNA dissolution, which contains 1 mM EDTA that can inhibit the PCR reaction (Demeke and Jenkins, 2010). We replaced this buffer with sterile bi-distilled water

DNA Integrity of Extracted Samples

Based on the electrophoretic profile of the extracted DNA (Fig. 2), the presence of a single band indicates that the DNA extracted is intact. Its integrity is maintained by a good sample preparation method, ensuring complete dehydration.

Yield and Purity of Extracted DNA

The average extraction yield needs to be calculated to compare different nucleic acid extraction techniques. This parameter is essential as it ensures that the amount of DNA obtained is sufficient for downstream applications (such as PCR), and the concentration calculation provides an idea of the signal obtained after electrophoretic migration.

According to Piccolo et al., (2012), the yield of genomic DNA extraction was very low, about 0.0045 ng/mg of frozen leaves at (-80 °C). In contrast, the results from the optimized protocol were both interesting and satisfactory, with a concentration of 3975 µg/ml and an average yield of 2385 µg/g.

Additionally, the modified protocol yielded high-purity DNA, with an average 260 nm/280 nm ratio of 1.90 ± 0.05, which is considered excellent, and a 260 nm/230 nm absorbance ratio of 1.99 ± 0.05.

Cost Estimation

The cost estimate for the optimized protocol for a single 100 mg sample is 0.39 €, compared to 0.10 € for the non-optimized protocol. Thus, 5 mg of dry leaves (equivalent to 50 extractions) costs 19.73 €. The price of the modified protocol is reasonable compared to the cost of a GenElute™-E Single Spin Plant DNA kit (SIGMA), which costs 242.00 € for 50 tests.

5. Conclusion

Our study aimed to optimize a genomic DNA extraction method for Algerian grapevine varieties, with a focus on achieving high yield and quality DNA using resources readily available in our laboratory. We compared the results obtained from our optimized protocol with those from the pre-optimization protocol.

The improved protocol shows promise in extracting genomic DNA from dehydrated grapevine leaves stored at room temperature. Our method, which employs simple preparation techniques such as oven drying and mortar grinding without liquid nitrogen, appears to be effective for the samples tested. We successfully improved the reference protocol, with satisfactory results. The optimized protocol resulted in a good DNA concentration (3975 µg/ml) and yield (2385 µg/g) with a low extraction cost (0.39 € per sample).

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. 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

2. Anđelković, M. Radovanović, B. Anđelković, A. M. and Radovanović, V. (2015). Phenolic compounds and bioactivity of healthy and infected grapevine leaf extracts from red

varieties Merlot and Vranac (*Vitis vinifera* L.). *Plant foods for human nutrition*. 70, 317-323. https://doi.org/10.1007/s11130-015-0496-3

3. Aubakirova, K. Omasheva, M. Ryabushkina, N. Tazhibaev, T. Kampitova, G. and Galiakparov, N. (2014). Evaluation of five protocols for DNA extraction from leaves of *Malus sieversii*, *Vitis vinifera*, and *Armeniaca vulgaris*. *Genetics and Molecular Research*. 13(1), 1278-1287. http://dx.doi.org/10.4238/2014.February.27.13

4. Benbouza, H. Baudoin, J.-P. and Mergeai, G. (2006). Amélioration de la méthode d'extractiond'ADN au CTAB appliquée aux feuilles de cotonnier. *Biotechnology, Agronomy, Society and Environment*. 10(2), 73-76.

5. Bienvenu, T. Meunier, C. Bousquet, S. Chiron, S. Richard, L. Gautheret-Dejean, A. Rouselle, J.-F. and Feldmann, D. (1999). Les techniques d'extraction de l'ADN à partir d'un échantillon sanguin. *Annales de biologie clinique*. 57(1), 77-84.

6. Bowers, J. E. Dangl, G. S. Vignani, R. and Meredith, C. P. (1996). Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). *Genome*. 39(4), 628-633. https://doi.org/10.1139/g96-080

7. Bokszczanin K. and Przybyla A. A. (2006). New simple and efficient method of DNA isolation from pear leaves rich in polyphenolic compounds. International Journal of Horticultural Science. 12(4):21-24.

8. Cantu, D. Walker, M. A. and Chittaranjan, K. (2019). The Grape Genome. 1er edition. Springer Nature, Switzerland, 385 p.

9. Chase, M. W. and Hills, H. H. (1991). Silica gel: An ideal material for field preservation of leaf samples for DNA studies. *Taxon*. 40(2), 215-220.

10. Dani, C. Oliboni, L. S. Agostini, F. Funchal, C. Serafini, L. Henriques, J. A. and Salvador, M. (2010). Phenolic content of grapevine leaves (*Vitis labrusca* var. Bordo) and its neuroprotective effect against peroxide damage. *Toxicology in Vitro*. 24(1), 148-153. http://dx.doi.org/10.1016/j.tiv.2009.08.006

11. Dellaporta, S. L. Wood, J. and Hicks, J. B. (1983). A plant DNA minipreparation: version II. *Plant molecular biology reporter*. 1, 19-21.

12. Demeke, T. and Jenkins, G. R. (2010). Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits. *Analytical and Bioanalytical Chemistry*. 396(6), 1977-1990.

13. Do, N. and Adams, R. P. (1991). A simple technique for removing plant polysaccharide contaminants from DNA. *BioTechniques*. 10(2), 162-164.

14. Doyle, J. J., and Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin*. 19(1):11-5.

15. 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.

16. Friar, E. A. (2005). Isolation of DNA from plants with large amounts of secondary metabolites. *Methods in enzymology*. 395, 1-12. https://doi.org/10.1016/S0076-6879(05)95001-5

17. Hamama, A. el Heit, K. Meghezzi, S. Sebki, S. Agouazi, O. and Cherfaoui, M. S. (2014). Étude des caractères ampélographiques et phyllométriques des cépages mineurs du tel Algérien. In *37th World Congress of Vine and Wine and 12th General Assembly of the OIV (Part 2)*. EDP Sciences. 6p.

18. Hanania, U. Velcheva, M. Sahar, N. and Perl, A. (2004). An improved method for isolating high-quality DNA from Vitis vinifera nuclei. *Plant Molecular Biology Reporter*. 22, 173-177.

19. Hasan, S. M. Z. Shafie, M. S. B. and Shah, R. M. (2008). Efficient method for the extraction of genomic DNA from wormwood (Artemisia capillaris). *African Journal of Biotechnology*, 7(18).

20. Henry R.J. 2008. Plant genotyping II: SNP technology. CAB International, Wallingford, UK, 296 p.

21. Inglis, P. W. Pappas, M. D. C. R. Resende, L. V. and Grattapaglia, D. (2018). Fast and inexpensive protocols for consistent extraction of high-quality DNA and RNA from challenging plant and fungal samples for high-throughput SNP genotyping and sequencing applications. *PloS one*. 13(10), e0206085. https://doi.org/10.1371/journal.pone.0206085

22. Isnard, H. (1951). La Vigne en Algérie. Etude géographique. France: Ophrys- Gap. 2: 537p.

23. Japelaghi, R. H. Haddad, R. and Garoosi, G. A. (2011). Rapid and efficient isolation of high-quality nucleic acids from plant tissues rich in polyphenols and polysaccharides. *Molecular biotechnology*. 49, 129-137. https://doi.org/10.1007/s12033-011-9384-8

24. Jobes, D. V., Hurley, D. L., & Thien, L. B. (1995). Plant DNA isolation: a method to efficiently remove polyphenolics, polysaccharides, and RNA. *Taxon*. 44(3), 379-386. https://doi.org/10.2307/1223408

25. Katterman, F. R. H. and Shattuck, V. I. (1983). An effective method of DNA isolation from the mature leaves of Gossypium species that contain large amounts of phenolic terpenoids and tannins. *Preparative Biochemistry*. 13(4), 347-359. https://doi.org/10.1080/00327488308068177

26. Khanuja, S. P. Shasany, A. K. Darokar, M. P. and Kumar, S. (1999). Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. *Plant Molecular Biology Reporter*. 17(1), 74-74.

27. Khouni, S. Laiadi, Z. Bertazzon, N. Angelini, E. and Migliaro, D. (2023). Preservation and sanitary status of Algerian grapevine germplasm: Management and improvement. *South African Journal of Botany*. 153, 346-356. https://doi.org/10.1016/j.sajb.2023.01.017

28. Laiadi, Z. Bentchikou, M. M. Bravo, G. Cabello, F. and Martínez-Zapater, J. M. (2009). Molecular identification and genetic relationships of Algerian grapevine cultivars maintained at the germplasm collection of Skikda (Algeria). Vitis. 48, 25-32.

29. Laiadi, Z. Bencharif, S. Lakhrif, Z. Bentchikou, M. M. and Mohand-Larbi, R. (2013). First ampelometric study of autochthonous grapevines in Algeria: Germplasm collection of Mascara. *Vitis*. 52(1), 21-27. https://doi.org/10.5073/VITIS.2013.52.21-27

30. Lefort, F., and Douglas, G. C. (1999). An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species Acer, Fraxinus, Prunus and Quercus. *Annals of Forest Science*. 56(3), 259-263.

31. Levadoux, L. Benabderrabou, A. and Douaouri, B. (1971). Ampelographie algerienne; cepages de cuve et de table cultives en Algerie. SNED.

32. Lewinsohn, E. Steel, C. I. and Croteau, R. (1994). Simple isolation of functional RNA from woody stems of gymnosperms. *Plant Molecular Biology Reporter*. 12, 20-25.

33. Lodhi, M. A. Ye, G. N. Weeden, N. F. and Reisch, B. I. (1994). A simple and efficient method for DNA extraction from grapevine cultivars and Vitis species. *Plant molecular biology Reporter*. 12, 6-13.

34. Lucas, M. S., Carvalho, C. D. S., Hypolito, G. B., & Côrtes, M. C. (2019). Optimized protocol to isolate high quality genomic DNA from different tissues of a palm species. *Hoehnea*. 46(02), e942018. https://doi.org/10.1590/2236-8906-94/2018

35. Marsal, G. Baiges, I. Canals, J. M. Zamora, F. and Fort, F. (2011). A fast, efficient method for extracting DNA from leaves, stems, and seeds of *Vitis vinifera* L. *American journal of enology and viticulture*. 62(3), 376-381. https://doi.org/10.5344/ajev.2011.10082

36. Marsal, G. Boronat, N. Canals, J. M. Zamora, F. and Fort, F. (2013). Comparison of the efficiency of some of the most usual DNA extraction methods for woody plants in different tissues of *Vitis vinifera* L. *OENO One*. 47(4), 227-237.

37. Migliaro, D. Morreale, G. Gardiman, M. Landolfo, S. and Crespan, M. (2013). Direct multiplex PCR for grapevine genotyping and varietal identification. *Plant Genetic Resources*. 11(2), 182-185. https://doi.org/10.1017/S1479262112000433

38. Nunes, C. F. Ferreira, J. L. Fernandes, M. C. N. Breves, S. Generoso, A. L. Soares, B. D. F. Dias, M. S. C., Pasqual, M. Borem, A. and Cançado, G. M. (2011). An improved method for genomic DNA extraction from strawberry leaves. *Ciência Rural*. 41(8), 1383-1389.

39. Öner, T. Ö. Temel, M. Pamay, S. Abacı, A. K. and Akkale, H. B. K. (2022). An Improved Method for Efficient DNA Extraction from Grapevine. *International Journal of Life Sciences and Biotechnology*. 6(1), 21-36. https://doi.org/ 10.38001/ijlsb.1150387

40. Palomera-Avalos, V. Castro-Felix, P. and Villalobos-Arambula, A. R. (2008). High yield and high-quality DNA from vegetative and sexual tissues of Mexican white pine (Pinus ayacahuite). *African Journal of Biotechnology*. 7(1). 51-54.

41. Pandey, R. N., Adams, R. P., & Flournoy, L. E. (1996). Inhibition of random amplified polymorphic DNAs (RAPDs) by plant polysaccharides. *Plant Molecular Biology Reporter*, *14*, 17-22.

42. Piccolo S.L., Alfonzo A., Conigliaro G., Moschetti G., Burruano S. et Barone A. 2012. A simple and rapid DNA extraction method from leaves of grapevine suitable for polymerase chain reaction analysis. *African journal of Biotechnology*. 11(45):10305-10309.

43. Pirttilä, A. M. Hirsikorpi, M. Kämäräinen, T. Jaakola, L. and Hohtola, A. (2001). DNA isolation methods for medicinal and aromatic plants. *Plant Molecular Biology Reporter*, *19*, 273-273. https://doi.org/10.1007/BF02772901

44. 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.

45. Rathnayake, A. S., Allué, J., Llugany, M., Puig-Pujol, A., Hirimburegama, K., & Poschenrieder, C. (2014). High quality DNA obtained from a single seed of Vitis vinifera L. using rapid DNA extraction method. *American Journal of Plant Sciences*. 5, 2023-2030. http://dx.doi.org/10.4236/ajps.2014.513217

46. Ribeiro, R. A. and Lovato, M. B. (2007). Comparative analysis of different DNA extraction protocols in fresh and herbarium specimens of the genus Dalbergia. *Genetics and Molecular Research*. 6(1), 173-187.

47. Russell, A. Samuel, R. Rupp, B. Barfuss, M. H. Šafran, M. Besendorfer, V. and Chase, M. W. (2010). Phylogenetics and cytology of a pantropical orchid genus Polystachya (Polystachyinae, Vandeae, Orchidaceae): evidence from plastid DNA sequence data. *Taxon*. 59(2), 389-404.

48. Rahali, M. Migliaro, D. Laiadi, Z. Bertazzon, N. Angelini, E. and Crespan, M. (2019) Genetic identification, origin and sanitary status of grapevine cultivars (*Vitis vinifera* L.) grown in Babar, Algeria. *Vitis*. 58, 153-158. https://doi.org/10.5073/vitis.2019.58.153-158

49. Sahu, S. K. Thangaraj, M. and Kathiresan, K. (2012). DNA Extraction Protocol for Plants with High Levels of Secondary Metabolites and Polysaccharides without Using Liquid Nitrogen and Phenol. *International Scholarly Research Network Molecular Biology*. 1-6.

50. Scaldaferri, M. M. Freitas, J.S. Santos, E. S. L. Vieira, J. G. P., Gonçalves, Z. S. and Cerqueira-Silva C. B. M. (2013). Comparison of protocols for genomic DNA extraction fromvelame pimenta (*Croton linearifolius*), a native species to the Caatinga, Brazil. *African Journal of Biotechnology*. 12(30), 4761-4766.

51. Schori, M. Appel, M. Kitko, A. and Showalter, A. M. (2013). Engineered DNA polymerase improves PCR results for plastid DNA. *Applications in Plant Sciences*. 1, 1-7.

52. Scobeyeva, V. A. Omelchenko, D. O. Dyakov, L. M. Konovalov, A. S. Speranskaya, A. S. and Krinitsina, A. A. (2018). Comparison of some plant DNA extraction methods. *Russian Journal of Genetics*. 54, 576-586.

53. Sharma A.D., Gill P.K., Singh P. (2002). DNA isolation from dry and fresh samples of polysaccharide-rich plants. Plant Molecular Biology Reporter. 20(4):415–415.

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

55. Surzycki, S. (2012). *Basic techniques in molecular biology*. Springer Science & Business Media. 444p.

56. This, P. Jung, A. Boccacci, P. Borrego, J. Botta, R. Costantini, L. Crespan, M. Dangl, G.S. Eisenheld, C. Ferreira-Monteiro, F. Grando, S. Ibáñez, J. Lacombe, T. Laucou, V. Magalhães, R. Meredith, C.P. Milani, N. Peterlunger, E. Regner, F. Zulini, L. and Maul, E.

(2004). Development of a standard set of microsatellite reference alleles for identification of grape cultivars. *Theoritical and Applied Genetics*. 109, 1448-1458. https://doi.org/10.1007/s00122-004-1760-3.

57. Thomson, D. and Henry, R. (1995). Single-step protocol for preparation of plant tissue for analysis by PCR. *Biotechniques*. 19(3), 394-7.

58. Varma, A. Padh, H. and Shrivastava, N. (2007). Plant genomic DNA isolation: an art or a science. *Biotechnology Journal: Healthcare Nutrition Technology*. 2(3), 386-392. https://doi.org/10.1002/biot.200600195

59. Wang, T. Y. Wang, L. Zhang, J. H. and Dong, W. H. (2011). A simplified universal genomic DNA extraction protocol suitable for PCR. *Genetics and Molecular Research*. 10(1), 519-525. https://doi.org/10.4238/vol10-1gmr1055

60. Xin, Z. Velten, J. P. Oliver, M. J. and Burke, J. J. (2003). High-throughput DNA extraction method suitable for PCR. *Biotechniques*. 34(4), 820-826. https://doi.org/10.2144/03344rr04