Akademik Ziraat Dergisi 14(1): 65-73 (2025)
ISSN: 2147-6403 e-ISSN: 2618-5881 DOI: http://dx.doi.org/10.29278/azd.1689058

Araştırma
(Research)

Determination of the Effects of Different DNA Isolation Methods on Quantity and Quality

Çağlar KAYA[1*], Esra ŞAHİN[1]

[1]Çanakkale Onsekiz Mart Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Çanakkale/TÜRKİYE

Alınış tarihi: 1 Mayıs 2025, Kabul tarihi: 16 Haziran 2025
Sorumlu yazar: Çağlar KAYA, e-posta: ckaya@stu.comu.edu.tr

Abstract

Objective: This study compared the effects of different DNA extraction methods (CTAB, phenol-chloroform, silica columns, magnetic beads) on the DNA yield and purity obtained from maize (*Zea mays* L.), bean (*P. vulgaris* L.), oak (*Q. robur* L.) and scots pine (*P. sylvestris* L.).

Materials and Methods: In this study, young apical leaves (the most distal fully expanded leaves) of maize, bean, oak and scots pine were used. For each species, 10 leaf tissue samples were collected from young apical leaves during the vegetation season of the year 2024 from plantations located at different sites within the Bayramiç district of Çanakkale province. Leaf tissue samples were collected during the vegetation season of the year 2024, specifically during the early phenological stages of each species. For maize and bean, samples were obtained in the early vegetative growth phase, when the apical leaves were fully expanded but still young and metabolically active. For oak and Scots pine, sampling was conducted during the early spring, shortly after bud burst, when newly emerged apical leaves were in the initial phase of leaf expansion. Young apical leaves were preferred as they contain fewer secondary metabolites and phenolic compounds, which can interfere with DNA extraction and downstream molecular applications. This sampling strategy ensured the collection of high-quality tissue suitable for genomic DNA isolation. DNA isolation was performed using four different methods: CTAB, phenol-chloroform, silica-based columns, and magnetic beads. These methods were compared in terms of their suitability for genetic analyses specific to each plant species.

Results: Using the phenol-chloroform method, a notably high DNA yield was obtained from *P. sylvestris* L. samples (371.75 ng/µL). Similarly, *Q. robur* L. also exhibited high yield (352.00 ng/µL), suggesting that these species are particularly compatible with this method. The CTAB method also yielded successful results. *P. sylvestris* L. achieved a high DNA yield (323.75 ng/µL), indicating that this species' genetic material is compatible with the CTAB method. A DNA yield of 308.00 ng/µL was observed for *Q. robur* L., which may necessitate additional steps for protein removal. The silica column method yielded DNA samples of a lower quantity than the other three methods. For *P. sylvestris* L. and *Q. robur* L., yields of (230.75 ng/µL) and (222.25 ng/µL), respectively, were recorded. Lower yields were observed in *P. vulgaris* L. (171.75 ng/µL) compared to *Zea mays* L. (211.75 ng/µL). The diminished DNA recovery may be attributable to the constrained binding capacity of silica columns or inadequate initial material. In the magnetic beads method, DNA yields for *P. sylvestris* L. (206.25 ng/µL), *Q. robur* L. (201.75 ng/µL), *Zea mays* L. (173.50 ng/µL) and *P. vulgaris* L. (151.75 ng/µL) were the lowest among all methods.

Conclusion: This study compares DNA isolation methods including CTAB, phenol-chloroform, silica columns, and magnetic beads for *Zea mays* L., *P. vulgaris* L., *Q. robur* L., and *P. sylvestris* L. The findings reveal that the differences in DNA yields and purity depend on the plant species and tissue characteristics. The phenol-chloroform method provided the highest DNA yields and purity across all plant species. This method excels in obtaining DNA free from proteins, polysaccharides, and phenolic compounds, ensuring high molecular integrity. The results demonstrate that *Q. robur* L. and *P. sylvestris* L., which are rich in lignin and phenolic compounds, had the highest yields and preserved molecular integrity. This supports the use of the phenol-

Kaya, Ç. & Şahin, E. (2025). Determination of the Effects of Different DNA Isolation Methods on Quantity and Quality. *Akademik Ziraat Dergisi*, 14(1), 65-73.

chloroform method in molecular analyses requiring high-quality DNA.

Keywords: DNA isolation, CTAB, phenol-chloroform, silica columns, magnetic beads

Farklı DNA İzolasyon Yöntemlerinin Miktar ve Kalite Üzerine Etkilerinin Belirlenmesi

Öz

Amaç: Bu çalışmanın amacı, farklı DNA ekstraksiyon yöntemlerinin (CTAB, fenol-kloroform, silika kolonlar, manyetik boncuklar) mısır (*Zea mays* L.), fasulye (*P. vulgaris* L.), meşe (*Q. robur* L.) ve sarıçamdan (*P. sylvestris* L.) elde edilen DNA verimi ve saflığı üzerindeki etkilerinin karşılaştırılmasıdır.

Materyal ve Yöntem: Bu çalışmada, mısır, fasulye, meşe ve sarıçam türlerine ait genç apikal yapraklar (bitkinin en uç kısmındaki tam olarak gelişmiş yapraklar) kullanılmıştır. Her bir türden, 2024 vejetasyon dönemi süresince Çanakkale ilinin Bayramiç ilçesinde yer alan farklı lokasyonlarındaki bitki plantasyonlarına ait genç apikal yapraklardan 10 adet yaprak doku örneği toplanmıştır. DNA izolasyonu, CTAB, fenol-kloroform, silika bazlı kolonlar ve manyetik boncuklar olmak üzere dört farklı yöntem kullanılarak gerçekleştirilmiştir. Bu yöntemler, her bir bitki türü için genetik analizlere uygunlukları açısından karşılaştırılmıştır.

Bulgular: Fenol-kloroform yöntemi kullanılarak *P. sylvestris* L. örneklerinden oldukça yüksek düzeyde DNA verimi elde edilmiştir (371.75 ng/µL). Benzer şekilde, *Q. robur* L. türü de yüksek bir DNA verimi (352.00 ng/µL) göstermiştir. Bu durum, her iki türün de özellikle bu yöntemle yüksek uyum sağladığını ortaya koymaktadır. CTAB yöntemi de başarılı sonuçlar vermiştir. *P. sylvestris* L., bu yöntemle 323.75 ng/µL DNA verimi göstererek CTAB yöntemine genetik materyal açısından oldukça uyumlu olduğunu kanıtlamıştır. *Q. robur* L. için elde edilen DNA verimi ise 308.00 ng/µL olup, bu sonuç protein uzaklaştırma için ek adımlar gerekebileceğine işaret etmektedir. Silika kolon yöntemi ile elde edilen DNA verimleri, genellikle diğer üç yöntemle karşılaştırıldığında daha düşük kalmıştır. Bu yöntemde, *P. sylvestris* L. için 230.75 ng/µL, *Q. robur* L. için ise 222.25 ng/µL DNA verimi kaydedilmiştir. *Zea mays* L. türünde 211.75 ng/µL DNA verimi gözlenirken, *P. vulgaris* L. türünde bu değer 171.75 ng/µL olarak ölçülmüştür. Düşük DNA verimi, silika kolonlarının sınırlı bağlama kapasitesinden ya da yetersiz başlangıç materyalinden kaynaklanmış olabilir. Manyetik boncuk yöntemi ile elde edilen DNA verimleri ise tüm yöntemler arasında en düşük seviyelerde kalmıştır. Bu yöntemde; *P. sylvestris* L. için 206.25 ng/µL, *Q. robur* L. için 201.75 ng/µL, *Zea mays* L. için 173.50 ng/µL ve *P. vulgaris* L. için 151.75 ng/µL DNA verimleri kaydedilmiştir.

Sonuç: Bu çalışmada *Z. mays* L., *P. vulgaris* L., *Q. robur* L. ve *P. sylvestris* L. türleri için CTAB, fenol-kloroform, silika kolonlar ve manyetik boncuklar gibi farklı DNA izolasyon yöntemleri karşılaştırılmıştır. Elde edilen bulgular, DNA verimi ve saflığındaki farklılıkların bitki türlerine ve doku özelliklerine bağlı olduğunu ortaya koymaktadır. Tüm bitki türlerinde en yüksek DNA verimi ve saflığı fenol-kloroform yöntemi ile elde edilmiştir. Bu yöntem proteinler, polisakkaritler ve fenolik bileşiklerden arındırılmış DNA izolasyonu sağlayarak yüksek moleküler bütünlükte DNA elde edilmesine olanak tanımaktadır. Sonuçlar, lignin ve fenolik bileşikler açısından zengin olan *Q. robur* L. ve *P. sylvestris* L. türlerinin en yüksek DNA verimine sahip olduğunu ve moleküler bütünlüğün korunduğunu göstermektedir. Bu durum, yüksek kaliteli DNA gerektiren moleküler analizler için fenol-kloroform yönteminin tercih edilmesini desteklemektedir.

Anahtar kelimeler: DNA izolasyonu, CTAB, fenol-kloroform, silika kolonları, manyetik boncuklar

Introduction

DNA isolation from plant cells represents a fundamental step in numerous molecular biology and genetic disciplines, including genetic analyses, biotechnological applications, and plant breeding. However, the complex cellular architecture, presence of secondary metabolites, and genetic diversity in plants pose significant challenges during this process (Murray and Thompson, 1980; Doyle and Doyle, 1987; Du et al., 2024). Polysaccharides, phenolic compounds, lignin, and other secondary metabolites found in plants frequently hinder the DNA isolation process (Fopp-Bayat, 2024). Therefore, selecting the appropriate DNA isolation method is critical for the success of genetic analyses. Given the variability in cellular structures and metabolic profiles among plant species, the most suitable isolation method should be determined for each species (Dogdu, 2025).

Woody plants, such as oak and pine, often present additional difficulties in DNA isolation due to their lignin and cellulose content, which are challenging to break down (Guldur et al., 2024). It is well-documented that phenolic compounds in woody plants may complicate DNA isolation, even when using conventional methods like phenol-chloroform or CTAB. Intensive purification steps may be required to counteract the effects of secondary metabolites in species such as pine and oak (Rehman et al., 2024).

In contrast, plant species with thinner cell walls, such as monocotyledons (e.g., maize) and dicotyledons (e.g., beans), generally allow faster and more efficient DNA isolation (Cetinkaya and Aysan, 2022). These species typically yield higher DNA quantities and purity, providing valuable data for comparative studies. However, factors such as the efficiency of protocols used, the purity of isolated DNA, and PCR success rates can vary significantly depending on the plant species (Dutta et al., 2024).

Commonly used protocols for plant DNA isolation include phenol-chloroform extraction, the CTAB (Cetyltrimethylammonium Bromide) method, silica column-based techniques, and more recently, magnetic bead-based isolations (Deka, 2024). Although the phenol-chloroform method ensures high yield and purity, its use is limited by the involvement of toxic chemicals and time-intensive procedures (Dogdu, 2025). The CTAB method is particularly effective in removing phenolic compounds from woody plants; however, secondary metabolites can still impact yield in some species (Kiy and Akpulat, 2024). While silica column-based methods stand out for their practicality, they often result in lower yields (Akbulut, 2024). Magnetic beads offer a rapid and straightforward alternative but typically produce lower purity and yield (Rezvantseva et al., 2024).

Comparing the efficiency of these different DNA isolation methods is crucial for identifying the most suitable protocols for each plant species. Key parameters, such as DNA purity, quality, and yield, are essential for genetic analyses (Jablonski et al., 2024). Low-yield or low-purity DNA samples can significantly compromise the accuracy of genetic analyses (Tatar et al., 2024). By evaluating DNA isolation across various plant species, it becomes possible to determine the most efficient and appropriate method for each, directly enhancing the success of genetic studies. This study aims to identify the most effective DNA isolation methods for obtaining high-yield, pure, and high-quality DNA from various plant species, and to contribute to the optimization of these protocols for genetic analyses. Such research will facilitate more reliable and accurate outcomes in plant biotechnology, genetic engineering, and plant breeding.

Material and Method

In this study, young apical leaves (the most distal fully expanded leaves) of maize (*Zea mays* L.), bean (*P. vulgaris* L.), oak (*Q. robur* L.) and Scots pine (*P. sylvestris* L.) were used as the source of plant material. Young leaves were preferred because they typically contain fewer secondary metabolites and phenolic compounds, which can interfere with DNA extraction and downstream applications.

For each species, 10 leaf tissue samples were collected from young apical leaves during the vegetation season of the year 2024 from plantations located at different sites within the Bayramiç district of Çanakkale province. DNA isolation was carried out using four different methods: CTAB, phenol-chloroform, silica-based columns, and magnetic beads. These extraction methods were compared to evaluate their suitability for obtaining high-quality DNA for genetic analyses specific to each plant species.

Material

The plant species used in this study included maize, bean, oak, and scots pine. These species exhibit significant diversity in terms of morphological and genetic characteristics, making them valuable for evaluating DNA isolation methods. Maize and bean are herbaceous plants with thinner and more soluble cell walls, while oak and scots pine are woody species requiring more challenging isolation processes due to their rigid cell walls and lignin content.

Healthy and young leaves were collected from all four species, frozen using liquid nitrogen, and transported to the laboratory. The frozen samples were stored at -20°C until further processing.

Method

DNA isolation was performed using four different methods: CTAB, phenol-chloroform, silica columns, and magnetic beads.

CTAB (Cetyltrimethylammonium Bromide) Method

The CTAB method is a widely used protocol for DNA isolation from plant cell walls, particularly effective in removing polysaccharides to obtain high-purity DNA. In this study, DNA extraction was carried out using the CTAB extraction protocol as described by Doyle and Doyle (1987).

The process began with leaf samples frozen in liquid nitrogen and ground into a fine powder using an OMNI vibrational grinder in 2 mL Eppendorf tubes. Young leaf tissues from the plant species were then transferred to 1.5 mL Eppendorf tubes, and 250 µL of CTAB buffer was added. After homogenization, an additional 250 µL of CTAB buffer was added, and the mixture was incubated at room temperature for 5 minutes. Subsequently, 250 µL of chloroform (IAA) was added to each tube, followed by vortexing for 1–2 minutes.

The tubes were centrifuged at 13.000 rpm for 12 minutes with proper balancing. After centrifugation, the upper phase was transferred to new Eppendorf tubes, and 0.7 volumes of isopropanol were added. The tubes were then incubated at room temperature for 5 minutes.

Following incubation, the tubes were centrifuged again at 13.000 rpm for 7 minutes, and the upper phase was discarded. The remaining pellet was washed with 1 mL of 70% ethanol. After washing, the tubes were centrifuged for another 5 minutes at 13.000 rpm. Ethanol was removed, and the pellet was allowed to air dry for 30–45 minutes.

Once completely dried, the pellet was resuspended in 50 µL of distilled water and incubated at 55°C for 5 minutes. Following this incubation, the tubes were centrifuged for 5 minutes at 13.000 rpm, and the resulting DNA solution was stored at -20°C for future use.

Phenol-Chloroform Method

The DNA isolation performed using the phenol-chloroform method starts with the addition of a lysis buffer to disrupt the cell walls of the samples. In this study, 600 µL of lysis buffer (composed of 100 mM Tris-HCl, pH 8.0, 100 mM NaCl, 10 mM EDTA, and 1% SDS) was added to the samples. Subsequently, 10 µL of Proteinase K was added, and the samples were incubated at room temperature for 1 hour to digest proteins.

Next, 600 µL of a phenol-chloroform mixture (1:1, pH 8.0) was added to the solution. Phenol dissolves proteins, while chloroform dissolves lipids. The mixture was vortexed for 10 seconds to ensure thorough mixing. The samples were centrifuged at 13.000 rpm for 10 minutes, resulting in the formation of three distinct phases; Upper phase: Contains DNA. Middle phase: Contains proteins and organelles. Lower phase: Contains the phenol and chloroform solutions.

The upper phase, containing the DNA, was carefully transferred to a new tube. To further purify the DNA, 600 µL of pure chloroform was added and mixed, ensuring the removal of any residual phenol and chloroform. The solution was centrifuged again at 13.000 rpm for 10 minutes, and the upper phase was carefully collected.

To precipitate the DNA, 600 µL of 96% ethanol was added, which facilitates DNA aggregation. After mixing, the samples were centrifuged at 12.000 rpm for 10 minutes, causing the DNA to precipitate. The DNA pellet was then washed with 70% ethanol, vortexed briefly, and centrifuged again. The ethanol supernatant was carefully removed, and the DNA pellet was air-dried for approximately 10–15 minutes.

To dissolve the DNA, 100 µL of sterile water was added, and the solution was incubated for a few minutes. The obtained DNA samples were stored at -20°C for long-term use.

Silica Column Method

he silica column method is an effective DNA isolation technique where DNA binds to the silica surface, allowing for its purification. Initially, 600 µL of lysis buffer was added to the homogenized plant tissue. The homogenized sample was incubated for approximately 30 minutes in a refrigerator. Prior to incubation, Proteinase K was added to the sample, with the aim of breaking down the cell membranes and releasing the DNA molecule. To avoid harsh physical effects, the lysis was carried out gently.

The lysis solution was then centrifuged at 13.000 rpm for 10 minutes. After centrifugation, the upper phase (supernatant) was carefully transferred to a new tube. This supernatant was then added to a silica column. The column and supernatant were mixed and centrifuged at 8.000 rpm for approximately 1 minute. During this step, the DNA binds to the silica surface.

After DNA binding, the column was washed using a washing buffer and centrifuged again. This washing step was repeated three times to remove contaminants and other unwanted components that were bound to the DNA. After washing, a brief centrifugation was performed to remove any remaining liquid from the column.

The DNA was eluted from the column using an elution buffer. The column was treated with 100 µL of elution buffer and incubated for 1 minute. Finally, the column was centrifuged to collect the DNA-containing elution solution.

Magnetic Beads Method

The magnetic beads method is a rapid DNA isolation technique where DNA binds to magnetic beads, enabling purification. In this method, 600 µL of lysis buffer was added to the sample. The lysis buffer contained Tris-HCl (50 mM, pH 8.0), NaCl (100 mM), EDTA (10 mM), and SDS (0.5%). Next, 10 µL of Proteinase K was added to break down proteins. The samples were incubated at room temperature for approximately 1 hour.

After the incubation, 50 µL of magnetic beads were added to the solution and mixed thoroughly for 5 minutes. A magnetic field was applied, drawing the DNA-bound magnetic beads to the side of the tube. No centrifugation was used in this step; instead, the beads were separated under the influence of the magnetic field.

Next, 500 µL of wash buffer was added to the sample. The wash buffer contained 75% ethanol and 10 mM Tris-HCl (pH 8.0). Washing was carried out by centrifuging at 8.000 rpm for 1 minute, and this washing step was repeated three times.

Subsequently, 100 µL of sterile water and 10 mM Tris-HCl solution were added. The solution was mixed for 1 minute, after which the magnetic beads were separated using the magnetic field. Finally, an elution buffer was added, and the solution was centrifuged at 8.000 rpm for 1 minute to collect the DNA-containing supernatant.

DNA Purity and Yield Measurements

The purity of the isolated DNA samples was assessed using a UV-Vis spectrophotometer (Brand: Shimadzu-Model: UV- 1800) by measuring the absorbance at 260 nm and 280 nm wavelengths. Each sample was measured in triplicate to ensure accuracy and reproducibility. The purity of DNA was evaluated by calculating the A260/A280 ratio, where a ratio between 1.8 and 2.0 is generally considered indicative of high-quality, protein-free DNA (Torres et al., 2021). Ratios below this range may suggest protein contamination, while higher ratios can indicate RNA contamination.

DNA yield was quantified by determining the concentration of DNA (ng/µL) from the absorbance at 260 nm, multiplied by the elution volume to calculate the total amount of DNA obtained from each isolation method. This allowed for comparison of the efficiency of different extraction protocols.

Preparation of Agarose Gel and Genomic DNA Gel Electrophoresis

The genomic DNA (gDNA) samples were subjected to electrophoresis on a 1% agarose gel. For this purpose, 1 gram of agarose was weighed using an analytical balance and added to 100 mL of 1x TBE buffer in an Erlenmayer flask. The mixture was heated in a microwave until the agarose was completely dissolved. Once the agarose was fully dissolved, the solution was allowed to cool. After cooling, 1 µL of ethidium bromide (EtBr) was added to the solution. EtBr intercalates between the DNA bases, making the DNA visible under UV light.

The agarose solution was then poured into the electrophoresis tank. Prior to pouring, combs were placed at the top of the tank to create wells for loading DNA samples. After pouring the agarose solution into the tank, it was allowed to polymerize for approximately 30 minutes. Once the gel had solidified, the combs were carefully removed, and the gel was ready for electrophoresis.

The prepared agarose gel was placed into the electrophoresis tank filled with 1x TBE running buffer. Prior to loading, DNA samples were mixed with 6x loading dye in a ratio of 5:1 (5 µL DNA sample + 1 µL loading dye). A total volume of 6 µL of this mixture was carefully loaded into each well of the gel. A 1 kb DNA ladder (Thermo Scientific, GeneRuler 1 kb DNA Ladder) was used as a molecular size marker and 5 µL of ladder was loaded into a separate well.

Electrophoresis was carried out at a constant voltage of 100 V for approximately 45 minutes. After electrophoresis, the gel was visualized under UV light using a gel documentation system to assess the integrity and size of the extracted genomic DNA samples.

Statistical Analysis

For each plant species and each DNA extraction method, three independent replicates were performed. The obtained DNA yield and purity values were statistically analyzed using one-way analysis of variance (ANOVA) to evaluate differences among the extraction methods. Prior to conducting ANOVA, the assumptions of normality and homogeneity of variances were tested. The Shapiro–Wilk test was used to assess the normality of the data distribution, and the results indicated that the data followed a normal distribution. Levene's test was employed to evaluate the homogeneity of variances, and the assumption was met. When statistically significant differences ($p < 0.05$) were detected by ANOVA, Tukey's Honest Significant Difference (HSD) post-hoc test was applied to determine which specific groups differed from each other. All statistical analyses were carried out using SAS software version 9.3.1.

Results and Discussion

The study evaluated and compared four different DNA isolation methods (CTAB, phenol-chloroform, silica columns, and magnetic breads) applied to four plant species (*Zea mays* L., *P. vulgaris* L., *Q. robur* L. and *P. sylvestris* L.). The results demonstrated that DNA yield and purity significantly varied depending on the plant species and the isolation method employed.

Using the phenol-chloroform method, a notably high DNA yield was obtained from *P. sylvestris* L. samples (371.75±2.36 ng/μL). Similarly, *Q. robur* L. also exhibited high yield (352.00±2.16 ng/μL), suggesting that these species are particularly compatible with this method. The high yield can be attributed to the effective removal of phenolic compounds and the preservation of DNA purity. Likewise, the method produced effective results for *Z. mays* L. (281.75±2.36 ng/μL) and *P. vulgaris* L. (251.75±2.36 ng/μL) (Table 1).

The CTAB method also yielded successful results. *P. sylvestris* L. achieved a high DNA yield (323.75±4.78 ng/μL), indicating that this species' genetic material is compatible with the CTAB method. For *Q. robur* L., a DNA yield of (308.00±5.41 ng/μL) was observed. However, in species such as *P. vulgaris* L. (214.75±5.25 ng/μL) and *Zea mays* L. (254.50±4.79 ng/μL), DNA yields were significantly lower compared to the phenol-chloroform method. This disparity suggests that the CTAB method may be less efficient in tissues with high protein and polysaccharide content, potentially necessitating additional steps for protein removal (Table 1).

DNA yields obtained using the silica column method were generally lower than those of the other three methods. For *P. sylvestris* L. and *Q. robur* L., yields of (230.75±0.95 ng/μL) and (222.25±2.06 ng/μL), respectively, were recorded. Lower yields were observed in *P. vulgaris* L. (171.75±2.36 ng/μL) compared to *Zea mays* L. (211.75±2.36 ng/μL). The lower DNA recovery might be due to the limited binding capacity of silica columns or insufficient initial material (Table 1).

In the magnetic beads method, DNA yields for *P. sylvestris* L. (206.25±7.5 ng/μL), *Q. robur* L. (201.75±2.36 ng/μL), *Zea mays* L. (173.50±3.10 ng/μL), and *P. vulgaris* L. (151.75±2.36 ng/μL) were the lowest among all methods. This low yield may indicate that the magnetic beads method is less effective in tissues with high phenolic content. Additionally, DNA isolated with this method was observed to have a higher risk of contamination and breakage. Known challenges with the magnetic beads method include the incomplete removal of contaminants such as proteins, polyphenols, and polysaccharides from plant tissues. These contaminants can act as inhibitors, particularly in sensitive downstream analyses.

The amount of DNA is one of the key parameters indicating the effectiveness of the isolation method used. As shown in Table 1., DNA yields obtained for each plant species vary depending on the isolation method used. The DNA yields from *Zea mays* L., *P. vulgaris* L., *Q. robur* L., and *P. sylvestris* L. using the CTAB method generally yielded better results compared to other methods.

The phenol-chloroform method is widely used for obtaining high DNA yield and purity. The highest yields were obtained from *P. sylvestris* L. (371.75±2.36 ng/μL) and *Q. robur* L. (352.00±2.16 ng/μL). High DNA yields were also obtained from *Zea mays* L. (281.75±2.36 ng/μL) and *P. vulgaris* L. (251.75±2.36 ng/μL). These results are consistent with studies by Sahu et al. (2012) and Pandit et al. (2007), indicating that phenolic components were effectively removed and the DNA was isolated with high purity. This method proved especially effective in plant tissues with high phenolic content, as seen in *P. sylvestris* L. and *Q. robur* L., which are rich in phenolic compounds (Table 1).

The silica column method generally resulted in lower DNA yields compared to the other three methods. The highest yields were obtained from *P. sylvestris* L. (230.75±0.95 ng/μL) and *Q. robur* L. (222.25±2.06 ng/μL), but *P. vulgaris* L. (171.75±2.36 ng/μL) and *Zea mays* L. (211.75±2.36 ng/μL) showed lower yields (Table 1).

These results align with Aljanabi and Martinez (1997), who observed that silica column methods are less effective in tissues with high polysaccharide content. Although silica columns are typically used for obtaining pure DNA, some species exhibited lower yields, possibly due to the presence of polysaccharides and other compounds in the tissues that interfere with DNA isolation. Additionally, the lower yields may be attributed to the limited capacity of the columns or insufficient starting material.

Table 1. DNA yield and purity obtained from different plant DNA isolation methods*

DNA isolation methods	Plant species	DNA quantity (ng/µL)	A260/A280	A260/A230
CTAB	*Zea mays* L.	254.50±4.79[c]	1.85±0.05[b]	2.05±0.02[b]
	P. vulgaris L.	214.75±5.25[d]	1.88±0.06[a]	2.10±0.03[a]
	Quercus robur L.	308.00±5.41[b]	1.87±0.04[ab]	1.98±0.04[c]
	Pinus sylvestris L.	323.75±4.78[a]	1.85±0.05[b]	2.00±0.03[b]
Phenol-chloroform	*Zea mays* L.	281.75±2.36[c]	1.90±0.04[ab]	2.15±0.02[a]
	P. vulgaris L.	251.75±2.36[d]	1.92±0.05[a]	2.10±0.03[b]
	Quercus robur L.	352.00±2.16[b]	1.89±0.03[b]	2.10±0.03[b]
	Pinus sylvestris L.	371.75±2.36[a]	1.90±0.04[ab]	2.12±0.03[b]
Silica columns	*Zea mays* L.	211.75±2.36[c]	1.80±0.05[b]	2.00±0.03[b]
	P. vulgaris L.	171.75±2.36[d]	1.82±0.06[ab]	2.05±0.02[ab]
	Quercus robur L.	222.25±2.06[b]	1.83±0.05[a]	2.05±0.02[ab]
	Pinus sylvestris L.	230.75±0.95[a]	1.84±0.06[a]	2.08±0.01[a]
Magnetic beads	*Zea mays* L.	173.50±3.10[c]	1.78±0.06[a]	2.00±0.03[a]
	P. vulgaris L.	151.75±2.36[d]	1.75±0.07[b]	1.90±0.04[b]
	Quercus robur L.	201.75±2.36[b]	1.77±0.06[ab]	1.95±0.03[ab]
	Pinus sylvestris L.	206.25±7.5[a]	1.76±0.06[ab]	1.98±0.03[ab]

*There are statistically significant differences among plant species in terms of the releated methods ($p<0.05$).
** The A260/A280 ratio indicates protein contamination, with pure DNA typically having a ratio between 1.8 and 2.0. Lower values suggest the presence of proteins or phenol. The A260/A230 ratio provides additional information about organic compound or salt contamination; ideal values range from 2.0 to 2.2. Ratios lower than this may indicate contamination with carbohydrates, phenol, or residual guanidine.

Among the four DNA extraction methods tested CTAB, phenol-chloroform, silica columns, and magnetic beads significant differences in DNA yield and purity were observed depending on the plant species and method used (Table 1). The phenol-chloroform method consistently provided the highest DNA yield and purity, particularly in the woody species *Q. robur* (352.00 ± 2.16 ng/µL) and *P. sylvestris* (371.75 ± 2.36 ng/µL), which are known to contain high levels of lignin and phenolic compounds. These results are supported by Toader et al. (2010), who emphasized the importance of reducing PCR inhibitors, such as phenolics, through DNA dilution (1:100), especially in oak tissues, to improve amplification efficiency.

The CTAB method also demonstrated favorable results, especially in *P. sylvestris* (323.75 ± 4.78 ng/µL) and *Q. robur* (308.00 ± 5.41 ng/µL). Its effectiveness in removing polysaccharides and phenolic contaminants from woody tissues aligns with previous studies (Doyle & Doyle, 1987; Lefort and Douglas., 1999). Notably, Lefort and Douglas (1999), achieved DNA yields of up to 950 µg/g FW from mature oak leaves using a modified CTAB protocol. The use of young apical leaves in our study may have further contributed to higher yields due to their lower content of inhibitory secondary metabolites.

In contrast, the silica column method yielded significantly lower DNA concentrations across all species, with *P. vulgaris* (171.75 ± 2.36 ng/µL) and *Q. robur* (222.25 ± 2.06 ng/µL) showing particularly low values. This may be due to limited DNA binding capacity and the negative effects of secondary metabolites that interfere with the column's efficiency. Similar challenges have been reported by Aljanabi and Martinez (1997), and corroborated by Toader et al. (2010), who found commercial kits less effective in mature Quercus tissues.

The magnetic bead method produced the lowest DNA yields across all species *P. vulgaris* yielded only 151.75 ± 2.36 ng/µL accompanied by reduced A260/A280 ratios, indicating possible contamination with proteins or phenolic residues. These findings are consistent with Rezvantseva et al. (2024), who reported high fragmentation and contamination risks associated with this method, particularly in phenolic rich or structurally rigid tissues.

DNA purity assessments further highlighted the advantages of phenol-chloroform and CTAB methods. A260/A280 ratios close to the ideal range (1.8–2.0) confirmed minimal protein contamination, while phenol-chloroform also achieved superior A260/A230 values, indicating effective removal of polysaccharides and phenols critical for downstream applications like PCR.

In summary, the phenol-chloroform method emerged as the most robust and reliable across all species, particularly for woody plants requiring high-quality DNA. Although more labor-intensive, it ensures superior yield and purity. The CTAB method remains a viable and cost-effective alternative, especially when complemented with purification steps. In contrast, the silica column and magnetic bead methods showed limitations, especially in phenolic-rich species. These findings underscore the need for species-specific optimization of DNA extraction protocols, particularly for woody taxa, to enhance the accuracy and reproducibility of molecular analyses in plant biotechnology and breeding studies.

Conclusion

This study compares DNA isolation methods including CTAB, phenol-chloroform, silica columns, and magnetic beads for *Zea mays* L., *P. vulgaris* L., *Q. robur* L., and *P. sylvestris* L. The findings reveal that the differences in DNA yields and purity depend on the plant species and tissue characteristics. The phenol-chloroform method provided the highest DNA yields and purity across all plant species. This method excels in obtaining DNA free from proteins, polysaccharides, and phenolic compounds, ensuring high molecular integrity. The results demonstrate that *Q. robur* L. and *P. sylvestris* L., which are rich in lignin and phenolic compounds, had the highest yields and preserved molecular integrity. This supports the use of the phenol-chloroform method in molecular analyses requiring high-quality DNA.

The CTAB method stands out for its cost-effectiveness and ease of application, showing good results, especially in species like *P. sylvestris* L. and *Q. robur* L., which have high phenolic content. However, the lower DNA yield from *P. vulgaris* L., which contains high protein content, suggests that additional optimization for protein removal may be needed for this method to be more effective.

The silica column method was found to be less effective due to its lower DNA yield and loss of molecular integrity. Despite this, it can still be useful for less demanding applications where ease of use and speed are prioritized, especially for tissues with low levels of contaminants. In contrast, the magnetic beads method showed poor performance with low DNA yields and contamination issues. The increased DNA breakage in tissues with high phenolic content further hampered its effectiveness.

Given these results, the phenol-chloroform method should be prioritized for genetic analyses, PCR-based studies, and genetic diversity projects where high-quality DNA is critical. It is particularly effective in tissues with high phenolic and lignin content. The CTAB method, with its cost and time advantages, can serve as an alternative but should be optimized for protein-rich tissues by including additional chemicals like proteases. The silica column method may be suitable for routine, small-scale DNA isolation, provided that the amount of starting material is carefully adjusted. The effectiveness of the magnetic beads method should be improved by developing new buffers and chemical modifications to reduce phenolic compound interference and DNA fragmentation.

This study underscores the importance of selecting the appropriate DNA isolation method for plant species in genetic and molecular biology research. Future studies should explore these methods in a wider range of plant species and further optimize them, leading to significant advancements in DNA isolation processes.

Conflict of Interest

The authors have declared no conflict of interest.

Author Contrubiton Statement

The authors have equal contributions.

References

Akbulut, H.M. (2024). *Hücre dışı DNA izolasyonlarında belirli parametrelerin optimizasyonu*. (Master's thesis). *Kırşehir Ahi Evran Üniversitesi-Sağlık Bilimleri Enstitüsü, Tıbbi Biyoloji Anabilim Dalı*, ss.57.

Aljanabi, SM. & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, *25*(22), 4692-4693.

Cetinkaya, R. & Aysan, Y. (2022). Doğu Akdeniz bölgesinde yetişen mısır (*Zea mays*) bitkisinin yeni bir bakteriyel hastalığı: Dickeya zeae'nın neden olduğu bakteriyel sap çürüklüğü. *Mustafa Kemal University Journal of Agricultural Sciences/Mustafa Kemal Universitesi Tarim Bilimleri Dergisi*, *27*(3), 493-501.

Deka, P.C. (2024). *Gene cloning.* In Research Anthology on Bioinformatics, Genomics and Computational Biology, 650-689. IGI Global.

Dogdu, E.M. (2025). Türkiye'de yayılış gösteren *pinus* türlerine özgü bir dna izolasyon protokolü geliştirilmesi (Doctoral dissertation), *Harran Üniversitesi, Fen Bilimleri Enstitüsü, Moleküler Biyoloji ve Genetik Anabilim Dalı*, ss.94

Doyle, J.J. & Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin*, *19*, 11-15.

Du, X., Lu, M., Lan, H., Cai, Z., Pan, D. & Wu, Y. (2024). Development of a high-throughput DNA isolation method for livestock and poultry meat based on mesoporous metal-organic framework-coated solid-phase microextraction device. *Journal of Food Composition and Analysis*, 127, 105977.

Dutta, M., Sharma, P., Raturi, V., Bhargava, B. & Zinta, G. (2024). SarCTAB: An efficient and cost-effective DNA isolation protocol from geophytes. *3 Biotech*, *14*(2), 36.

Fopp-Bayat, D., Raczyński, T. & Kuciński, M. (2024). Applicability of an in-house Chelex-100 DNA isolation method for extracting genetic material from sterlet embryos. *Fisheries & Aquatic Life*, *32*(1), 1-8.

Guldur, M., Dikilitaş, M., Kılınç, B. & Şimşek, E. (2024). Şanlıurfa peyzaj alanlarında çam ağaçlarında sürgün kurumalarına ve geriye doğru ölümlere neden olan Alternaria alternata etmeninin Türkiye'deki ilk kaydı. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi*, *29*(1), 212-223.

Jablonski, B., Meier, NA., Chatwin, W., Coggeshall, MV., Romero-Severson, J. & Revord, RS. (2024). Quantitative trait loci for bearing habit in a 'Sparrow'×'Schessler'Juglans nigra mapping population. *Tree Genetics & Genomes*, *20*(5), 30.

Kiy, D. & Akpulat, H.A. (2024). Yüksek oranda fenolik bileşen içeren Thymus (*Lamiaceae*) türlerinin modifiye edilmiş CTAB yöntemiyle genomik DNA izolasyonu. *Turkish Journal of Biodiversity*, *7*(1), 11-16.

Lefort, F. & 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.

Murray, M.G. & Thompson, W. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic acids research, 8*(19), 4321-4326.

Pandit, S.S., Mitra, S.S., Giri, A.P. & Gupta, V.S. (2007). A quick method for isolating rna from raw and ripe fleshy fruits as well as for co-isolating DNA and RNA from polysaccharideand polyphenol-rich leaf tissues. *Journal of Plant Biology*, *50*, 60-64.

Rehman, A., Khan, I. & Farooq, M. (2024). Secondary metabolites mediated reproductive tolerance under heat stress in plants. *Journal of Plant Growth Regulation*, *43*(9), 2993-3011.

Rezvantseva, Y., Zybin, D., Prostyakova, A., Oleinikov, V. & Kapustin, D. (2024). Sorption properties of pristine and functionalized magnetic carbon nanotubes in relation to double-stranded DNA. *Journal of Biotechnology*, 385, 13-22.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. & Allard, R. (1984). Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences*, *81*(24), 8014-8018.

Sahu, S.K., Thangaraj, M. & 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 Notices*, *2012*(1), 205049.

Tatar, M., Bildirici, A.E., Ölmez, F., Mustafa, Z. & Tozlu, G. (2024). Eski ve yeni böcek örneklerinde farklı DNA izolasyon protokollerinin karşılaştırılması. *Journal of the Institute of Science & Technology/Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, *14*(2), 135-148.

Toader, V.A., Moldovan, I.C., Sofletea, N., Abrudan, I.V. & Curtu, AL. (2010). DNA isolation and amplification in oak species (*Quercus* spp.). Bulletin of the Transilvania University of Brasov. *Series II: Forestry Wood Industry Agricultural Food Engineering*, 45-50.

Torres, S., Dailey, Y. & White, K. (2021). Application of biomedical molecular techniques in environmental sciences. *Practical Applications of Medical Geology*, 287-317.