

SPECIFICS OF GENOMIC DNA ISOLATION FROM PLANTS OF THE GENUS *ROSA* USING VARIOUS PROTOCOLS AND THEIR MODIFICATIONS

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Abstract. The *Rosa* genus includes several species of ecological and conservation importance. However, obtaining high-quality genomic DNA from *Rosa* leaf extracts can be difficult because secondary metabolites can interfere with DNA extraction and subsequent molecular analysis. This study evaluated the performance and versatility of the CTAB-based DNA extraction protocol for leaves of *Rosa acicularis* Lindl., *Rosa alberta* Regel and *Rosa spinosissima* L. from the Altai and Tarbagatai regions of Kazakhstan. Genomic DNA was extracted using a CTAB-based method and compared with the DNA obtained using the primary extraction method. DNA concentrations were measured spectrophotometrically. Differences between extraction methods were analyzed using Welch's t-test.

The CTAB protocol yielded the highest DNA amounts in the analysed samples. Average DNA concentration ranged from 188.95 to 385.24 ng/μl, depending on species and location. The highest average concentration was observed in *R. acicularis* from the Altai Mountains (385.24 ± 142.22 ng/μl), and the lowest in *R. spinosissima* (188.95 ± 89.38 ng/μl). Significant differences were found between individual samples, especially in *R. alberta* from Tarbagatai and *R. spinosissima* from the Altai Mountains. Samples processed with the primary extraction method generally showed lower and more variable DNA content; differences between extraction methods were statistically significant ($p < 0.05$).

The CTAB-based protocol provided sufficient DNA extraction for molecular analysis of the studied *Rosa* species and proved applicable to plant material from different regions of Kazakhstan. However, the large variability between samples suggests that DNA concentration alone does not fully reflect DNA quality. Further verification of DNA purity and PCR amplification efficiency is recommended for subsequent molecular biological applications.

Keywords: *Rosa acicularis*, *Rosa alberta*, *Rosa spinosissima*, CTAB-based method, DNA

ӘРТҮРЛІ ПРОТОКОЛДАРДЫ ЖӘНЕ ОЛАРДЫҢ МОДИФИКАЦИЯЛАРЫН ҚОЛДАНУ АРҚЫЛЫ *ROSA* ТУЫСЫНА ЖАТАТЫН ӨСІМДІКТЕРДЕН ГЕНОМДЫҚ ДНҚ БӨЛІП АЛУДЫҢ ЕРЕКШЕЛІКТЕРІ

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Аңдатпа. *Rosa* тұқымдасына экологиялық және табиғатты қорғауда маңызды бірнеше түрлер кіреді. Дегенмен, итмұрын жапырағының сығындыларынан жоғары сапалы геномдық ДНҚ алу екінші реттік метаболиттердің болуына байланысты қиын болуы мүмкін, бұл ДНҚ экстракциясына және кейінгі молекулалық талдауға кедергі келтіруі мүмкін. Бұл зерттеуде Қазақстанның Алтай және Тарбағатай аймақтарында жиналған *Rosa acicularis* Lindl., *Rosa alberta* Regel және *Rosa spinosissima* L. жапырақтары үшін СТАВ (цетилтриметиламмоний бромиді) көмегімен ДНҚ экстракция протоколының тиімділігі мен әмбебаптығы бағаланды. Зерттеу барысында геномдық ДНҚ СТАВ көмегімен бөлініп алынды және нәтижелер стандартты экстракция әдісімен алынған нәтижелермен салыстырылды. ДНҚ концентрациясы спектрофотометриялық түрде өлшенді. Экстракция әдістері арасындағы айырмашылықтар Уэлчтің 't'-тестін пайдаланып талданды.

СТАВ протоколы талданған үлгілерде ДНҚ-ның статистикалық тұрғыдан айтарлықтай жоғары мөлшерін берді. ДНҚ-ның орташа концентрациясы түрге және жинау орнына байланысты 188,95-тен 385,24 нг/мкл-ге дейін болды. Ең жоғары орташа концентрация Алтай аймағынан алынған *R. acicularis*-те байқалды ($385,24 \pm 142,22$ нг/мкл), ал ең төменгісі *R. spinosissima*-да байқалды ($188,95 \pm 89,38$ нг/мкл). Жеке үлгілер арасында, әсіресе Тарбағатайдан алынған *R. alberta* мен Алтайдан алынған *R. spinosissima* арасында айтарлықтай айырмашылықтар анықталды. Стандартты әдісті қолданып анализденген үлгілер, әдетте, төмен және тұрақсыз ДНҚ концентрациясын көрсетті, экстракция әдістері арасындағы айырмашылық статистикалық тұрғыдан маңызды болды ($p < 0,05$).

СТАВ негізіндегі протокол зерттелген *Rosa* түрлерін молекулалық талдау үшін жеткілікті ДНҚ өнімділігін берді және Қазақстанның әртүрлі аймақтарынан алынған өсімдік материалымен жұмыс істеуге жарамды болып шықты. Дегенмен, үлгілер арасындағы айтарлықтай өзгергіштік ДНҚ концентрациясының өзі оның сапасын толық көрсетпейтінін көрсетеді. Молекулалық биология зерттеулерінде одан әрі пайдалану үшін ДНҚ тазалығын және ПТР күшейту тиімділігін қосымша тексеру ұсынылады.

Кілт сөздер: *Rosa acicularis*, *Rosa alberta*, *Rosa spinosissima*, СТАВ негізіндегі әдіс, ДНҚ

ОСОБЕННОСТИ ВЫДЕЛЕНИЯ ГЕНОМНОЙ ДНК ИЗ РАСТЕНИЙ РОДА *ROSA* С ИСПОЛЬЗОВАНИЕМ РАЗЛИЧНЫХ ПРОТОКОЛОВ И ИХ МОДИФИКАЦИЙ

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Аннотация. Род *Rosa* включает ряд видов, имеющих важное экологическое и природоохранное значение. Однако получение высококачественной геномной ДНК из экстрактов листьев шиповника может быть затруднено из-за наличия вторичных метаболитов, способных препятствовать выделению ДНК и последующему молекулярному анализу. В данном исследовании оценивались эффективность и универсальность протокола выделения ДНК с использованием СТАВ (цетилтриметиламмоний бромид) для листьев *Rosa acicularis* Lindl., *Rosa alberta* Regel и *Rosa spinosissima* L., собранных в Алтайском и Тарбагатайском регионах Казахстана. Геномную ДНК выделяли методом с использованием СТАВ, а полученные результаты сравнивали с данными, полученными при использовании базового метода экстракции. Концентрацию ДНК измеряли спектрофотометрически. Различия между методами выделения анализировали с помощью t-критерия Уэлча.

Протокол с использованием СТАВ позволил получить наибольшее количество ДНК в анализируемых образцах. Средняя концентрация ДНК варьировала от 188,95 до 385,24 нг/мкл в зависимости от вида и места сбора. Наивысшая средняя концентрация была отмечена у *R. acicularis* из Алтайского региона ($385,24 \pm 142,22$ нг/мкл), а самая низкая — у *R. spinosissima* ($188,95 \pm 89,38$ нг/мкл). Были выявлены значительные различия между отдельными образцами, особенно у *R. alberta* из Тарбагатай и *R. spinosissima* с Алтая. Образцы, обработанные базовым методом, в целом демонстрировали более низкое и более вариабельное содержание ДНК, различие между методами выделения было статистически значимым ($p < 0,05$).

Протокол на основе СТАВ обеспечил выход ДНК, достаточный для молекулярного анализа изученных видов *Rosa*, и оказался пригодным для работы с растительным материалом из различных регионов Казахстана. Однако значительная вариабельность показателей между образцами свидетельствует о том, что одна лишь концентрация ДНК не в полной мере отражает ее качество. Для дальнейшего использования в молекулярно-биологических исследованиях рекомендуется дополнительная проверка чистоты ДНК и эффективности ПЦР-амплификации.

Ключевые слова: *Rosa acicularis*, *Rosa alberta*, *Rosa spinosissima*, метод на основе СТАВ, ДНК

Introduction. Obtaining high-quality genomic DNA is a fundamental prerequisite for a wide range of molecular genetic studies in plants, including polymerase chain reaction (PCR), DNA barcoding, phylogenetic analysis, population genetics, and DNA sequencing. The reliability of subsequent analyses depends largely on the quantity, purity, and integrity of the extracted DNA. Therefore, developing efficient, reproducible DNA extraction methods is a crucial step in molecular research on plant species (Csaikl et al., 1998; Lutz et al., 2011; Xin & Chen, 2012; Aboul-Maaty &

Oraby, 2019).

Isolating high-quality DNA from plant tissues can be challenging because plant cells contain a complex mixture of primary and secondary metabolites. In particular, polyphenolic compounds, polysaccharides, proteins, pigments, and other secondary metabolites may co-extract with nucleic acids during tissue disruption and DNA purification (Khanuja et al., 1999; Friar, 2005; Varma et al., 2007; Dehestani & Tabar, 2007; Japelaghi et al., 2011; Sahu et al., 2012). Polyphenols can oxidise and form complexes with DNA and proteins (Jobes et al., 1995; Hadi et al., 2017; Heikrujam et al., 2020), while polysaccharides can increase the viscosity of DNA preparations and interfere with enzymatic reactions (Perlman et al., 1987; Lodhi et al., 1994; dos Santos et al., 2012). Consequently, DNA preparations containing such impurities may lack sufficient purity and inhibit downstream PCR and sequencing reactions. Thus, DNA extraction efficiency can vary significantly by plant species, tissue type, and developmental stage (Rezadoost et al., 2016; Ruggieri et al., 2016).

Species of the genus *Rosa* are of particular interest for molecular genetic research due to their significant morphological diversity, close interspecific relationships, and taxonomic complexity (Matsumoto et al., 1998; Ritz et al., 2005; Koopman et al., 2008; Gaurav et al., 2022; Ergashov et al., 2026). Molecular methods serve as valuable tools for species identification, phylogenetic reconstruction, assessment of genetic diversity, and population studies of members of this genus (Steele et al., 2011; Yang et al., 2012; Patwardhan et al., 2014; Dissanayake et al., 2020). However, successfully applying molecular markers for *Rosa* species requires DNA of appropriate quality and purity to ensure reliable amplification and sequencing. DNA extraction from wild *Rosa* species can be particularly challenging because of the chemical composition of their tissues and compounds that interfere with DNA purification and PCR amplification (Wissemann & Ritz, 2005; Kaul et al., 2009; Debener & Linde, 2009; Maloupa et al., 2021). Among the species found in Kazakhstan, *Rosa acicularis*, *Rosa alberta*, and *Rosa spinosissima* are important representatives of the genus (Vdovina et al., 2024; Danilova et al., 2025; Yermagambetova et al., 2025); working with them may require optimising standard DNA isolation methods. Obtaining DNA suitable for reliable amplification is crucial for the subsequent molecular-genetic characterisation and species identification of these plants.

The cetyltrimethylammonium bromide (CTAB) method is one of the most common approaches to plant DNA extraction because the reagent separates nucleic acids from various cellular components (Rogers & Bendich, 1988; Allen et al., 2006; Liu et al., 2025). However, standard CTAB protocols may need modification depending on the plant material and the concentration of interfering compounds. It has been reported that adding purification steps and using substances such as polyvinylpyrrolidone (PVP), reducing agents, or optimised salt concentrations can improve the quality of DNA extracted from plants rich in secondary metabolites (Porebski et al., 1997; Yadav et al., 2018; Buljević et al., 2025). Nevertheless, an extraction protocol optimised for a specific taxon or tissue type does not necessarily yield satisfactory results when applied to other species (Yockteng et al., 2013; Jones et al., 2023).

Despite the increasingly widespread use of molecular methods to study plant diversity in Kazakhstan, DNA isolation procedures optimised for a range of wild *Rosa* species remain insufficiently documented. Consequently, developing a reproducible method for obtaining DNA suitable for PCR amplification is a vital prerequisite for further molecular-genetic research on these species. This study compared a standard DNA extraction method with a CTAB-based method, optimised a protocol for DNA extraction from *Rosa acicularis*, *Rosa alberta*, and *Rosa spinosissima*, and evaluated the quality and suitability of the extracted DNA for subsequent molecular analysis via PCR. The optimised procedure is intended to provide a reproducible approach to molecular studies of these species, and potentially other plants that are difficult to analyse due to taxonomic or chemical characteristics.

Materials and Methods. The modified (Porebski et al., 1997) original CTAB-based method (Doyle, 1987) was applied to the leaves of *Rosa acicularis*, *Rosa alberta*, and *Rosa spinosissima*. They were collected during the active growing season from Tarbagatai and Altai Mountains in eastern Kazakhstan. They were subsequently air-dried, then transported to the laboratory in paper bags and stored at -80°C. The dry leaves underwent further DNA extraction. 20-25 mg of each plant tissue

sample taken from only healthy, young, non-browning, undamaged leaves was placed in a 2 mL round-bottom microcentrifuge tube. Veins and petioles were removed as well. The tubes were labelled with a permanent marker, indicating the sample number, species, and date. All manipulations were performed using sterile equipment and nitrile gloves to prevent contamination.

The whole experiment took 4 days, but could be done in less time. Two metal balls were added to each 2 mL round-bottom microcentrifuge tube with plant samples for mechanical tissue disruption. Before homogenization, the tissues were placed in liquid nitrogen for 10-15 minutes to freeze the samples. This was followed by a grinding process in a homogeniser under a fume hood by placing the tubes symmetrically in a chilled tube rack. The maximum speed was set.

Prior to DNA extraction, the lysis buffer solution was prepared for two batches under a fume hood, each containing 30 mL of CTAB, 0.6 g of PVP, and 60 μ L of β -mercaptoethanol. Preheated on a heating block at 65°C, 60 mL of CTAB Lysis Buffer BioChemica was separated into two tubes. 0.6 g of PVP was weighed for two tubes each and added. The solutions were vortexed. Finally, 2 portions of 60 μ L of β -mercaptoethanol were used to finish preparing the solutions. Total concentrations for 2 portions should contain 60 mL of CTAB, 120 μ L of β -mercaptoethanol, 1.2 g of PVP.

The next step, called lysis, started with adding 1100 μ L of prepared CTAB solution to the sample tubes, each followed by vortexing and incubation in 4 cycles for 15 minutes each at 65°C. It is important to note that after the first cycle, the metal balls should be discarded using a magnet, and the fourth cycle can be done on the second day; thus, the incubated samples (3rd cycle of incubation) were stored in a refrigerator.

On the next day, the samples were heated in a heating block at 65°C (4th cycle of incubation). 600-700 μ L of chloroform was added to each sample and vortexed. If the solution remained heterogeneous, the tubes were inverted several times to homogenise the mixture. The samples were centrifuged at 12000 rpm for 5 minutes at 4°C, after which the supernatants (300-350 μ L) were transferred to newly labelled 1.5 mL microcentrifuge tubes. DNA was precipitated by adding 3 M sodium acetate (pH 5.5) and 600 μ L of 96% ethanol. The tubes were gently inverted 10-20 times until visible DNA precipitation appeared, followed by overnight incubation at -20°C.

On the 3rd day, the samples were centrifuged at 12000 rpm for 15 minutes at 4°C. The supernatants were discarded, and the tubes were briefly air-dried before incubating in a concentrator at 60°C for 1 minute. DNA pellets were resuspended in 200 μ L of RNase-free ddH₂O; subsequently, 100 μ L of 5 M NaCl was added, and the mixture was then vortexed thoroughly. Next, 600 μ L of 96% ethanol was added, and the samples were mixed by gentle inversion, followed by incubation at -20°C for 30 minutes. The samples were centrifuged at 12000 rpm for 10 minutes, after which the supernatant was removed. The tubes were dried in a concentrator at 30°C for 2 minutes. 150 μ L ddH₂O (RNase-free) and 1 μ L RNase A (DNase- and Protease-free) were added to each sample and mixed by gentle inversion. Before storing the samples in the refrigerator, they were incubated for 30 minutes at 37°C.

On day 4, the DNA samples were measured using the NanoDrop UV/Vis Spectrophotometer (U2 MicroDigital, Republic of Korea). First, the tubes were vortexed and briefly centrifuged. The spectrophotometer pedestal was cleaned with a lint-free tissue prior to and between measurements, and 1.5 μ L of the DNA sample was used. DNA concentrations and absorbance ratios were recorded.

The standard method via the DNA extraction kit (Jena Bioscience, Germany) (Olawuyi et al., 2020) was also conducted to compare it with the CTAB-based method on *Rosa acicularis* and *Rosa spinosissima*. Sample collection and preparation were done the same way as in the above-mentioned protocol. Plant tissues were transferred to 2 mL microcentrifuge tubes containing two metal homogenization beads and frozen in liquid nitrogen for 10-15 minutes. The powdered material was homogenised at high speed using a bead homogeniser. Subsequently, 600 μ L of Cell Lysis Solution was added to each sample, followed by vortexing. The samples were incubated at 65°C for 60 minutes, with occasional vortexing to maximise particle lysis. After thawing, the tissue was removed using a magnet, and the lysates were stored at -20°C until the following day.

After thawing, 1.5 μ L of RNase A solution was added to each lysate. The samples were gently inverted and incubated at 37°C for 60 minutes, with occasional mixing. The lysates were then

concentrated, and 100 µL of protein precipitation solution was added. After mixing and cooling on ice for 10–15 minutes, the samples were centrifuged at 15,000 rpm for 15 minutes at -4°C.

The resulting supernatant was transferred to clean 1.5 mL microcentrifuge tubes, and 300 µL of chloroform was added. The samples were vortexed for 20–30 seconds and centrifuged at 15,000 rpm for 10 minutes and carefully transferred the upper aqueous phase containing genomic DNA to new tubes. The DNA was precipitated with an equal volume (300 µL) of pre-warmed isopropanol (>99%), then vigorously inverted. The samples were incubated overnight at -20°C and then centrifuged at 15,000 rpm for 5 minutes at -4°C. The supernatant was removed, and the DNA pellets were air-dried and washed with 500 µL of wash buffer. The pellets were resuspended by vortexing and incubated at 60°C until completely dissolved. The samples were centrifuged at 5,000 rpm for 5 minutes, after which the supernatant was removed. A wash step was performed once with 500 µL of wash buffer. The pellets were then washed with 500 µL of ultrapure water and centrifuged at 5,000 rpm for 5 minutes. Excess water was removed, and the DNA pellets were allowed to dry for at least 10 minutes. The DNA was then resuspended in 100 µL of DNA hydration solution and incubated at 65 °C for 60 minutes, with periodic agitation and brief centrifugation. The isolated DNA samples were stored at -20 °C until further analysis.

The concentration of the synthetic genomic DNA was measured using a NanoDrop micro-volume spectrophotometer. DNA quality was assessed based on concentration and spectrophotometric purity (Olawuyi et al., 2020). The quality of DNA samples was statistically compared with the quality of DNA samples extracted with CTAB-based methods to determine whether the sample purity is acceptable for further genetic analyses.

Results. Using the CTAB-based method, DNA yield varied significantly by *Rosa* species and sampling region (Table 1). For *Rosa acicularis*, the mean DNA yield was 385.24 ± 142.22 ng/µL in the Altai region and 230.68 ± 119.02 ng/µL in the Tarbagatay region; for *Rosa alberta*, the yield was 231.84 ± 109.41 ng/µL in the Altai region and 316.52 ± 220.64 ng/µL in the Tarbagatay region. Additionally, for *Rosa spinosissima*, the mean yield was 334.27 ± 186.43 ng/µL in the Altai region and 188.95 ± 89.38 ng/µL in the Tarbagatay region (Table 1).

Table 1. Results of DNA concentrations of *Rosa acicularis*, *Rosa alberta*, and *Rosa spinosissima* leaves by the CTAB-based method.

Species	Area	n	Mean (ng/µl)	SD (ng/µl)	Minimum	Maximum
<i>Rosa acicularis</i>	Altai	8	385.24	142.22	171.68	531.98
<i>Rosa acicularis</i>	Tarbagatai	8	230.68	119.02	51.84	409.72
<i>Rosa alberta</i>	Altai	8	231.84	109.41	42.85	373.94
<i>Rosa alberta</i>	Tarbagatai	8	316.52	220.64	3.83	654.75
<i>Rosa spinosissima</i>	Altai	7	334.27	186.43	96.89	682.24
<i>Rosa spinosissima</i>	Tarbagatai	8	188.95	89.38	37.89	292.76

Overall, DNA yield for samples extracted using the CTAB method ranged from 3.83 to 682.24 ng/µL. The highest mean yield was observed in *Rosa acicularis* from the Altai region (385.24 ng/µL), while the lowest was in *Rosa spinosissima* from the Tarbagatay region (188.95 ng/µL). Significant variability within groups was also observed, particularly for *Rosa alberta* from the Tarbagatay region (standard deviation [SD] = 220.64 ng/µL) and *Rosa spinosissima* from the Altai region (SD = 186.43 ng/µL). Some groups showed high standard deviation values (Table 1).

Samples processed using the original DNA extraction method exhibited higher variability (Table 2). The mean DNA concentration in *Rosa acicularis* samples from the Tarbagatai region was 37.93 ± 44.45 ng/µL. In contrast, the mean concentration in *Rosa spinosissima* samples from the Altai region was significantly lower, at only 6.80 ± 11.46 ng/µL (Table 2).

Table 2. Results of DNA concentrations of samples of *Rosa acicularis* and *Rosa spinosissima* leaves by the original DNA extraction method.

Species	Area	n	Mean (ng/μl)	SD (ng/μl)	Minimum	Maximum
<i>Rosa acicularis</i>	Tarbagatai	2	37.93	44.45	6.499	69.356
<i>Rosa spinosissima</i>	Altai	3	6.80	11.46	-2.765	19.5

DNA concentrations obtained using the CTAB and standard extraction methods differed significantly (Welch's t-test, $p = 5.94 \times 10^{-9}$) (Fig. 1). The CTAB method yielded significantly higher DNA concentrations than the standard method. The box plot also revealed considerable variability among samples obtained via the CTAB protocol, characterised by a wide interquartile range and overall distribution of DNA concentrations. In contrast, DNA concentrations obtained using the standard method were much lower and showed a narrower distribution. Several samples obtained via the CTAB method exhibited exceptionally high DNA concentrations exceeding 600 ng/μL, whereas concentrations obtained via the standard method generally remained below approximately 70 ng/μL ($p < 0.001$).

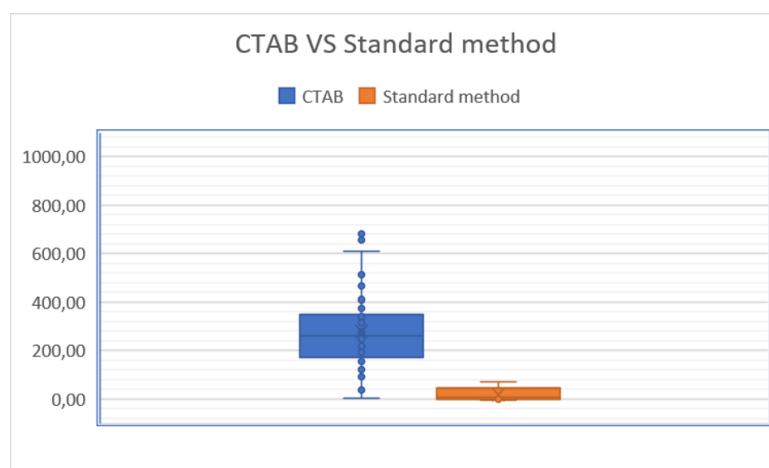


Figure 1. Box plot diagram showing the statistical difference between CTAB and the standard method ($p < 0.001$ ***; significant by Welch t-test)

The resulting DNA underwent PCR analysis using specific primers (Fig. 2).

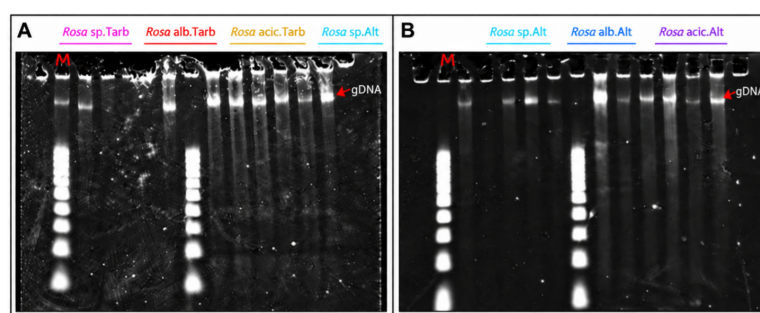


Figure 2. Agarose gel electrophoresis of genomic DNA (gDNA) from *Rosa* samples. (A) PCR amplification of samples from Tarbagatai (Tarb) and Altai (Alt) regions. (B) PCR amplification of samples from the Altai (Alt) region. M - DNA molecular weight marker; gDNA – genomic DNA

Discussion. The results indicate substantial differences in DNA yield from *Rosa* samples by extraction method. The CTAB method generally produced DNA concentrations above the overall mean; average values for the various groups (categorised by species and region) ranged from

188.95 to 385.24 ng/ μ L. However, the high standard deviation values recorded in some groups indicate significant variability among individual samples. The high variability among CTAB samples may reflect differences in how individual plant samples respond to the extraction procedure. Such variability may be linked to differences - across species or tissues - in the abundance of polysaccharides, polyphenolic compounds, pigments, and other secondary metabolites. These compounds can complicate the isolation and purification of plant genomic DNA and contribute to variation in DNA recovery among samples (Darling, 2022).

Significant differences in DNA concentration may reflect the specific biochemical composition of the leaf samples. Species of the genus *Rosa* contain high levels of polysaccharides, polyphenolic compounds, and other secondary metabolites that can interfere with DNA extraction and subsequent molecular analysis (Aboul-Maaty & Oraby, 2019). CTAB-based extraction protocols are widely used for plant samples because CTAB removes polysaccharides, while the addition of PVP and β -mercaptoethanol helps neutralise the effects of phenolic compounds (Porebski et al., 1997; Yadav et al., 2018; Buljević et al., 2025). Thus, the high DNA yield observed in several groups demonstrates the effectiveness of this CTAB-based protocol for most of the analysed *Rosa* samples (Xu et al., 2004; Jabbarzadeh et al., 2009).

However, DNA concentration alone does not fully reflect the quality of the extracted DNA. This is especially important for plant samples containing secondary metabolites, as co-extracted compounds can inflate spectrophotometer readings without necessarily indicating the presence of high-quality genomic DNA (Siegel et al., 2017; Tuncay & Uzun, 2025). When comparing primary extraction results from the two methods using Welch's t-test, we found statistically significant differences in DNA yield ($p < 0.001$). This suggests that the improved DNA recovery with the CTAB procedure may be due to the extraction chemistry's ability to facilitate cell lysis and separate DNA from a complex mixture of plant compounds. This is particularly important for *Rosa* species, which can contain high levels of phenolic compounds and other secondary metabolites. Modified CTAB procedures incorporating components such as PVP have previously been developed for plant tissues rich in polyphenols and polysaccharides (Porebski et al., 1997; Yadav et al., 2018; Buljević et al., 2025).

A significant limitation of this comparative study is the relatively small number of samples and the insufficient amount of reagents used for the primary extraction method. Specifically, the standard method sample included only two *R. acicularis* specimens from Tarbagatai and three *R. spinosissima* specimens from the Altai region. Future research should be conducted on a larger number of samples.

The results also suggest that extraction efficiency may vary by species and geographic origin. The results indicate that extraction efficiency can vary depending on the species and geographical origin of the samples. For instance, when using the CTAB protocol, *R. acicularis* samples from the Altai region yielded higher average DNA concentrations than those from the Tarbagatai region. In contrast, the opposite was true for *R. alberta*. A similar pattern was observed for *R. spinosissima*; DNA concentrations were higher in the Altai samples than in the Tarbagatai samples. These differences could reflect variation in leaf tissue properties, physiological state, environmental conditions, or secondary metabolite content in plants grown under different conditions (Yang et al., 2018; Reshi et al., 2023). However, because species and geographic groups were unevenly represented across the two sampling methods, this dataset cannot disentangle the influence of these factors from the effect of the sampling protocol itself.

Overall, the CTAB protocol yielded sufficient DNA for a significant proportion of the *Rosa* samples and represents a viable method for DNA extraction from this genus. For samples consistently yielding low DNA concentrations or unsatisfactory amplification results, further optimisation of parameters - such as the amount of plant material, grinding method, incubation conditions, and purification steps - may be required.

Conclusion. The results showed that modified CTAB was suitable for extracting genomic DNA from leaves of *Rosa acicularis*, *Rosa alberta*, and *Rosa spinosissima* collected in the Altai and Tarbagatay regions of Kazakhstan.

The CTAB method yielded significantly higher DNA concentrations than samples that

underwent a standard DNA extraction method. Therefore, the modified CTAB protocol can be considered a more effective method for extracting genomic DNA from *Rosa* leaves for subsequent analysis. However, further investigation with larger sample quantities is needed.

References

- Aboul-Maaty, N. A. F., & Oraby, H. A. S. (2019). Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. *Bulletin of the National Research Centre*, 43(1), 1-10.
- Allen, G. C., Flores-Vergara, M. A., Krasynanski, S., Kumar, S., & Thompson, W. F. (2006). A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nature Protocols*, 1(5), 2320-2325.
- Buljević, N., Preiner, D., Šikuten, I., & Tomaz, I. (2025). Comparison of Different DNA Isolation Methods from Grapevine (*Vitis vinifera*) Leaves. *Separations*, 12(11), 316.
- Csaikl, U. M., Bastian, H., Brettschneider, R., Gauch, S., Meir, A., Schauerte, M., ... & Ziegenhagen, B. (1998). Comparative analysis of different DNA extraction protocols: a fast, universal maxi-preparation of high quality plant DNA for genetic evaluation and phylogenetic studies. *Plant Molecular Biology Reporter*, 16(1), 69-86.
- Danilova, A. N., Vdovina, T. A., Kotukhov, Y. A., Anufriyeva, O. A., Vinokurov, A. A., Isakova, E. A., ... & Sumbembayev, A. A. (2025). Study of the Geographical Distribution, Ecological-Biological Characteristics, and Economic Value of *Rosa acicularis* Lindl., *Rosa laxa* Retz., and *Rosa spinosissima* L.(Rosaceae) in Kazakhstan's Part of the Altai Mountains. *Diversity*, 17(7), 441.
- Darling, H. S. (2022). Do you have a standard way of interpreting the standard deviation? A narrative review. *Cancer Research, Statistics, and Treatment*, 5(4), 728-733.
- Debener, T., & Linde, M. (2009). Exploring complex ornamental genomes: the rose as a model plant. *Critical reviews in plant sciences*, 28(4), 267-280.
- Dehestani, A., & Tabar, S. K. (2007). A rapid efficient method for DNA isolation from plants with high levels of secondary metabolites. *Asian J. Plant Sci*, 6(6), 977-981.
- Dissanayake, A. J., Bhunjun, C. S., Maharachchikumbura, S. S. N., & Liu, J. K. (2020). Applied aspects of methods to infer phylogenetic relationships amongst fungi. *Mycosphere*, 11(1), 2652-2676.
- dos Santos, S., Piculell, L., Medronho, B., Miguel, M. G., & Lindman, B. (2012). Phase behavior and rheological properties of DNA-cationic polysaccharide mixtures. *Journal of colloid and interface science*, 383(1), 63-74.
- Doyle, J. J. L. (1987). Genomic plant DNA preparation from fresh tissue □ CTAB method. *Phytochemical bulletin*, 19(11), 11.
- Ergashov, I., Ismonova, G., Ibrokhimova, G., Yusupov, Z., Mamasoliev, S., Berdiyev, M., & Naralieva, N. (2026). Comparative chloroplast genome analysis and phylogeny of the genus *Rosa* (Rosaceae). *Analele Universităţii din Oradea, Fascicula Biologie*, 33(1), 24-32.
- Friar, E. A. (2005). Isolation of DNA from plants with large amounts of secondary metabolites. In *Methods in enzymology* (Vol. 395, pp. 1-12). Academic Press.
- Gaurav, A. K., Namita, Raju, D. V. S., Ramkumar, M. K., Singh, M. K., Singh, B., ... & Sevanthi, A. M. (2022). Genetic diversity analysis of wild and cultivated *Rosa* species of India using microsatellite markers and their comparison with morphology based diversity. *Journal of Plant Biochemistry and Biotechnology*, 31(1), 61-70.
- Hadi, S. M., Bhat, S. H., Azmi, A. S., Hanif, S., Shamim, U., & Ullah, M. F. (2007, October). Oxidative breakage of cellular DNA by plant polyphenols: a putative mechanism for anticancer properties. In *Seminars in cancer biology* (Vol. 17, No. 5, pp. 370-376). Academic Press.
- Heikrujam, J., Kishor, R., & Mazumder, P. B. (2020). The chemistry behind plant DNA isolation protocols. In *Biochemical analysis tools-Methods for bio-molecules studies*. IntechOpen.
- Jabbarzadeh, Z., Khosh-Khui, M., Salehi, H., & Saberivand, A. (2009). Optimization of DNA extraction for ISSR studies in seven important rose species of Iran.
- Japelaghi, R. H., Haddad, R., & Garoosi, G. A. (2011). Rapid and efficient isolation of high quality nucleic acids from plant tissues rich in polyphenols and polysaccharides. *Molecular biotechnology*, 49(2), 129-137.
- Jobes, D. V., Hurley, D. L., & Thien, L. B. (1995). Plant DNA isolation: a method to efficiently remove polyphenolics, polysaccharides, and RNA. *Taxon*, 44(3), 379-386.
- Jones, M. M., Nagalingum, N. S., & Handley, V. M. (2023). Testing protocols to optimize DNA extraction from tough leaf tissue: A case study in *Encephalartos*. *Applications in plant sciences*, 11(3), e11525.
- Kaul, K., Dhyani, D., & Sharma, R. K. (2009). Evaluation of DNA extraction methods for RAPD, SSR and AFLP analyses of wild rose species. *Floriculture and Ornamental Biotechnology*, 3(1), 25-30.
- Khanuja, S. P., Shasany, A. K., Darokar, M. P., & Kumar, S. (1999). Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. *Plant molecular biology Reporter*, 17(1), 74-74.
- Koopman, W. J., Wissemann, V., De Cock, K., Van Huylbroeck, J., De Riek, J., Sabatino, G. J., ... & Smulders, M. J. (2008). AFLP markers as a tool to reconstruct complex relationships: a case study in *Rosa* (Rosaceae). *American Journal of Botany*, 95(3), 353-366.
- Liu, B., Wu, H., Cao, Y., Ma, G., Zheng, X., Zhu, H., ... & Sui, S. (2025). Reducing costs and shortening the cetyltrimethylammonium bromide (CTAB) method to improve DNA extraction efficiency from wintersweet and some other plants. *Scientific Reports*, 15(1), 13441.
- Lodhi, M. A., Ye, G. N., Weeden, N. F., & Reisch, B. I. (1994). A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Plant molecular biology Reporter*, 12(1), 6-13.
- Lutz, K. A., Wang, W., Zdepski, A., & Michael, T. P. (2011). Isolation and analysis of high quality nuclear DNA with reduced organellar DNA for plant genome sequencing and resequencing. *BMC biotechnology*, 11(1), 54.
- Maloupa, E., Karapatzak, E., Ganopoulos, I., Karydas, A., Papanastasi, K., Kyrkas, D., ... & Krigas, N. (2021). Molecular authentication, phytochemical evaluation and asexual propagation of wild-growing *Rosa canina* L.(Rosaceae) genotypes of Northern Greece for

sustainable exploitation. *Plants*, 10(12), 2634.

Matsumoto, S., Kouchi, M., Yabuki, J., Kusunoki, M., Ueda, Y., & Fukui, H. (1998). Phylogenetic analyses of the genus *Rosa* using the matK sequence: molecular evidence for the narrow genetic background of modern roses. *Scientia Horticulturae*, 77(1-2), 73-82.

Olawuyi, O. J., Erhonsele, O. O., Chukwuka, K. S., & Faneye, A. O. (2020). Morphological and Genomic responses of *Pennisetum glaucum* L. (Pearl Millet) to Lead nitrate. *Advances in Environmental Biology*, 14(7), 31-46.

Patwardhan, A., Ray, S., & Roy, A. (2014). Molecular markers in phylogenetic studies-a review. *Journal of Phylogenetics & Evolutionary Biology*, 2(2), 131.

Perlman, D., Chikarmane, H., & Halvorson, H. O. (1987). Improved resolution of DNA fragments in polysaccharide-supplemented agarose gels. *Analytical biochemistry*, 163(1), 247-254.

Porebski, S., Bailey, L. G., & Baum, B. R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant molecular biology reporter*, 15(1), 8-15.

Reshi, Z. A., Ahmad, W., Lukatkin, A. S., & Javed, S. B. (2023). From nature to lab: A review of secondary metabolite biosynthetic pathways, environmental influences, and in vitro approaches. *Metabolites*, 13(8), 895.

Rezadoost, M. H., Kordrostami, M., & Kumleh, H. H. (2016). An efficient protocol for isolation of inhibitor-free nucleic acids even from recalcitrant plants. *3 Biotech*, 6(1), 61.

Ritz, C. M., Schmutz, H., & Wissemann, V. (2005). Evolution by reticulation: European dogroses originated by multiple hybridization across the genus *Rosa*. *Journal of Heredity*, 96(1), 4-14.

Rogers, S. O., & Bendich, A. J. (1988). Extraction of DNA from plant tissues. In *Plant molecular biology manual* (pp. 73-83). Dordrecht: Springer Netherlands.

Ruggieri, J., Kemp, R., Forman, S., & Van Eden, M. E. (2016). Techniques for nucleic acid purification from plant, animal, and microbial samples. In *Sample Preparation Techniques for Soil, Plant, and Animal Samples* (pp. 41-52). New York, NY: Springer New York.

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.

Siegel, C. S., Stevenson, F. O., & Zimmer, E. A. (2017). Evaluation and comparison of FTA card and CTAB DNA extraction methods for non-agricultural taxa. *Applications in plant sciences*, 5(2), 1600109.

Steele, P. R., & Pires, J. C. (2011). Biodiversity assessment: State-of-the-art techniques in phylogenomics and species identification. *American Journal of Botany*, 98(3), 415-425.

Tuncay, H. O., & Uzun, F. S. (2025). Effects of Drying Methods, Tissue Types and Grinding Methods on Plant DNA Quality. *European Journal of Biology*, 84(1), 103-113.

Varma, A., Padh, H., & Shrivastava, N. (2007). Plant genomic DNA isolation: an art or a science. *Biotechnology Journal: Healthcare Nutrition Technology*, 2(3), 386-392.

Vdovina, T. A., Lagus, O. A., Isakova, E. A., & Vinokurov, A. A. (2024). State of coenopopulations of wild berry plants in the territory of Kazakhstan Altai. *Fundamental and Experimental Biology*, 11629(4), 129-134.

Wissemann, V., & Ritz, C. M. (2005). The genus *Rosa* (Rosoideae, Rosaceae) revisited: molecular analysis of nrITS-1 and atp B-rbc L intergenic spacer (IGS) versus conventional taxonomy. *Botanical journal of the Linnean Society*, 147(3), 275-290.

Xin, Z., & Chen, J. (2012). A high throughput DNA extraction method with high yield and quality. *Plant methods*, 8(1), 26.

Xu, Q., Wen, X., & Deng, X. (2004). A simple protocol for isolating genomic DNA from chestnut rose (*Rosa roxburghii* Tratt) for RFLP and PCR analyses. *Plant Molecular Biology Reporter*, 22(3), 301-302.

Yadav, S., Ram, C., Singh, S., & Rana, M. K. (2018). Plant genomic DNA isolation—the past and the present, a review. *Indian Journal of Plant Genetic Resources*, 31(03), 315-327.

Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. *Molecules*, 23(4), 762.

Yang, Z., & Rannala, B. (2012). Molecular phylogenetics: principles and practice. *Nature reviews genetics*, 13(5), 303-314.

Yermagambetova, M., Imanbayeva, A., Ishmuratova, M., Sumbembayev, A., & Almerikova, S. (2025). Characterization of the four *rosa* L. Species from Kazakhstan based on complete plastomes and nuclear ribosomal internal transcribed spacer (ITS) sequences. *Genes*, 16(8), 852.

Yockteng, R., Almeida, A. M., Yee, S., Andre, T., Hill, C., & Specht, C. D. (2013). A method for extracting high-quality RNA from diverse plants for next-generation sequencing and gene expression analyses. *Applications in plant sciences*, 1(12), 1300070.