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IMPROVEMENTS TO A COMMERCIAL DNA EXTRACTION METHOD FOR HIGH-QUALITY NUCLEIC ACID EXTRACTIONS FROM *Populus tremuloides* Michx. VEGETATIVE BUDS

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Mejoras en un método comercial de extracción de ADN para obtener extractos de ácido nucleico de alta calidad a partir de yemas vegetativas de *Populus tremuloides* Michx.

Improvements to a commercial DNA extraction method for high-quality nucleic acid extractions from *Populus tremuloides* Michx. vegetative buds

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ABSTRACT: Quaking aspen (*Populus tremuloides*) is a tree species of interest due to the potential discovery of an adaptive history in its genome, which is why the correct isolation of its DNA is essential. In this study, two extraction methods based on the NucleoSpin™ Plant II kit were investigated using vegetative buds from eight paired samples. Method 1 used buffer PL1, a homogenizer, and a 30-minute incubation with RNase; Method 2 employed buffer PL2, glass beads, and a 45-minute incubation with RNase. DNA quality and quantity were assessed by spectrophotometry, gel electrophoresis, and PCR amplification. A paired *t*-test revealed that Method 1 yielded a significantly higher DNA concentration than Method 2 (mean difference = 71.98 ng/µL; 95% CI 17.3–126.7; $p = 0.017$; dz = 1.10). In contrast, no significant differences in the 260/280 ratio were observed, either by the paired *t*-test (mean difference = 0.10; 95% CI −0.08–0.28; $p = 0.219$) or by the Wilcoxon signed-rank test (median difference = +0.02; 95% CI −0.01–0.295; $p = 0.25$). Method 1 produced higher yields but with DNA fragmentation visible on the gel, which may interfere with some analyses. Method 2 showed a lower concentration but slightly clearer bands on the gel, indicating better DNA integrity. PCR was successful in both cases, but the higher DNA integrity in Method 2 suggests that this method is more suitable for studies requiring intact DNA. Although Method 1 yields a larger amount, Method 2 is preferable when DNA integrity is critical for genomic analysis.
Key words: quaking aspen, isolation, PCR amplification, DNA integrity, genetics
RESUMEN: El álamo temblón (*Populus tremuloides*) es una especie arbórea de interés debido al potencial descubrimiento de una historia adaptativa en su genoma, por lo que el aislamiento correcto de su ADN resulta esencial. En este estudio se investigaron dos métodos de extracción basados en el kit NucleoSpin™ Plant II, utilizando yemas de

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crecimiento de ocho muestras apareadas. El método 1 empleó el tampón PL1, un homogeneizador y una incubación de 30 minutos con RNasa; el método 2 utilizó el tampón PL2, perlas de vidrio y una incubación de 45 minutos con RNasa. La calidad y cantidad del ADN se evaluaron mediante espectrofotometría, electroforesis en gel y amplificación por PCR. Una prueba t apareada reveló que el método 1 produjo una concentración de ADN significativamente mayor que el método 2 (diferencia media = 71.98 ng/µL; IC 95%: 17.3–126.7; $p = 0.017$; dz = 1.10). En contraste, no se observaron diferencias significativas en la relación 260/280, ya fuera mediante la prueba t apareada (diferencia media = 0.10; IC 95%: −0.08–0.28; $p = 0.219$) o la prueba de rangos con signo de Wilcoxon (diferencia mediana = +0.02; IC 95%: −0.01–0.295; $p = 0.25$). El método 1 produjo mayores rendimientos, pero con fragmentación del ADN visible en el gel, lo que puede interferir en algunos análisis. El método 2 mostró una menor concentración, pero bandas ligeramente más definidas en el gel, lo que indica mejor integridad del ADN. La PCR fue exitosa en ambos casos, aunque la mayor integridad del ADN en el método 2 sugiere que este método es más adecuado para estudios que requieren ADN intacto. Aunque el método 1 genera una mayor cantidad, el método 2 es preferible cuando la integridad del ADN es crítica para el análisis genómico.
Palabras claves: álamo temblón, aislamiento, amplificación por PCR, integridad del ADN, genética

INTRODUCTION

DNA extraction is a critical step in molecular biology studies, particularly for species of ecological and evolutionary interest such as quaking aspen (*Populus tremuloides*) (Goessen *et al.*, 2022). This species is widely distributed across North America, from Alaska to central Mexico, and plays a fundamental role in forest ecosystems due to its clonal reproduction and the presence of ancient DNA in some populations (Mitton & Grant, 1996; Mock *et al.*, 2008). Southern populations of *P. tremuloides*, particularly those in Mexico, have been hypothesized to represent relict lineages, remnants of ancient genetic diversity that have persisted in isolation following glacial retreats. These populations may harbor unique allelic combinations shaped by long-term climatic and geographic constraints, making them valuable for studying local adaptation and evolutionary history (Goessen *et al.*, 2022; Hernández-Velasco *et al.*, 2024).

Understanding its genetic composition requires high-quality DNA for applications such as PCR amplification, sequencing, and genomic analyses (Saiki *et al.*, 1988; Zhu *et al.*, 2020) as well as differentiation and hybridization studies in tree species (Sánchez-Hernández *et al.*, 2022; Wehenkel *et al.*, 2020). However, DNA extraction from plants is challenging due to the presence of secondary metabolites, polysaccharides, and phenolic compounds that can compromise DNA purity and integrity (Mitchell, *et al.*, 2023; Porebski *et al.*, 1997; Schenk *et al.*, 2023). In the case of *P. tremuloides,* it has been reported that modifications to commercial DNA extraction protocols are necessary (Goessen *et al.*, 2022).

To overcome these challenges, various DNA extraction protocols have been developed for *Populus,* with the cetyltrimethylammonium bromide (CTAB) method being one of the most commonly used, originally proposed by (Doyle & Doyle, 1990) and later modified (Qiu *et al.*, 2019; Zhou *et al.*, 2023). Additionally, commercial kits have been used for microsatellite analysis (Blonder *et al.*, 2020), and some studies have developed custom protocols based on phenol-chloroform extraction (Bagley *et al.*, 2020). The CTAB method is a cost-effective and widely used alternative, although its efficiency depends on the type of tissue and sample conditions (Schenk *et al.*, 2023). In contrast, commercial kits such as the Macherey-Nagel™ Nucleo Spin™ Plant II offer standardized protocols with reagents designed to minimize contaminants. However, these kits may not always provide the highest DNA quality for all research applications (Méndez-Cea *et al.*, 2019; Shields *et al.*, 2013). In some cases, developing a custom protocol or adapting commercial procedures may be preferable (Jiao *et al.*, 2015).

This study compares two variations of the NucleoSpin™ Plant II kit protocol for DNA extraction from *P. tremuloides*, which differ in homogenization techniques and lysis buffers. The kit manual recommends homogenizing samples with liquid nitrogen or laboratory ball mills, resources that are not always available. In addition, it offers two alternative buffers for cell lysis (PL1 based on CTAB-based and Sodium Dodecyl Sulfate, SDS-based PL2), which have each different degrees of effectiveness depending on the properties of the plant tissue. Testing these options was therefore important in order to establish an optimized and accessible protocol for extracting DNA from buds of *P. tremuloides*.

The objective is to assess how these modifications impact DNA yield, purity (260/280 ratios), and fragment integrity, which are critical aspects for applications requiring high-quality DNA. Unlike previous studies that have compared multiple extraction methods (Verbylaite *et al.*, 2010), this study focuses on evaluating adjustments within a widely used commercial kit to determine whether these modifications can improve the quality of extracted DNA.

By analyzing DNA concentration, purity, and PCR amplification success, this study seeks to determine the suitability of these variations for genomic applications in *P. tremuloides*, thus contributing to the optimization of DNA extraction strategies for genetic and evolutionary studies of the species.

MATERIALS AND METHODS

Samples studied and their genomic DNA extraction

In April 2022, vegetative buds were collected from three *P. tremuloides* trees in Santiago Papasquiaro, *Durango*, Mexico located at 25°1'40.5" Lat. N and 105°42'8.5" Long. W, at an elevation of 2568 m. The collected population is referred to as SAA, the number that follows indicates the sampled tree, and the orientation (north, south, east, west) indicates the position of the collected buds. The samples were transported in airtight bags for approximately eight hours and then stored at -18°C in a Daewoo refrigerator-freezer, model DFR-N141D, for eight months before being processed in the laboratory (due to restrictions on access to instruments and the laboratory). DNA was isolated using two adaptations of the Macherey-Nagel™ Nucleo Spin™ Plant II Mini kit protocol for plant DNA. By focusing on buds, we were therefore able to ensure both a high DNA yield and biological relevance for future epigenetic applications.

DNA isolation protocol

1. Homogenization of the sample and cell lysis

Variation Method 1

The Nucleo Spin™ Plant II manual recommends using up to 100 mg wet weight or 20 mg dry weight of plant tissue for DNA extraction. In this study, the lignified hull of the buds was removed, leaving the central part, which was weighed to an optimal 30-40 mg. Initial tests showed that sample weights below 20-30 mg or more than 40–50 mg resulted in lower DNA concentration and purity. The buds were ground in a mortar and placed in 1.5 mL microtubes.

An aliquot of 400 µL of lysis buffer PL1, containing CTAB (which was included as part of the prepared buffer in the NucleoSpin™ Plant II kit, Macherey-Nagel, Germany), was added to each sample. CTAB releases nucleic acids and forms an insoluble complex when NaCl concentration is reduced to ~0.5 M. Phenolic compounds, polysaccharides, and other contaminants are removed with the supernatant as they do not precipitate under these conditions (Ausubel *et al.*, 2003).

The samples and buffer were mixed in a vortex mixer (Vortex-Genie 2, Scientific Industries, Inc.) for one minute at maximum speed and shaking at approximately 3200 rpm. The mixture was then homogenized for 10 sec (D-160 DLAB® tissue homogenizer) and centrifuged (Legend Micro 21R, Centrifuge, Thermo Fisher Scientific, 2011, Germany) at 11000 rpm for two minutes. The aqueous phase was transferred to a new tube, and 10 µL of RNase (which was included as part of NucleoSpin™ Plant II kit, Macherey-Nagel, Germany) was then added to each sample. The RNase contained in the kit was prepared by addition of 600 µL of nuclease-free water and

incubation (Incubator H_2O Bath series, Sheldon Manufacturing, Inc., Shel Lab, USA) at 65 °C for 30 minutes.

Variation Method 2

For the second DNA extraction process, 6 to 8 sample buds, with a total weight of 30–40 mg, were used. The samples were ground in a mortar and placed in 1.5 mL microtubes. Five mg of 0.1 mm glass beads and 400 µL of PL2 lysis buffer (SDS-based, provided in the NucleoSpin™ Plant II kit, Macherey-Nagel, Germany) were added to each sample. SDS is an anionic detergent that solubilizes cell membranes and denatures proteins, facilitating the release of nucleic acids.
The sample was mixed with the buffer and the glass beads in a vortex mixer (Vortex-Genie 2, Scientific Industries Inc.) for five minutes at maximum speed and shaking at approximately 3200 rpm. After centrifugation for two minutes, the aqueous phase was placed in a new 1.5 mL tube, avoiding the concentrated mass at the end of the tube, and 10 µL of RNase (previously prepared) was added to each sample.
The samples were incubated at 65 °C for 45 minutes (Incubator H2O Bath series, Sheldon Manufacturing, Inc., Shel Lab, USA) and then allowed to cool for five minutes at room temperature, before 75 µL of PL3 buffer (protein precipitation buffer, potassium acetate-based, provided in the kit) was added. The mixtures were processed in the vortex mixer and incubated (Incubator H2O Bath series, Sheldon Manufacturing, Inc., Shel Lab, USA) at -80 ºC for five minutes. The samples were then left at room temperature to thaw.

2. Filtration, adjustment of DNA binding conditions, DNA binding to silica membrane

After incubation, the samples underwent a filtration and lysate clarification process using the violet column (supplied with the kit), followed by the addition of buffer PC under different conditions depending on the method:

Variation Method 1

The lysate was applied to the violet column and centrifuged at 13,000 rpm for two minutes. The column was discarded, and 450 µL of buffer PC (guanidine hydrochloride-based) was added directly to the clarified lysate in the collection tube. The mixture was gently vortexed for 5 seconds before proceeding to DNA binding on the silica column.

Variation Method 2

Thawed samples were also clarified using the violet column and centrifuged at 13,000 rpm for two minutes. In this case, the aqueous phase was transferred to a new tube, and 450 µL of buffer PC was added to the recovered supernatant. The mixture was vortexed briefly before being loaded onto the silica column for DNA binding.

Application of PC Buffer and Green Column:

In both methods, an aliquot of 450 µL of buffer PC (which was included as part of the buffers in the NucleoSpin™ Plant II kit, Macherey-Nagel, Germany) was then added to the tubes containing the samples. Buffer PC contains guanidine hydrochloride, which completely removes RNA, metabolites and other PCR inhibitors. The mixture was done by gently vortexing for 5 sec. The provided green column (NucleoSpin™ filter) was placed inside a 2 mL collection tube. Then, 700 µL of the sample mixed with PC buffer was added to this assembly. The tube was centrifuged at 13000 rpm for one minute. After centrifugation, the liquid was discarded, while the column, which includes the filter, was retained.

3. Washing and drying of the silica membrane

In both methods, the green column was washed three times:

1. Add 400 µL of PW1 buffer, centrifuge at 13000 rpm for one minute, discard the liquid.
2. Add 700 µL of PW2 buffer, centrifuge at 13000 rpm for one minute, discard the liquid. PW2 was prepared by adding 100 µL of 96-100 % ethanol. Keep the vial tightly closed to prevent evaporation.
3. Add 200 µL of PW2 buffer, centrifuge at 13000 rpm for two minutes, discard the fluid.

4. Eluting the DNA

The filter was placed in a new 1.5 mL microtube, and 30 µL of PE buffer with 13 µL of 1 mM EDTA (CTR scientific, México) was added to moisten the filter membrane. After incubating at

65 ºC for five minutes, the tube was centrifuged at 13000 rpm for one minute. The filter was discarded, and the eluted DNA was stored at -20 ºC. EDTA was added to the PE buffer to prevent DNA degradation by chelating Mg^{2+} ions needed by DNAases, as previous tests showed low DNA concentrations and 260/280 ratios below 1.8.

DNA quantification

The DNA was analyzed by spectrophotometry (in a NanoDrop 2000 spectrophotometer) to determine both the concentration (ng μL^{-1}) and the ratio of absorbance at 260 nm and 280 nm (260/280 ratio), an indicator of purity.

The electrophoresis system included an agarose gel prepared with 30 mL of 1X TAE, 0.3 g of standard electrophoresis agarose and 1 µL of ethidium bromide (EtBr). Aliquots of 3 µL of the samples were loaded using 3 µL of loading buffer, and a Promega brand of 10000 bp molecular size marker was added. Gels were visualized on a photodocumenter (ChemiDoc XRS+ from Bio-Rad) including Image Lab software (Bio-Rad Laboratories, 2017).

Statistical analysis

We analyzed n = 8 paired samples, each processed using Method 1 and Method 2, respectively. In this design, both methods were applied to the same biological unit, so each sample serves as its own control. This approach accounts for variability among trees and sampling positions, which would otherwise inflate error terms if methods were compared across independent groups. Since we did not generate technical replicates, the data structure consists of pairs rather than repeated measurements or factorial combinations; therefore, a paired comparison is the most appropriate statistical framework (McDonald, 2014). For each variable (DNA concentration and 260/280 ratio), we first tested the normality of the paired differences using the Shapiro–Wilk test. If the differences followed a normal distribution, we applied a paired *t*-test; otherwise, we used the Wilcoxon signed-rank test. For all tests, we reported two-sided *p*-values, 95% confidence intervals of the mean or median difference, and paired effect size (dz). Statistical analyses were performed in R Core Team, 2021.

Amplification of the protein tyrosine and serine/threonine kinase gene by PCR

After verifying the quality of the extracted DNA, the *AT3G09010* gene (encoding a protein tyrosine and serine/threonine kinase, PTK/PSK) was amplified, and a polymorphism was detected in Mexican *P. tremuloides*, identified as *Potrs00121211g02063* homologous to the *AT3G09010* gene (see more https://www.ncbi.nlm.nih.gov/gene/820053) (Goessen *et al.*, 2022). The following 5' to 3' primers were used to perform PCR for the amplification of 1,613 base pairs of the *PTK/PSK* gene: forward primer (Fwd-Potrs): GAACTGGTACCACATATCGTGCATAGAGAC, and reverse primer (Rev-Potrs): CCTGGAGCAGTGAGTAACTTGTCATTTAGCC. The Promega kit was used with a total reaction volume of 25 µL consisting of 5X buffer (5 µL), $MgCl_2$ (2 µL), dNTPs (0.25 µL), forward primer (0.25 µL), reverse primer (0.25 µL), Taq DNA polymerase (0.2 µL), DNA sample (1 µL), and nuclease-free water (16.05 µL).

The positive and negative controls, consisting of a previously analyzed DNA sample and nuclease-free water, were analyzed together with the prepared samples. PCR amplification was performed in a thermocycler under the following conditions: initial denaturation at 95°C for 1 minute, followed by 30 cycles at 95°C for 45 seconds, hybridization at 65°C for 45 seconds, and 72°C for 1 minute. A final extension was performed at 72°C for 5 minutes, and the reaction was kept at 4°C. During this process, the primers specifically bound to their complementary DNA sequences: the sense primer to the positive (+) strand and the antisense primer to the negative (−) strand.

Subsequently, the amplified fragments were visualized on agarose gel by using the same methodology described above. Gels were prepared with only three samples (SAA-9 South, SAA-9 West and SAA-5 South) for each method used (Method 1; Method 2), plus one positive and one negative marker.

RESULTS

DNA quantification

The DNA concentration and absorbance values for each sample are listed in Table 1. Normality was evaluated on the paired differences using the Shapiro–Wilk test. DNA concentration differences were normally distributed (W = 0.92, p = 0.124), whereas absorbance differences (260/280 ratio) were not (W = 0.55, p < 0.001). For DNA concentration, Method 1 yielded significantly higher values than Method 2 (mean paired difference = 71.98 ng/µL; 95% CI 17.3–126.7; t(7) = 3.11, p = 0.017; dz = 1.10) (Figure 1). This pattern was consistent across all samples and is clearly illustrated in the paired difference plot (Figure 2).

Tabla 1. Concentración y absorbancia (relación 260/280) del ADN de cada muestra extraída con el método 1 y el método 2, según análisis espectrofotométrico (Nanodrop 2000).
Table 1. DNA concentration and absorbance (260/280 ratio) of each sample extracted with Method 1 and Method 2, according to spectrophotometric analysis (Nanodrop 2000).

Sample	Conc. (ng/µL) M1	Conc. (ng/µL) M2	260/280 M1	260/280 M2
SAA-10-east	124	74.8	1.83	1.86
SAA-10-north	56.4	13.5	1.91	1.89
SAA-5-west	122.3	91	1.86	1.84
SAA-5-south	160.6	48.4	1.91	1.89
SAA-9-east	106.5	90.7	1.91	1.92
SAA-9-north	66.6	56.2	1.88	1.28
SAA-9-west	199	86.4	1.79	1.8
SAA-9-south	268	66.6	1.93	1.72

Note: "Conc." indicates DNA concentration (ng/µL). "260/280" indicates absorbance ratio (optimal values 1.8–1.9). Statistical comparisons were conducted with paired tests (paired t-test or Wilcoxon signed-rank test, depending on normality of paired differences).

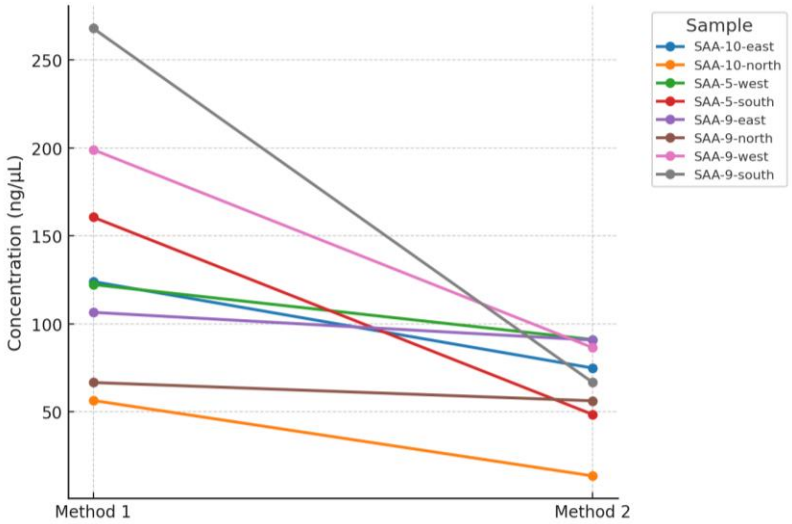

Figure 1. DNA concentration (ng/µL) for each sample processed with Method 1 and Method 2. Lines connect paired observations from the same sample (n = 8). Colors denote individual samples.
Figura 1. Concentración de ADN (ng/µL) para cada muestra procesada con el método 1 y el método 2. Las líneas conectan observaciones apareadas de la misma muestra (n = 8). Los colores indican las muestras individuales.

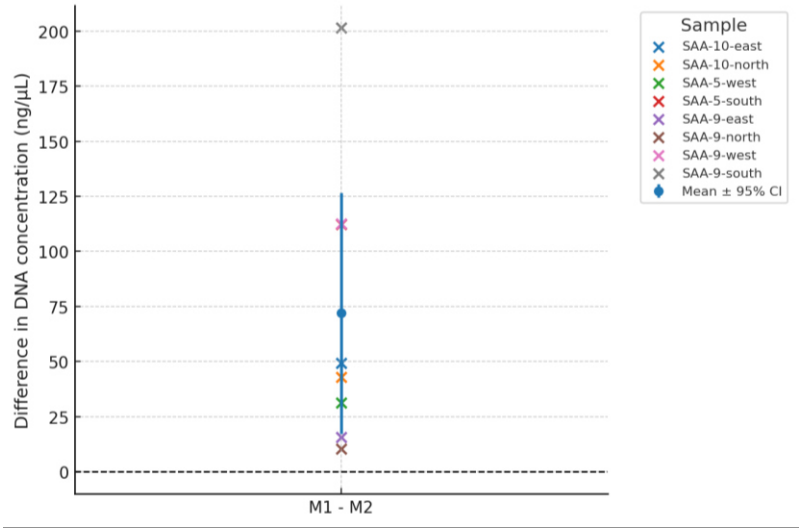

Figure 2. Paired differences in DNA concentration (Method 1 – Method 2). Gray dots represent individual differences, colors indicate sample identity, and the blue marker with error bars represents the mean difference ± 95% CI. A dashed line marks zero difference.
Figura 2. Diferencias apareadas en la concentración de ADN (método 1 – método 2). Los puntos grises representan diferencias individuales, los colores indican la identidad de la muestra y el marcador azul con barras de error muestra la diferencia media ± IC95%. La línea discontinua indica diferencia cero.

In contrast, for the 260/280 ratio, no significant differences were observed between methods. A paired t-test suggested no difference (mean difference = 0.10; 95% CI −0.08–0.28; $p = 0.219$; Figure 3), and the Wilcoxon signed-rank test confirmed this result (W = 9.0, $p = 0.25$). The Hodges–Lehmann median difference was +0.02 with a 95% CI of −0.01–0.295 (Figure 5). Both analyses converge on the conclusion that DNA purity was equivalent between the two methods.

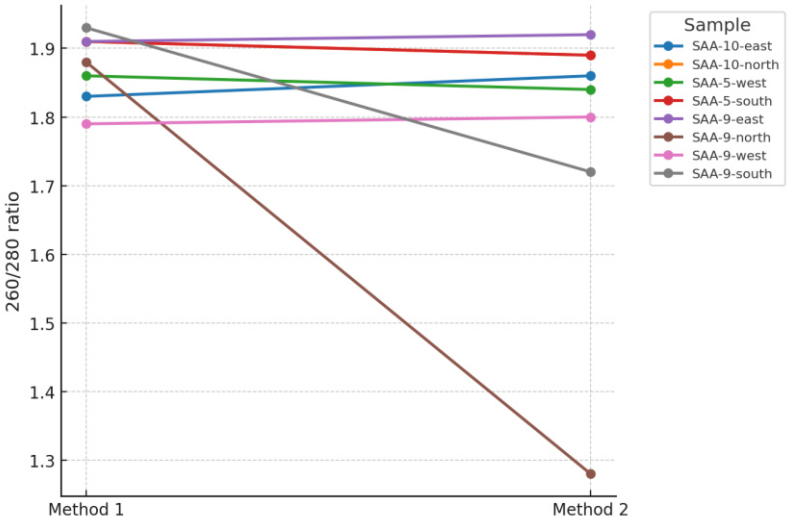

Figure 3. DNA purity (260/280 ratio) for each sample processed with Method 1 and Method 2. Lines connect paired observations from the same sample (n = 8). Colors denote individual samples.
Figura 3. Pureza del ADN (relación 260/280) para cada muestra procesada con el método 1 y el método 2. Las líneas conectan observaciones apareadas de la misma muestra (n = 8). Los colores indican las muestras individuales.

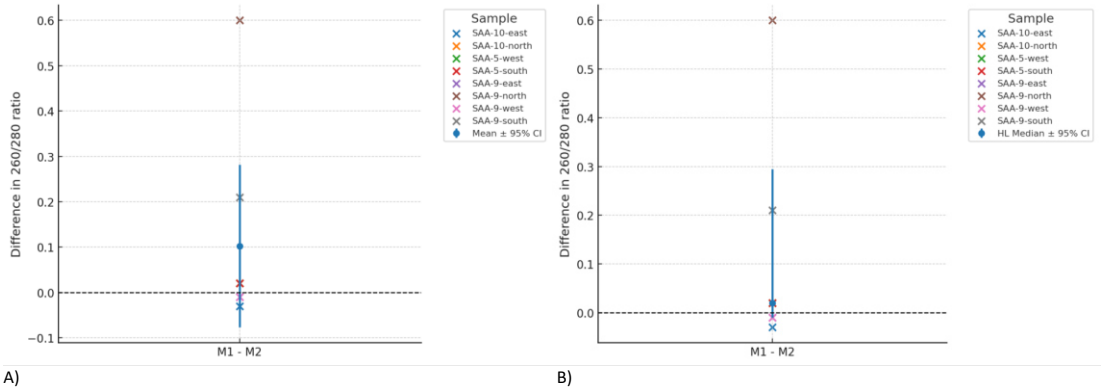

Figure 4. Paired differences in DNA purity (260/280 ratio, Method 1 – Method 2). Colored dots represent individual differences, and blue markers with error bars indicate central tendency estimates ± 95% CI. A) Mean difference (paired t-test). B) Hodges–Lehmann median difference (Wilcoxon signed-rank test). A dashed line marks zero difference.
Figura 4. Diferencias apareadas en la pureza del ADN (relación 260/280, método 1 – método 2). Los puntos coloreados representan diferencias individuales, y los marcadores azules con barras de error muestran las estimaciones de tendencia central ± IC95%. A) Diferencia media (prueba t apareada). B) Diferencia mediana de Hodges–Lehmann (prueba de rangos con signo de Wilcoxon). La línea discontinua indica diferencia cero.

The comparison between methods showed that method 1 yielded a statistically significant higher concentration of DNA than method 2. The 260/280 ratio was higher for Method 1 than for Method 2, but the difference was not statistically significant.
In addition, the products were visualized on agarose gels to corroborate the integrity of the DNA. For method 1, intense smearing is observed at the lower part of the gel (<500 bp), indicating that most of the extracted DNA is fragmented into very small sizes, while for method 2 bands are observed in the 500–3000 bp range, indicating the presence of slightly larger DNA fragments with less fragmentation compared to the first gel. (Figure 5 and 6).

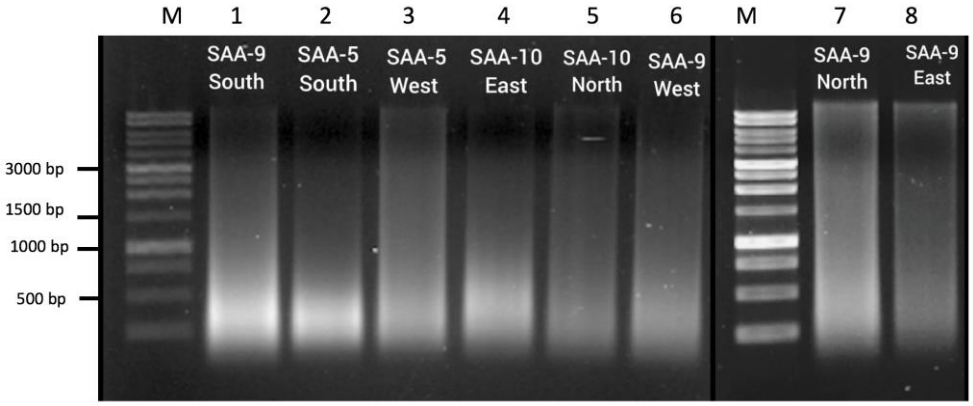

Figure 5. Electrophoresis of DNA samples obtained using Method 1, with PL1 buffer, a tissue homogenizer and a 30 minutes incubation with RNase. In lane M the 10000 bp marker is observed, while lanes 1 to 8 show eight DNA samples with the absence of any bands.
Figura 5. Electroforesis de muestras de ADN obtenidas mediante el método 1, con tampón PL1, un homogeneizador de tejidos y una incubación de 30 minutos con ARNasa. En el carril M se observa el marcador de 10 000 pb, mientras que los carriles 1 a 8 muestran ocho muestras de ADN sin bandas.

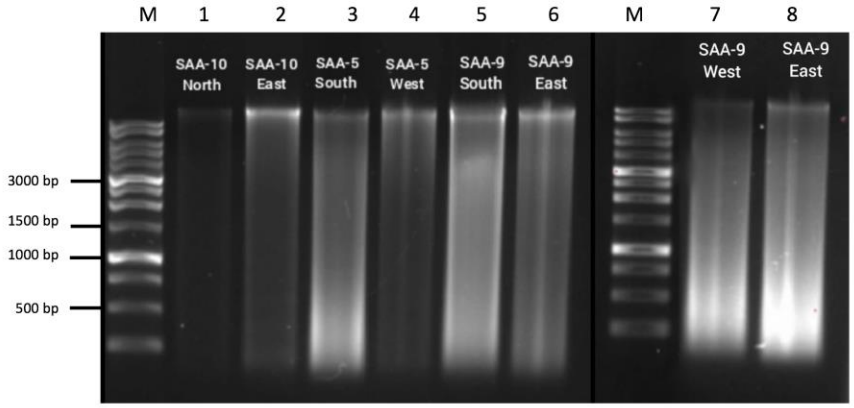

Figure 6. Electrophoresis of DNA samples obtained using Method 2, with PL2 and PL3 buffer, glass beads and incubation for 45 min with RNase. In lane M the 10000 bp marker is observed, while lanes 1 to 8 show eight DNA samples with a well-defined band.
Figura 6. Electroforesis de muestras de ADN obtenidas mediante el método 2, con tampón PL2 y PL3, microesferas de vidrio e incubación durante 45 min con ARNasa. En la línea M se observa el marcador de 10 000 pb, mientras que las líneas 1 a 8 muestran ocho muestras de ADN con una banda bien definida.

Amplification of the protein tyrosine and serine/threonine kinase gene by PCR

The PCR results are shown in Figure 7. In lane C+, the positive control was observed with a band of the expected size of 1600 bp, and in lane C- no amplification band was observed, as expected. In lanes 1 to 4, amplification at 1600 bp of three samples obtained using method 1 was observed. In the subsequent lanes 5 to 8, three samples obtained using method 2 were visualized (all amplified at 1600 bp).

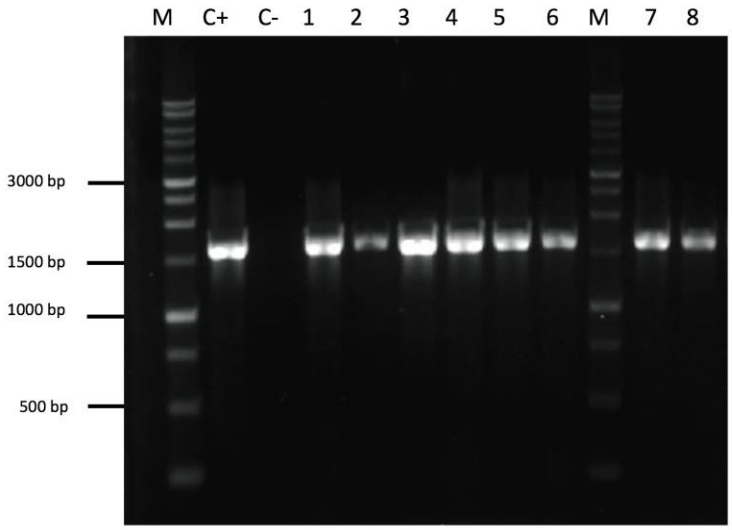

Figure 7. PCR amplification of three DNA samples obtained by each method. Lane M include the 10000 bp marker, C+, the positive control and C- the negative control. Lanes 1 to 4, include samples obtained using Method 1; lane 5 to 8 include samples obtained using Method 2. The DNA obtained by both methods was successfully amplified at 1600 bp.
Figura 7. Amplificación por PCR de tres muestras de ADN obtenidas con cada método. El carril M incluye el marcador de 10 000 pb, C+ el control positivo y C- el control negativo. Los carriles 1 a 4 incluyen muestras obtenidas con el método 1; los carriles 5 a 8 incluyen muestras obtenidas con el método 2. El ADN obtenido con ambos métodos se amplificó correctamente a 1600 pb.

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DISCUSSION

Vegetative bud samples were selected for this study because they contain constantly dividing meristematic cells in which DNA synthesis is active and which yield larger amounts of genomic DNA compared to mature tissues. In *Populus*, different tissues were used for molecular studies depending on the research objective, including leaves for assessing fungi in litter and for speciation and evolutionary analyses (Li *et al.*, 2021; Yang & Vinatzer, 2021), as well as wood for studying secondary metabolites and nutrients (Birkemoe *et al.*, 2022).

According to the results of this study, in Method 1, the PL1 lysis buffer contained cetyltrimethylammonium bromide (CTAB), which is widely used in plant DNA extraction due to its ability to remove polysaccharides and yield high-purity DNA (Aboul-Maaty & Oraby, 2019; Rogers & Bendich, 1994). This method generated higher DNA concentrations, while 260/280 ratio values did not differ significantly between methods. However, agarose gel electrophoresis revealed the lack of a defined band for Method 1, despite NanoDrop 2000 results indicating high DNA concentration. The absence of a band may indicate DNA degradation, as degradation results in fluorescent smears instead of the defined bands observed in high-quality DNA samples (Crespo *et al.*, 1999) (Figures 5).

There are homogenization methods, such as the use of pestles, which break down the sample by friction against the tube wall, as well as abrasive materials like powdered glass or resins. In these cases, it is recommended to add a small amount of lysis buffer before starting the process to denature proteins and stabilize DNA (Alejos *et al.*, 2014). Mechanical disruption in Method 1 was performed using a manual homogenizer, however, excessive DNA fragmentation was observed, suggesting that cell lysis may have been too aggressive (Demkina *et al.*, 2023).

On the other hand, Method 2 used PL2 buffer (SDS-based), glass beads, and vortex homogenization. Although the DNA concentration obtained with this method was lower, agarose gel electrophoresis revealed relatively higher quality compared to Method 1, with bands that were more clearly defined and indicative of larger fragments. Nevertheless, some degree of degradation was still evident, suggesting that while Method 2 improved DNA integrity, it did not completely eliminate fragmentation (García-Godos & Cueva-Castillo, 2021) (Figures 1 and 6).

In this method, the use of SDS, which contains sodium dodecyl sulfate (SDS), a detergent that facilitates membrane dissolution and the removal of proteins and polysaccharides (Dairawan & Shetty, 2020), while subsequent treatment with PL3 (a potassium acetate-based protein precipitation buffer) helped remove proteins and other contaminants. Protein precipitation with potassium acetate has been reported as an effective step to improve DNA purity by eliminating denatured proteins and residual polysaccharides (Chiong *et al.*, 2017). However, SDS-based extractions have also been reported to yield lower DNA quantities, so its use is recommended only in lysis buffer when an excess of secondary metabolites is present (Carey *et al.*, 2023). To mitigate these risks, its use in controlled concentrations, such as the 1% concentration suggested by (Kotchoni *et al.*, 2011), is recommended.

During the DNA extraction process, cell walls and membranes must be broken down in order to release cellular constituents and genetic material. This is usually done using detergents such as SDS, which solubilizes membranes and denatures proteins, and CTAB, which precipitates polysaccharides and removes secondary metabolites (Ausubel *et al.*, 2003). In fact, some protocols have reported that the combined use of SDS and CTAB can be particularly efficient for DNA extraction from difficult plant tissues, especially when liquid nitrogen or specialized homogenization equipment is not available (Ambawat *et al.*, 2020).

We assume that the original protocol of the NucleoSpin™ Plant II kit provides good results, as it has been successfully used in other genomic studies of *Populus* (Verbylaite *et al.*, 2010) (Scobeyeva *et al.*, 2018). Additionally, comparative studies in other plant systems, such as oilseed rape, have shown that DNA isolated with this kit is of comparable quality to that obtained with the classical Doyle and Doyle CTAB protocol (Dobrzycka *et al.*, 2014). This supports the reliability of the original kit protocol as a valid baseline for DNA extraction.

Comparing the results of this study with the CTAB protocol, some commercial kits have been observed to significantly reduce DNA extraction time by streamlining or simplifying steps such as nucleic acid precipitation and purification, thereby improving process efficiency (Scobeyeva *et al.*, 2018). In the NucleoSpin™ Plant II kit, for example, the addition of buffer PL3 (potassium acetate-based) promotes protein precipitation, while nucleic acids are efficiently bound to the silica membrane, replacing the traditional ethanol precipitation step used in CTAB protocols. Furthermore, modifications to the lysis and precipitation steps have overcome contamination problems, such as the formation of insoluble complexes between DNA and polysaccharides, which were reported in earlier studies (Rache-Cardenal *et al.*, 2022). Moreover, the use of CTAB- or SDS-based buffers, either separately or in combination, as described in some protocols, allows for sample processing without the need of specialized equipment or liquid nitrogen, which are costly and pose handling risks, as well as in processes such as lyophilization (Kotchoni & Gachomo, 2009; Sharma *et al.*, 2010). However, recent advances have led to the development of toxin-free methods that leverage the physicochemical properties of nucleic acids (El-Ashram *et al.*, 2016; Ueno *et al.*, 2024).

Using PCR, the DNA samples obtained with both methods were successfully amplified, producing a 1,600 bp fragment. Although Method 1 showed good spectrophotometric yields, gel electrophoresis results revealed lower DNA quality. This finding aligns with the report by (Abubakar *et al.*, 2021), who observed that DNA extracted with the NucleoSpin™ Plant II kit showed some degradation detected in electrophoresis, but without affecting PCR amplification. Additionally (Särkinen *et al.*, 2012), analyzed degraded plant DNA and concluded that DNA purity is a more critical factor than yield in predicting amplification success, emphasizing the need for extraction techniques that maximize DNA purity rather than just yield.

Interestingly, the amplified fragment corresponds to the *AT3G09010* gene, which encodes a protein tyrosine and serine/threonine kinase (PTK/PSK) and has previously been associated with a polymorphism specific to Mexican populations of *Populus tremuloides* (Goessen *et al.*, 2022). The successful amplification of this gene, even from partially degraded DNA, highlights its potential as a marker for understanding local adaptations in these populations. In this sense, the presence of this polymorphism underscores the biological importance of Mexican populations of *P. tremuloides*, which harbor unique genetic signatures that reflect their historical persistence under past climatic conditions.

CONCLUSION

This study compared two DNA extraction methods in *Populus tremuloides*, revealing significant differences in DNA concentration and integrity. Method 1, which used PL1 buffer (CTAB), a mechanical homogenizer, and a 30-minute RNase incubation, resulted in higher DNA concentrations but severe DNA fragmentation, with no significant differences in the 260/280 ratio compared to Method 2. In contrast, Method 2, which used the PL2 buffer (SDS), glass beads, an extended incubation time (45 minutes), and subsequent protein precipitation with PL3 (potassium acetate buffer), preserved DNA integrity better, as evidenced by more clearly defined fragments in electrophoresis.

Importantly, these findings demonstrate that modifications to commercial extraction protocols can lead to different outcomes in terms of DNA quantity and quality, highlighting the need to tailor protocols to both the available equipment and the downstream applications. Both methods enabled successful amplification of a 1600-bp fragment via PCR, confirming the suitability of the extracted DNA for molecular analyses. However, since DNA integrity is a key factor for sequencing applications and genetic and epigenetic studies in plants, Method 2 is considered the most suitable option for studies requiring high-quality DNA with minimal fragmentation.

RECOMMENDATIONS

When selecting a DNA extraction method, it is important to consider the available equipment and the sample homogenization process. The use of a mechanical homogenizer may be too aggressive for *P. tremuloides* bud tissues and may compromise DNA integrity. For studies requiring intact DNA, less invasive homogenization methods, such as with glass beads, may be preferable.
In addition, the intended use of the extracted DNA should be taken into account when choosing a protocol. While higher DNA concentrations may be beneficial for some studies, DNA integrity is crucial for analyses involving sequencing or epigenetic studies. Our findings show that even small modifications to commercial protocols can substantially influence both DNA yield and quality, underscoring the importance of tailoring the protocol to specific research needs. The use of vegetative buds as study material also highlights the relevance of meristematic cells, which can provide valuable insights for genetic and epigenetic studies in trees.
Finally, although this study focused on the 260/280 ratio as a standard indicator of DNA purity, it is also important to consider the 260/230 index in future studies as a complementary measure. Due to a lack of complete records, this parameter could not be systematically assessed here; however, we recommend its inclusion in future studies to enable a more comprehensive evaluation of DNA quality.

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