Comparison of Ten DNA Extraction Protocols from Wood of European Aspen (*Populus tremula* L.)

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Abstract

It is difficult to extract pure high quality DNA from tree tissues, which may not be amenable to advances in extraction methods suitable for other plants. This is especially true for wood samples, that are easy to collect from mature trees, but difficult to handle afterwards. We have compared ten different DNA extraction techniques that are known to be effective in plant genomic DNA isolation. We have used six well known DNA extraction techniques as well as four commercially available kits for DNA extraction from European aspen (*Populus tremula* L.) trees grown in the forest. The quality of DNA was tested by spectrophotometry and PCR amplification of the chloroplast intergenic spacer region between tRNR L-F genes. The results indicate the success using SDS, protein precipitation and CTAB DNA extraction techniques, while other methods (CTAB precipitation, Guanidinium isothiocyanate and alkaline isolation) provided DNA of poor quality or contaminated DNA not suitable for PCR. Commercially available kits also gave different results: DNA isolation reagent for genomic DNA with Plant AC reagent (Applichem) provided DNA with strong contamination, while Nucleospin Plant II (Macherey-Nagel), Genomic DNA purification kit (Fermentas) and innuPREP Plant DNA Kit (Analytikjena) yielded good quality and satisfactory concentration of genomic DNA.

Key words: European aspen/ Populus tremula/ DNA extraction/ wood/ PCR amplification

Introduction

DNA extraction is crucial for any molecular investigation. DNA extraction from microorganisms, human or animal tissue is already a routine procedure, while plants proved to be an exception of the rule. There are numerous protocols for DNA extraction from various plant species and tissues published (Murray and Thompson 1980, Dellaporta et al. 1983, Rogers and Bendich 1985, Doyle and Doyle 1987, Wagner et al. 1987, Bousquet et al. 1990, Devey et al. 1991, 1996, Stewart and Via 1993, Nelson et al. 1994, Jobes et al. 1995, Kim et al. 1997, Lin and Kuo 1998, Tibbits et al. 2006). Many of these protocols recommend DNA extraction from needles, leaves or buds. These tissues are the best source for DNA from mature trees, but a considerable amount of time and effort has to be spent to collect this type of samples, while mature trees are generally tall and sample collection requires firearms or climbing. Sample collection from mature trees often limits the number of samples taken and narrows down the scope of investigation, particularly for population approaches. Tibbits et al. (2006) described a method for DNA extraction using cambium tissue, but this method is not useful for forensic uses, such as timber theft cases.

An additional problem associated with tree DNA extraction is contamination by other molecular substances. Trees possess high levels of endogenous tannins, phenolics and polysacharides, and contamination of extracted DNA by these cellular components can inhibit subsequent molecular reactions. Removals of these components are crucial for molecular-based techniques. Some authors (Murray and Thompson 1980, Doyle and Doyle 1987, Wagner et al. 1987) suggest the use of extraction buffers containing CTAB (cetyltrimethylammonium bromide), others (Stewart and Via 1993, Devey et al. 1996, Kim et al. 1997) introduce PVP-40 (polyvinylpyrrollidone; mol wt. 40.000). Other methods based on sodium dodecyl sulfate (Nelson et al. 1994, Jobes et al. 1995) and guanidine detergent (Lin and Kuo 1998) are also published, but these protocols are rarely used for DNA extraction from tree species, mostly because of many cleaning steps that are required to separate DNA from co-extracting contaminants (Tibbits et al. 2006).

Populus species have acquired an important place in basic and applied research of woody plants. The role of *Populus* species in world forestry and their importance for research as a woody-plant model have led to increasing interest in this genus (Tzfira et al. 1997). *Populus tremula* (European aspen) is the only natural *Populus* species in Lithuania, and we have selected this species as a model tree for our research.

This article summarizes the results of a comparison of techniques with regard to good amplification and concentration of obtained DNA from European aspen wood tissue.

Materials and methods

Plant material

Plant material was collected from 2 mature *Populus tremula* trees taking wood and leaf samples. Wood samples were taken using a Pressler borer, as this method is time saving, simple and preserves the tree from serious damage. Leaf samples were taken using the Big Shot tool (SherrillTree, USA). Fresh leaf samples were dried with silica gel (approximately 10-15 g self indicating silica gel per 1 g plant material), while wood samples were collected in sterile containers, and kept in the freezer (-20°C) until use.

We have used six well known DNA extraction techniques (SDS isolation, Protein precipitation, CTAB isolation, CTAB precipitation, Guanidinium isothiocyanate, Alkaline isolation) (names and description given as described in Milligan, 1998) as well as four commercially available kits for plant genomic DNA extraction (DNA isolation reagent for genomic DNA with Plant AC reagent (Applichem), Nucleospin Plant II (Macherey-Nagel), Genomic DNA purification kit (Fermentas), innuPREP Plant DNA Kit (Analytikjena)). For each method used as a first step we grinded plant tissue in liquid nitrogen using mortal and pestle, and transferred the resulting powder to a tube. Each time the same amount of leaf tissue (10 mg) and wood tissue (100 mg) were used.

DNA extraction

Total genomic DNA was extracted from dried wood tissue (100 mg) and for comparison from silica gel dried leaf tissue (10 mg) from the same individuals by means of the following 10 procedures:

1. SDS isolation of total DNA (Edwards et al. 1991, Goodwin and Lee 1993)

Protocol:

• Transfer the resulting powder to tube and add 4 ml extraction buffer (200mM Tris pH 7.5, 25mM EDTA, 250mM NaCl, 0.5% (w/v) SDS) for each 10 mg of tissue (e.g. for leaf sample add 400µl).

• Vortex the sample for 5 sec.

• Centrifuge at 12 000 x g for 1 min to pellet cellular debris.

• Transfer 3 ml (300µl) of the supernatant to a new tube. Add 3 ml (300µl) of isopropanol and incubate at 20-25°C for 2 min.

• Centrifuge at 12 000 x g for 5 min.

• Dry the DNA pellet in a 65°C incubator, or at 20-25°C.

• Dissolve the DNA in a 100µl TE.

• Use 2.5µl of the dissolved DNA for a typical PCR reaction.

• The dissolved DNA may be stored at 4°C for over one year.

2. Isolation of total DNA by protein precipitation (Fang et al. 1992, Dellaporta et al. 1983)

Protocol:

• Transfer the resulting powder to tube containing 1.2 ml (600µl) extraction buffer (100mM Tris pH 8.0, 50mM EDTA pH 8.0, 500mM NaCl, 2% (w/v) SDS, 1% (w/v) PVP-360, 0.1% (v/v) β-mercaptoethanol (added immediately prior to use in a fume hood)) and incubate at 65°C for 20 min.

• Add one third the volume potassium acetate. Shake vigorously and incubate on ice for 5 min. Most proteins and polysaccharides are removed as a complex with the insoluble potassium dodecyl sulfate precipitate.

• Spin at 12 000 x g for 20 min at 4°C.

• Pipette the supernatant into a clean micro centrifuge tube. Try to avoid as much of the particulate material as possible. Add 0.5 vol. isopropanol. Mix and incubate the solution for 1 h at 4°C.

• Pellet the DNA at 12 000 x g for 15 min at 4°C. Gently pour off the supernatant and lightly dry the pellets either by inverting the tubes on paper towels for 10 min or as long as necessary, or by centrifuging it in Speed Vac for 1-2 min.

• Incubate the DNA in 200-500 µl TE at 65°C for 30 min to re-suspend it.

• Transfer the solution to a micro centrifuge tube and spin for 5 min at 4°C to remove any insoluble debris.

• Transfer the supernatant to another micro centrifuge tube. Add 0.1 vol. sodium acetate and two-thirds of the volume of cold isopropanol. Mix well, incubate at 4°C for 1 h, and pellet the DNA for 10 min in a micro centrifuge at 4°C.

• Wash the pellet with 200-500µl cold 80% ethanol for 10 min and centrifuge again for 1 min at 4°C. Dry the pellet for 10 min in a Speed Vac.

• Re-dissolve the DNA in TE using small increments (e.g. 10-100µl) depending on the size of the pellet.

3. CTAB isolation of total DNA (Doyle and Doyle 1987) with modifications described by (Doyle and Dick-

son 1987, Fang et al. 1992, Lodhi et al. 1994, Murray and Tompson 1980, Saghai-Maroof et al. 1984, Rogers and Bendich 1985, Rogers and Bendich 1988, Milligan 1998)

Protocol:

• Heat the extraction buffer ((50mM Tris pH 8.0, 0.7 M NaCl, 10mM EDTA, 1% (w/v) CTAB, 0.5% (v/v) β-mercaptoethanol (added immediately prior to use in a fume hood)) to 60°C.

• Immediately transfer the resulting powder to tube containing 1 ml (500µl) extraction buffer. Mix well.

• Incubate at 60°C for 30-120 min with periodic gentle swirling.

• Extract once with 1 ml (500µl) chloroform: isoamyl alcohol. Mix gently but thoroughly. Spin at 12 000 x g for 30 sec. at 20-25°C to separate the phases.

• Avoiding the interface, pipette the aqueous (top) phase into new tubes.

• Add 0.5 vol. of 5 M NaCl. Add cold isopropanol to 40%. Mix gently to precipitate nucleic acids. If no precipitate is visible, place at -20°C for 20 min or longer.

• Spin at 12 000 x g for 1 min at 20-25°C. If no pellet or precipitate is visible, place on ice for 20 min and spin again. In the worst case, spin for 10 min at 12 000 x g.

• Gently pour off as much of the supernatant as possible without losing the nucleic acid pellet. Add 0.5-1.0 ml of wash buffer (76% (v/v) ethanol, 10mM ammonium acetate) and swirl gently to wash the pellet. Let the nucleic acids sit in the wash buffer for 15-20 min. Generally, nucleic acids will become much whiter (cleaner) at this step.

• Spin at 12 000 x g for 1 min at 20-25°C. If this is not sufficient, spin harder and longer as before. Pour off wash buffer and allow the pellet to dry briefly (2-4 min) by inverting the tube on a paper towel. Be careful that the pellet does not slide out.

• Re-suspend DNA in re-suspension buffer (10mM ammonium acetate, 0.25mM EDTA pH 8.0) in small increments (e.g. 10-100µl) depending on the size of the pellet.

4. CTAB precipitation of total DNA (Bellamy and Ralph 1968, Murray and Tompson 1980, Rogers and Bendich 1985, Rogers and Bendich 1988)

Protocol:

• Follow steps 1-6 from the protocol 3.

• Add 0.1 vol. of 10% CTAB solution and mix.

• Perform a second chloroform extraction as in steps 5 and 6 of protocol 3.

• Add an equal volume of precipitation buffer (50mM Tris pH8.0, 10mM EDTA, 1% (w/v) CTAB) to reduce the concentration of NaCl to 0.35 M. Mix gently and incubate at 20-25°C for 30 min. Note that it is important to measure the sample volume so that the concentration of NaCl is reduced to the proper level.

• Recover the precipitated DNA by centrifugation at 12 000 x g at 20-25°C for 10-60 sec.

• Re-hydrate the DNA pellet in 200?l re-suspension buffer (10mM Tris pH 8.0, 1mM EDTA pH 8.0, 1mM NaCl).

• Add 2 vol. of cold 100% ethanol and mix gently to precipitate the nucleic acids. Recover the precipitated DNA by centrifugation at 12 000 x g at 4°C for 5-15 min.

• Wash the DNA pellet in 200?l cold 80% ethanol and centrifuge at 12 000 x g at 4°C for 5 min.

• Re-suspend the DNA pellet in re-suspension buffer in small increments (e.g. 10-100µl) depending on the size of the pellet.

• At this point it may be necessary to purify the DNA further in a cesium chloride gradient. This is especially true for those tissues that contain tannins or other secondary compounds, although more than one chloroform extraction or a final phenol extraction may be sufficient.

5. Guanidinium isothiocyanate isolation of total DNA (Cox 1968, Chomczynski 1993, Bowtel 1987, Chomszynski and Sacchi 1987, Jeanpierre 1987, Puissant and Houdebine 1990, Chomczynski and Mackey 1995)

Protocol:

• Transfer the resulting powder to the tube containing 5 ml (500µl) extraction buffer (6 M guanidinium isothiocyanate, 100mM sodium acetate, pH 5.5).

• Incubate at 20-25oC for 10 min. Longer incubation over 1 h with mixing may be necessary. Additionally, the procedure may be interrupted at this step by storing the extract at 20-25°C for as long as 18 h or at 4oC for as long as 3 days.

• Centrifuge at 12 000 x g for 10 min at 4°C to pellet the cellular debris.

• Precipitate the DNA from the supernatant by adding 10 ml (1 ml) 100% ethanol at 20-25°C. Mix by inversion and incubate at 20-25°C for 1-3 min. The DNA should become visible as a fibrous or cloudy precipitate.

• Collect the DNA by spooling on a pipette tip or by centrifugation at 1000 x g for 1-2 min at 4°C.

• Wash the DNA precipitate twice with 0.5-1.0 ml of 80% ethanol. The procedure can be interrupted during the washes by storing the DNA for 1 week at 20-25°C or for 3 month at 4°C.

• Remove the ethanol wash and allow the DNA precipitate to dry for 5-15 min at 20-25°C.

• Dissolve the DNA to a concentration of 0.25µg/µl in TE or 8mM NaOH; typically this entails addition of 200µl solvent. The alkaline solvent may solubilize the DNA faster and more completely.

• If necessary, centrifuge the sample at 12 000 x g for 10 min to remove insoluble material such as polysaccharides.

• If NaOH was used to dissolve the DNA, adjust the pH of the solution to a desired pH by adding Tris-HCl or Hepes (free acid).

6. Alkaline isolation of total DNA (Wang and Cutler 1993)

Protocol:

• Add 100 mg of wood tissue (or 10 mg of leaf tissue) sample to a mortal, and grind using liquid nitrogen.

• Transfer the resulting powder to the tube and add 1 ml (100 µl) of 0.5 M NaOH. Mix well.

• Transfer 5 µl quickly to a new tube containing 495µl storage buffer (100mM Tris pH 8.0, 1mMEDTA pH8.0). Mix well.

• Use 1 µl directly in a PCR reaction.

• Store the isolated DNA at -20ºC.

7. DNA isolation reagent for genomic DNA with Plant AC reagent (Applichem)

Protocol:

Approximately 100 mg of wood tissue sample and 10 mg of leaf tissue sample were ground in liquid nitrogen. Other steps were according to the Applichem protocol.

8. Nucleospin Plant II (Macherey-Nagel)

Protocol:

Approximately 100 mg of wood tissue sample and 10 mg of leaf tissue sample were ground in liquid nitrogen. Other steps were according to Nucleospin Plant II protocol.

9. Genomic DNA purification kit (Fermentas)

Protocol:

Approximately 100 mg of wood tissue sample and 10 mg of leaf tissue sample were ground in liquid nitrogen. Other steps were according to the Fermentas protocol.

10. innuPREP Plant DNA Kit (Analytikjena)

Protocol:

Approximately 100 mg of wood tissue sample and 10 mg of leaf tissue sample were ground in liquid nitrogen. Other steps were according to the innuPREP Plant DNA kit protocol.

DNA quantification

The concentration of extracted DNA was measured using Biophotometer (Eppendorf) at 260 nm wave length. As well the purity measures were taken to estimate the possible contaminants. The purity was measured at 260/280 and 260/230 nm wave lengths.

PCR amplification

PCR was performed in total 25 µl volume, and consisted of 2µl of extracted DNA (concentration as originally obtained), 13.8 µl ddH_2O, 2.5 µl dNTP mix (2mM), 2 µl $MgCl_2$, 2.5 µl PCR buffer (10x), 0.2 µl Taq polymerase (5U/µl) and 1 µl of each $trnL^{UAA}$ F (CGA AAT CGG TAG ACG CTA CG) – $trnF^{GAA}$ (ATT TGA ACT GGT GAC ACG AG) primers (10 µM). The chosen primers were originally described in Shaw et al., (2005). Reaction mixture was covered with mineral oil (10 µl). PCR conditions were as follows: initial denaturation step 80ºC for 5 min; denaturation step 94º C for 1 min; primer annealing step 50º C for 1 min; elongation step 72º C for 2 min; final elongation step 72º C for 5 min. Steps 2- 4 were repeated 35 times.

Results

We summarize methods, useful for DNA isolation from *Populus tremula* wood tissue, and compare these results with leaf tissue from the same individuals. DNA concentration and purity results (Table 1) indicate that CTAB precipitation, guanidinium isothiocyanate method and Applichem DNA isolation reagent (also based on guanidinium isothiocyanate reagent) are not suitable in our case for DNA extraction from *Populus tremula* tissues, irrespective of the type of tissue used. The simplest, three-step based alkaline isolation technique was successful only for DNA isolation from European aspen wood tissue, while leaf tissue gave no satisfactory results (DNA in the sample was not detected). This might be due to the fact that leaf contains much more polyphenolics and tannins as well as other substances that can prevent DNA extraction.

Table 1. DNA concentration and purity

DNA extraction method	Wood tissue sample			Leaf tissue sample		
	DNA conc. (ng/µl)	Purity 260/280 nm	Purity 260/230 nm	DNA conc. (ng/µl)	Purity 260/280 nm	Purity 260/230 nm
SDS	219	1.24	0.53	264	1.17	0.5
	52	2.60	0.29	206	1.15	0.55
Protein precipitation	26	1.96	0.12	185	2.02	2.02
	149	1.11	0.45	96	2.05	1.63
CTAB	63	1.24	0.41	307	1.01	0.35
	19	1.48	0.17	203	1.04	0.3
CTAB precipitation	—	—	—	—	—	—
	—	—	—	—	—	—
Guanidinium isothiocyanate	—	—	—	—	—	—
	—	—	—	—	—	—
Alkaline isolation	45	1.15	0.46	—	—	—
	33	1.10	0.44	—	—	—
DNA isolation reagent for genomic DNA with Plant AC reagent (Applichem)	—	—	—	—	—	—
	—	—	—	—	—	—
Nucleospin Plant II (Macherey-Nagel)	56	1.11	0.44	194	1.62	0.65
	50	1.32	0.41	245	1.34	0.72
Genomic DNA purification kit (Fermentas)	38	1.30	0.56	364	1.8	1.48
	56	1.27	0.41	506	1.56	1.07
InnuPREP Plant DNA Kit (Analytikjena)	216	1.14	0.16	132	1.5	0.22
	82	1.44	0.1	204	1.49	0.55

The other six methods gave satisfactory results (DNA concentrations vary from 20 to 220ng/µl from wood tissue and 100 - 310ng/µl from leaf tissue) according to DNA concentration and purity.

As well we have tested the quality of extracted DNA by PCR amplification (Figure 1). We have amplified 820bp chloroplast DNA fragment that is of sufficient length as a haplotype probe (Deguilloux et al. 2003a, Deguilloux et al. 2003b, Hatziskakis et al. 2008). This fragment length allows employing wood DNA extracts in many different molecular studies. Chloroplast tRNR L-F intergenic spacer amplification results show, that the most effective extraction technique is protein precipitation, while CTAB precipitation, guanidinium isothiocyanate method and Applichem DNA isolation reagent gave no amplification at all, confirming DNA quantification results. Alkaline extracted DNA from wood tissue gave no amplification results as well, even though some DNA is present in the sample. It is possible, that there are some PCR inhibitory substances left, or extracted DNA is degraded or oxidized. Other methods (CTAB and SDS) gave satisfactory results (DNA concentration range from 88ng/µl on average for wood tissue sample up to 245ng/µl on average for leaf tissue), and can be used for DNA extraction from wood tissue. The same quality results as for protein precipitation technique were obtained and using commercial kits (Nucleospin Plant II (Macherey-Nagel); Genomic DNA purification kit (Fermentas); InnuPREP Plant DNA Kit (Analytikjena)).

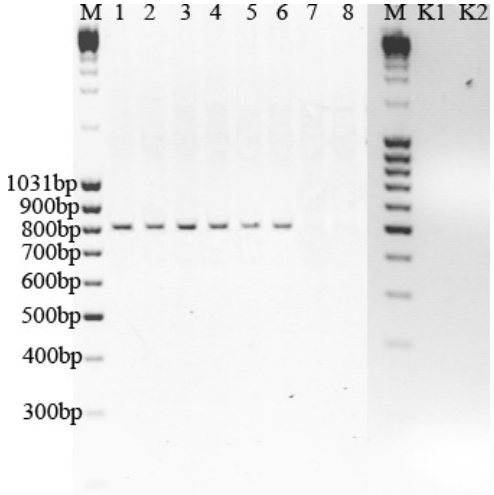

Figure 1. PCR products obtained after amplification using trnLUAA F - trnFGAA primers. M stands for molecular size marker (MassRuller DNA ladder mix, Fermentas), number 1 to 8 designates plant samples that corresponds to DNA extracted using protein precipitation method from wood (1-2) and leaf (3-4) as well as alkaline isolation method from wood (5-6) and leaf samples (7-8); K1 is the PCR control and K2 is the negative DNA control using fungal DNA sample

In Table 2 we compare DNA extraction methods that proved to be suitable for DNA extraction from wood tissue. Here we describe DNA extraction method, present the number of cleaning steps, as well as time required for extraction. Some methods (protein precipitation and CTAB) require a long time for extraction, but these involves several steps that hardly require any action (e.g. cooling, long centrifugation, precipitation) and in total the amount of effort is almost equal as compared to commercial kits.

Table 2. Comparison of successful DNA extraction procedures

DNA extraction method	Time required for DNA isolation	No. of cleaning steps	DNA quality	Cost
SDS	25 min per sample	1 alcohol precipitation step	medium	low
Protein precipitation	~ 4 - 4.5 h (possibility to handle several samples at the same time)	3 steps, protein and 2x alcohol precipitation	high (for wood sample)	low
CTAB	~ 2.5 h (possibility to handle several samples at the same time)	2 steps, chloroform and alcohol precipitation	high	low
Nucleospin Plant II (Macherey-Nagel)	30 min per sample	4 steps, precipitation (binding to membrane), 3x wash	high	high
Genomic DNA purification kit (Fermentas)	25 min per sample	3 steps, chloroform, precipitation, ethanol precipitation	high	medium
InnuPREP Plant DNA Kit (Analytikjena)	40 min per sample	5 steps, precipitation (binding to the membrane), protein digestion and 3x wash	high	high

Discussion and conclusions

Many genetic studies concerning tree species depend on the sampling availability. The ease of sampling is the important step in many investigations. This is especially true when investigating mature trees. Nondestructive sampling from mature tree generally involves the collection of foliage using firearm or climbing. There is a possibility to sample the cambium tissue of the tree and to use it for DNA extraction (Tibbits et al. 2006). However this method is not suitable for some of methods of tree sampling (because of the damage it causes) and for various forensic applications (as it is not always possible to collect fresh cambium tissue). Forensic use in world forestry mostly deals with timber trade control. Timber trade control can be achieved applying chloroplast sequences

using DNA extracted from wood, as described in Deguilloux et al. (2003b); but prior to this a wide diversity study describing haplotype variability in multiple places has to be made.

Wood sample collection by the use of a Pressler borer, is a simple, fast and almost nondestructive method, the wound using this drill is small and causes only minor damage to the tree trunk. In this case a small wood sample is collected that can be used for subsequent investigations.

An important aspect of any molecular investigation is successful DNA extraction. DNA extraction success is largely determined by the number of steps involved in the extraction procedure, and the ease and efficiency of DNA purification. Tree tissues often contain large amounts of polysaccharides and phenolic compounds that are difficult to separate from DNA (Murray and Thompson 1980, Katterman and Shattuck 1983, Mannerlöf and Tenning 1997, Ostrowska et al. 1998). Wood tissue is especially complicated for DNA extraction, for obtaining high quality DNA it has to undergo several cleaning steps (Asif and Cannon 2005, Rachmayanti et al. 2009). In our investigation we have used several DNA extraction techniques with different number of cleaning steps. We have started with the alkaline isolation technique, that hardly requires any cleaning step, and finally we have used several DNA extraction kits, that contain several different cleaning steps as well as enzymatic RNA and protein digestion. Our results suggest that for large scale investigations the best method for DNA extraction would be CTAB or protein precipitation techniques, although these require more time. Despite the time required these methods yield good quality and pure DNA suitable for PCR (see Table 1 and Figure 1). The length of the amplification product is one of the quality measurements of the extracted DNA, as the possible to amplify length is crucial for some molecular investigations (Deguilloux et al. 2003b, Rachmayanti et al. 2009). Our amplified PCR fragment was 820bp length and thus we can conclude that these methods are well suited for DNA extraction. The SDS method, even if it takes the same amount of time as commercially available kits gives DNA of low quality and is not recommended for routine use. Any of the three commercially available kits successfully used in this study could be employed in molecular research that will be using wood tissue sample. The usefulness of commercially available kits was also confirmed by other authors (Deguilloux et al. 2003b, Rachmayanti et al. 2006, Rachmayanti et al. 2009). These kits are easy to handle, simple and fast, but more expensive; for large scale investigation the price for DNA extraction needs to be considered.

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СРАВНЕНИЕ ДЕСЯТИ ПРОТОКОЛОВ ДЛЯ ЭКСТРАКЦИИ ДНК ИЗ ДРЕВЕСИНЫ ОСИНЫ ОБЫКНОВЕННОЙ (*POPULUS TREMULA* L.)

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Резюме

Нелегко получить чистую, высококачественную ДНК из тканей деревьев, для которых не всегда годится современные методы извлечения ДНК из тканей растении. Это самая распространенная проблема для образцов древесины, которые легко собрать с растущих деревьев, но к которым потом трудно применить молекулярные методы. Мы сравнили десять методов, которые известны как эффективные методы экстракции ДНК из тканей растений. Мы использовали шесть хорошо известных техник экстракции ДНК, а также четыре коммерчески доступных комплекта для экстракции ДНК из растущей в лесу осины обыкновенной (*Populus tremula* L.). Качество выделенной ДНК было проверенно с помощью спектрофотометрии и PCR - амплификации внутригенного промежуточного региона ДНК хлоропласта между тРНК генами *L-F.* Положительные результаты показали методы выделения ДНК с помощью SDS (ДСН), осаждения белков и CTAB, остальные методы (осаждения с помощью CTAB, Guanidinium isothiocyanate и изолирования щелочью) позволили получить только ДНК плохого качества или контаминированный ДНК, не подходящий для ПЦР. Коммерчески доступные комплекты тоже дали разные результаты: выделение ДНК с помощью реагента для геномной ДНК «Plant AC reagent» (Applichem) в результате дало сильно контаминированное ДНК, а используя «Nucleospin Plant II» (Macherey-Nagel), «Genomic DNA purification kit» (Fermentas) и «innuPREP Plant DNA Kit» (Analytikjena) – геномное ДНК хорошего качества и достаточной концентрации.

Ключевые слова: Осина обыкновенная /*Populus tremula*/ экстракция ДНК/ древесина/ PCR- амплификация