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A METHOD FOR TOTAL RNA EXTRACTION FROM SPECIES OF COUSINIA CASS

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ABOUT ARTICLE

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Abstract: This study investigated the extraction of total RNA from plant species of the genus Cousinia Cass. and the assessment of its quality. These plants are important components of the Central Asian flora and are characterized by their adaptation to various environmental stress factors. The main objective of the study was to develop an effective method for obtaining high-quality RNA from plant tissues and to evaluate its suitability for subsequent molecular analyses. RNA extraction was performed using a commercial RNA extraction kit. Samples were ground in liquid nitrogen, subjected to lysis, and purified using a spin-column technology. The quality and quantity of the extracted RNA were assessed using a NanoDrop spectrophotometer and agarose gel

electrophoresis. The results demonstrated high RNA purity (A260/A280 = 1.9–2.1) and the absence of substantial degradation. The method was found to be suitable for gene expression studies, RT-PCR, and RT-qPCR analyses in Cousinia species. These results provide an important basis for future studies of the molecular mechanisms underlying stress tolerance at the molecular level.

Introduction. The adaptation of plants to various environmental factors is closely associated with their genetic and molecular characteristics. In particular, abiotic stress factors such as drought, salinity, and high temperature directly affect plant growth, development, and distribution. Investigating the molecular basis of these processes is an important area of modern plant biology and molecular research [1–3]. Molecular biological approaches, particularly RNA-based analyses, have been widely applied to investigate plant responses to environmental stress and to identify stress-responsive genes [1–3].

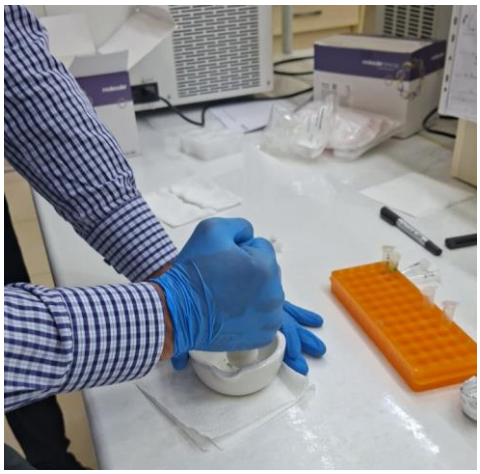
Species of the genus Cousinia Cass., which are widely distributed in the flora of Central Asia, are ecologically important and are characterized by adaptation to diverse natural conditions. These species represent suitable study organisms for investigating genetic diversity, stress tolerance, and adaptability. The analysis of gene expression at the RNA level provides valuable information for understanding the molecular mechanisms underlying plant responses to environmental factors [1,3,6]. Therefore, obtaining high-quality RNA from plant tissues is essential for reliable downstream molecular analyses, including RT-PCR and RT-qPCR [2,3,6].

A variety of methods for total RNA extraction from plant tissues have been described, including classical CTAB-based protocols and commercial purification systems based on spin-column technology [4,8–10]. However, obtaining high-quality RNA from plant tissues rich in phenolic compounds, polysaccharides, and other secondary metabolites remains challenging [5–7,11–15]. Such compounds may interfere with RNA purification and downstream molecular analyses; therefore, maintaining RNA purity and integrity and preventing RNA degradation are important considerations during RNA extraction [4–7,11,12,14,15].

Plants of the family Asteraceae, including representatives of the genus Cousinia, may contain substantial amounts of secondary metabolites, which can complicate nucleic-acid extraction. Previous studies have demonstrated that the use of reducing agents such as β-mercaptoethanol and additives such as polyvinylpyrrolidone (PVP) can improve RNA quality in plant tissues rich in secondary metabolites [6,11,15]. RNA quality is commonly assessed

using spectrophotometric parameters such as the A260/A280 and A260/A230 ratios, together with electrophoretic assessment of RNA integrity, including the 28S and 18S rRNA bands [5,7,12–14]. High-quality RNA is essential for accurate downstream applications such as RT-PCR and RT-qPCR [2,5–7,11,15].

Despite the importance of RNA-based molecular analyses, an effective and reproducible RNA extraction procedure specifically applicable to Cousinia species is needed. Therefore, the aim of this study was to develop an effective method for total RNA extraction from Cousinia species, assess the quality of the extracted RNA, and determine its suitability for subsequent molecular analyses. The results provide a methodological basis for future studies of gene expression and the molecular mechanisms underlying stress tolerance in Cousinia species.



Materials and methods

Figure 1. Laboratory process of RNA extraction from Cousinia Cass. leaves.

RNA Extraction Method

RNA extraction protocol used in the study. In this study, total RNA was extracted from Cousinia Cass. plant tissues using a commercial RNA extraction kit. This method enables the isolation of high-quality, non-degraded RNA and is suitable for subsequent molecular analyses, including RT-PCR and RT-qPCR.

For the experiment, 50–100 mg of freshly collected or dried plant material was placed in sterile porcelain mortars. A total of 500 µL of Lysis Buffer was added to each sample, followed by 5 µL of 2-mercaptoethanol (or DTT and PVP) to prevent RNA degradation and reduce oxidation. 2-Mercaptoethanol and PVP help inactivate phenolic compounds present in plant tissues and thereby contribute to maintaining RNA quality (Figure 1).

Homogenization: The sample was thoroughly homogenized mechanically using a sterile pestle. The resulting homogenate was transferred into sterile 1.5-mL Eppendorf tubes and mixed thoroughly on a vortex mixer for 10–20 seconds.

Lysis and Incubation: The homogenized samples were incubated at 56 °C for 3–5 minutes. During this step, cellular structures are disrupted and RNA is released into the solution.

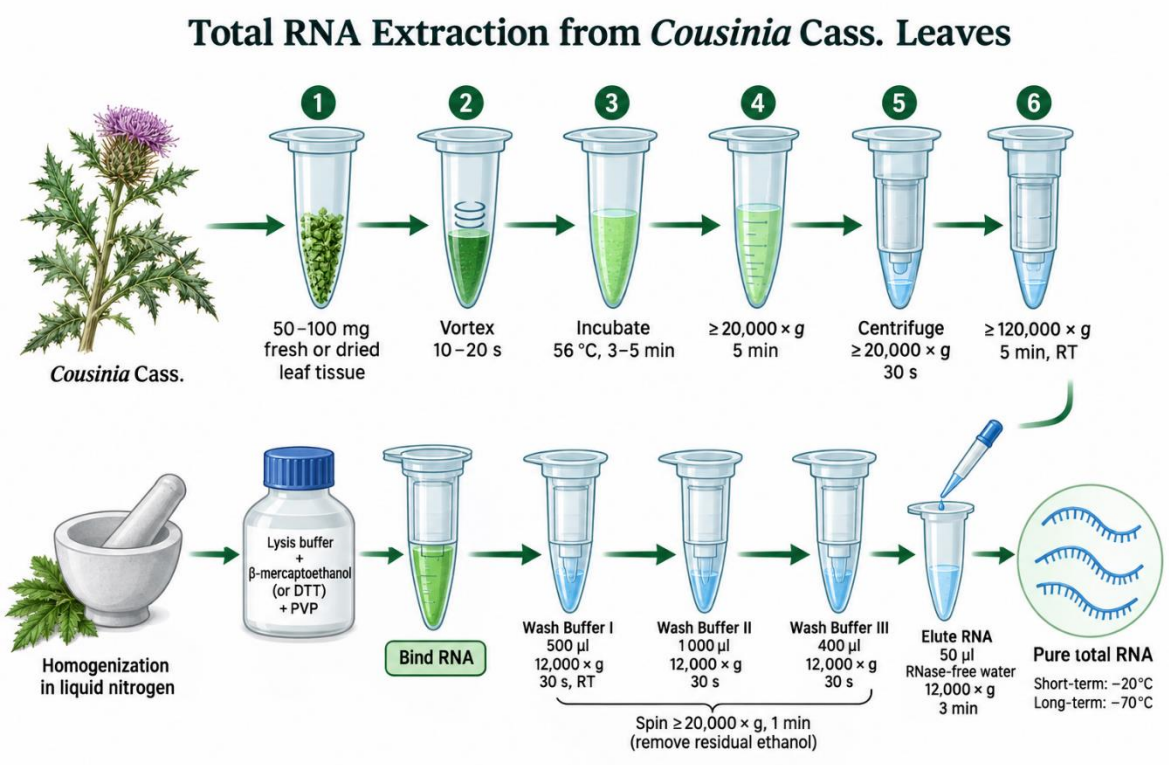


Figure 2. Schematic representation of the total RNA extraction procedure.

Centrifugation: The samples were centrifuged at ≥20,000 × g for 5 minutes at room temperature. As a result, cellular debris formed a pellet, while the RNA-containing supernatant remained in the upper phase. Approximately 450–550 µL of supernatant was carefully transferred to a new sterile tube.

RNA Purification Using a Spin Column: A total of 300 µL of 96% ethanol was added to the extracted supernatant and mixed thoroughly. This facilitates binding of RNA to the silica membrane. From the resulting mixture, 400 µL was transferred to a spin column and

centrifuged at 12,000 × g for 1 minute; the flow-through was discarded. The remaining mixture was loaded onto the same column and centrifuged at 12,000 × g for 30 seconds.

Table 1. Spectrophotometric measurements and quality assessment of RNA samples

Sample	Concentration (ng/μL)	A260/A280	A260/A230	Scientific assessment
Sample 1	171.80	1.89	3.689	Pure RNA; A260/A280 is good. A260/A230 is unusually high, possibly due to PVP or salt residues.
Sample 2	240.45	2.066	2.469	A260/A280 > 2, which may indicate an admixture of other nucleic acids. Overall quality is good.
Sample 3	165.45	1.818	2.720	The most favorable quality indicator. RNA appears stable, with low contamination.
Sample 4	412.25	1.909	2.477	Very high concentration. Quality is good; dilution is recommended before PCR-based analyses.

Washing Steps: The following steps were performed to remove proteins and residual salts from the RNA: 500 μL of Wash Buffer I was added, followed by centrifugation at 12,000 × g for 30 seconds; the flow-through was discarded. Then, 400 μL of Wash Buffer II was added and the column was centrifuged at 12,000 × g for 30 seconds. If necessary, the second washing step was repeated.

The empty column was then centrifuged at ≥20,000 × g for 1 minute to completely remove residual ethanol.

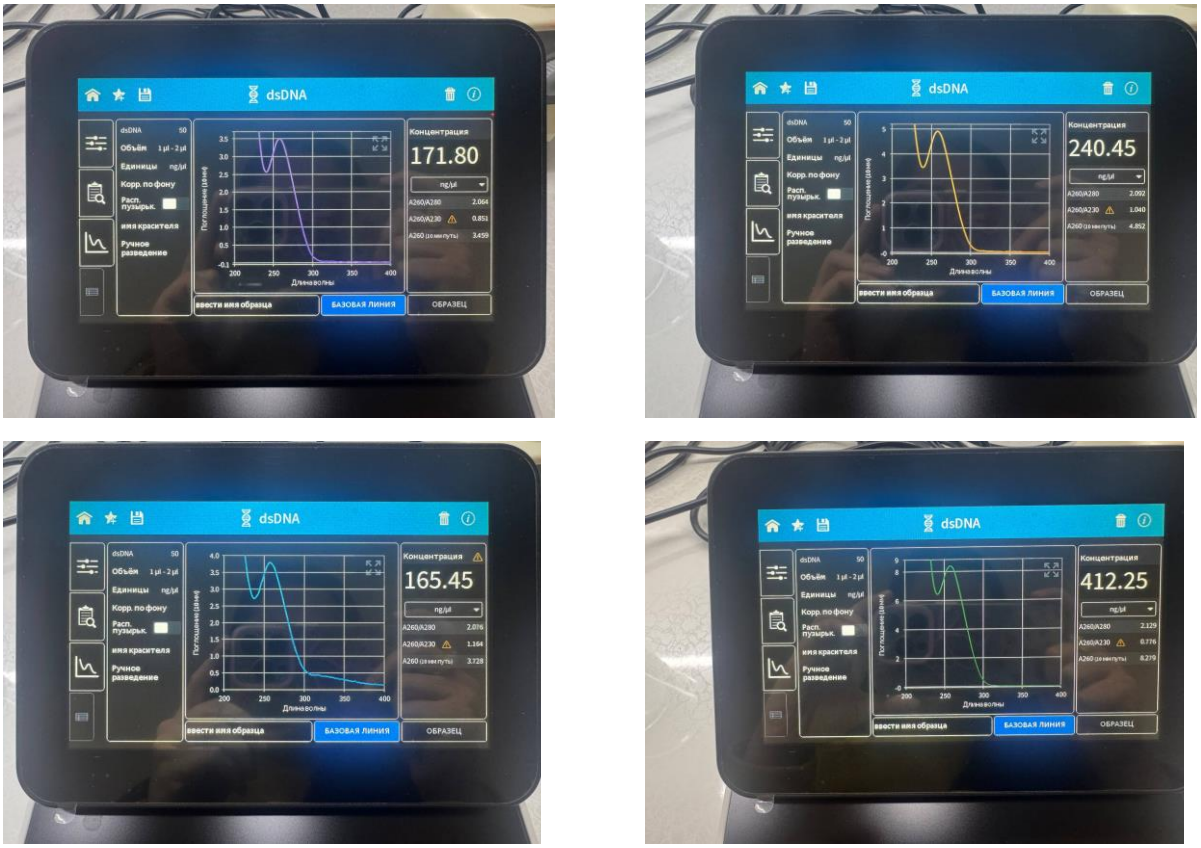


Figure 3. Absorption spectra and quality parameters of the extracted RNA samples measured using a spectrophotometer.

RNA Elution

The cleaned columns were transferred to new sterile tubes. A volume of 50 µL of RNase-free water was applied to the center of the membrane and allowed to stand for 1 minute, followed by centrifugation at 12,000 × g for 3 minutes. High-quality, purified total RNA was obtained.

RNA Storage and Quality Assessment

The extracted RNA was stored at −20 °C for short-term storage and at −70 °C for long-term storage (Figure 2).

RNA quality and concentration were assessed using a NanoDrop spectrophotometer (A260/A280 = 1.9–2.1) and 1% agarose gel electrophoresis by evaluating the 28S and 18S rRNA bands [5,7].

Assessment of RNA Concentration and Purity

The concentration and purity of the extracted RNA samples were assessed using spectrophotometry (Table 1). The measured RNA concentrations ranged from 165.45 ng/µL to

412.25 ng/μL, indicating that the samples contained sufficient RNA for subsequent molecular analyses.

The A260/A280 ratios ranged from 1.818 to 2.066 across all samples, indicating low or minimal protein contamination. In Sample 2, the ratio exceeded 2.0, which may indicate the presence of other nucleic acids, such as DNA, in the RNA preparation. In Sample 3, the A260/A280 ratio of 1.818 was considered relatively optimal, indicating that the RNA sample was stable and of good quality.

The A260/A230 ratios ranged from 2.469 to 3.689. Values above the conventional range in some samples, particularly the value of 3.689 in Sample 1, may be associated with background effects during spectrophotometric measurement or residual extraction reagents, such as salts or polyphenol-binding compounds. Nevertheless, the overall purity of the RNA samples was considered satisfactory.

Sample 4 had the highest RNA concentration (412.25 ng/μL), while its quality indicators were also good. However, because of the high concentration, dilution of the sample before subsequent molecular analyses, particularly PCR-based applications, is advisable. Overall, the results demonstrate that the extracted RNA samples had sufficient concentrations and satisfactory purity and could be used in subsequent molecular biological studies (Figure 3).

Advantages of the Method

The method is characterized by the following advantages: efficient extraction of high-quality RNA from plant tissues; effectiveness with samples rich in phenolic compounds and polysaccharides; suitability for RT-PCR and RT-qPCR; and high reproducibility.

Conclution. This study investigated the extraction of total RNA from plant species of the genus Cousinia Cass. and evaluated the quality of the extracted RNA. The methodology based on a commercial RNA extraction kit enabled the isolation of high-quality, non-degraded RNA from plant tissues. According to NanoDrop spectrophotometric analysis, the RNA samples showed high purity (A260/A280 = 1.9–2.1), while the A260/A230 ratios were generally within acceptable ranges. Agarose gel electrophoresis confirmed the preservation of RNA integrity. These results indicate that the extracted RNA was suitable for subsequent RT-PCR and RT-qPCR analyses.

Important advantages of the method include its applicability to plant samples rich in phenolic compounds, high reproducibility, and reliability for molecular analyses. The results provide an important scientific basis for studying gene expression, identifying molecular mechanisms involved in responses to abiotic stress, and evaluating stress tolerance in Cousinia species. In the future, the results of this study may serve as a methodological basis for

investigating the molecular mechanisms of stress adaptation in plant species of the Central Asian flora, as well as for breeding and biotechnological research.

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