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# Magnetic nanoparticles: a promising component in RNA extraction process

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## ABSTRACT

Magnetic nanoparticles separation technology is a method for quick and easy extraction biomolecules such as proteins, DNA and RNA. The present work describes total RNA isolation procedure from transformed rose petals in our laboratory using magnetic nanoparticles as a solid phase absorbant. Petals are the main sources of secondary metabolites, i.e. carotenoids, anthocyanins, flavonoids and phenolic compounds, which interfere with nucleic acids isolation. The physical basis of this technique relies on the interaction with external magnetic fields, and therefore the magnetic moment of the particles and nucleic acid plays the main role. The present work showed that, quantity and quality of extracted RNA by magnetic procedure were higher than that of the conventional method in all tested samples. Additionally, preparing RNA samples, take less than 50 minutes as against several hours taken by common protocols. Furthermore, successful RNA isolation was found to follow-up reactions such as PCR amplification and restriction endonuclease digestion especially in colorful petals. The solid-phase extraction method for the isolation of RNA in this research offers several advantages over the conventional methods using phenol-chloroform extraction: it is convenient to use, rapid, time-saving and reducing the consumption of toxic organic solvents; therefore, making it more amenable to automation.

**Keywords:** Magnetic nanoparticles; PCR; RNA extraction; Transformed Rose petal

## Introduction

The amount of high quality RNA isolation is an important and critical procedure and can be a limiting factor in plant molecular biology experiments, such as cDNA synthesis and cDNA library construction, PCR and RT-qPCR amplification technologies, northern blot hybridization and microarray method (1). RNA extraction from some plant tissues is especially difficult due to high levels of polysaccharides and secondary metabolites contaminants such as carotenoids, anthocyanins, flavonoids and poly-phenolic compounds that co-precipitate with RNA and result in low yields of poor quality (2, 3). The RNA isolation

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from petals of transformed rose (*Rosa hybrida* L.), may be particularly difficult because of the presence of large quantities of secondary metabolites that constitute the major obstacle of extraction and purification (4, 5, 6). It has been reported that phenolic compounds oxidize to quinone and this aromatic compound covalently binds to RNA, that cause brown colour and reduces maintenance time; thereby hinders downstream applications (7, 8). Also, co-precipitated polysac-charides with RNA conta-minate extraction process (9, 10, 11). Additionally, polysaccharides contaminants make isolated nucleic acid more viscous and inhibit Taq polymerase and restriction enzyme activity (12, 13, 14). Therefore, a reliable method is necessary for the isolation of high yields of excellent quality of RNA from rose petals tissues.

Several RNA extraction procedures from various plant sources have been described. The hot borate method and its modifications (15) were extensively used in different laboratories, but these methods are time consuming (16). A large number of protocols for RNA isolation are based on toxic organic solvents such as phenol or phenol/chloroform (17, 18, 19) that do not always provide adequate quantity and quality of RNA. Furthermore, phenol is readily absorbed through the skin and can cause nausea, burns and death (20). Additionally, there are numerous RNA isolation kits, but the main problem with these commercially available kits, is their high cost per sample (8, 21, 22). Most of the above-mentioned methods are time-consuming, expensive and result in a low yield of RNA. Therefore, the aim of this study was to introduce RNA isolation by magnetic nanoparticles separation technology from transformed rose petals tissues as a simple, rapid, economic and convenient method for subsequent experiments.

## Materials and Methods

*Plant samples for RNA isolation*

Transformed red-color roses were obtained from Iranian Research Organization for Science and Technology (IROST), Tehran. Transgenic explants are result of transferring target genes to somatic embryogenesis calli of cut-roses by *Agrobacterium* co-cultivation.

*Extraction method*

In the present work, isolation procedure was performed by GABIT (Genetics and Agricultural Biotechnology Institute of Tabarestan) Kit (patent No. 87847). Initially, the samples (10 mg petal tissue) were ground in liquid nitrogen in a sterile pre-chilled mortar and pestle. Afterwards, according to the protocol, lysis buffer (guanidinium thiocyanate, triton X-100, sodium acetate, sodium citrate, sodium chloride, tris hydrochloride, ethylene diamine tetra acetic acid, potassium acetate, glucose, diethyl pyrocarbonate, polyvinylpyrrolidone, N-lauryl sarco-sine, pH 3.5) was added to the tube. Then, protein kinase K (2 mg $ml^{-1}$) with DTT or 2-ß-mercapto-ethanol was added to the mixture and the tubes were vortexed. Then, the tubes were incubated in a 65°C water bath for 10 min and then centrifuged at 10000 g for 10 min at 4°C. The supernatant was transferred to a new test tube including magnetic nanoparticles. Then, the tubes inverted slowly such a way that nanoparticles form suspension in solution, completely. Binding buffer (polyethylene glycol, sodium chloride, diethyl pyrocarbonate) was added in this step and then tubes were shaken vigorously. The tubes were centrifuged at 8000 g for 5 min at 4°C and the supernatant was discarded. Then washing buffer (ethylene diamine tetra acetic acid, tris hydrochloride, ethanol, guanidine hydrochloride, diethyl pyro-carbonate, pH 5.6) was added to the tubes which were shaken vigorously. After centrifugation at 8000 g for 5 min at 4°C, the supernatant was discarded and tubes including magnetic nanoparticles were dried. The next step, elution buffer (tris hydrochloride, ethylene diamine tetra acetic acid, diethyl pyrocarbonate, pH 8.9) was added to tubes such a way that magnetic nanoparticles are completely suspended. Then tubes were incubated in a 60°C water bath for 15 min and after that centrifuged at 8000 g for 5 min at 4°C, supernatant containing the RNA was transferred to a fresh tube and incubated at –80°C.

*Assessment of RNA quantity and quality*

Purity and concentration of RNA samples were assessed using a spectrophotometer (Eppendorf BioPhotometer ®D30). Integrity of total RNA was evaluated on a 1% agarose gel using universal

markers of 28S rRNA, 18S rRNA and 5S rRNA (23) (Fig. 1a).

PCR was carried out with Rh-alpha tubulin primers following initial denaturing template at 94°C for 2 min. The cyclic parameters were 94°C, 30s; 59°C, 30 s; 72°C, 30s for 35 cycles, followed by a final extension period at 72°C for 5 min. The amplified product (10–15 µl) was checked by agarose gel electrophoresis which was subsequently stained with ethidium bromide and visualized under UV light (Fig. 1b).

The primers (accession no. AF394915) with forward (5´-CCACCTACACCAATCTCAATC-3´) and reverse (5´-CTGAATGTGGATGTGACTGAG-3´) were designed by AlleIID software (Version: 7.5) to produce amplicons size 98 bp.

## Results

The high quality of extracted RNA by the magnetic nanoparticles protocol has been shown by the intensity of 28S, 18S and 5S rRNA markers on agarose gel with no DNA contamination in all samples (Fig. 1a). Additionally, high quantity of extracted RNA was obtained according to spectrophotometric measurements (Table 1).

RNA preparations from different transformed rose lines did not reveal high level of RNA degradation that may occur during isolation. The A260/280 ratios of RNA extracts indicated that there was no protein, phenol or other contaminants that absorb strongly at or near 280 nm. Suitability of the purified RNA for downstream applications was validated by the PCR after synthesis of cDNA. A fragment of about 98 bp was successfully amplified from cDNA using house-keeping gene primers (Figure 1b), whereas a very fade 28S and 18S band was observed on agarose gel from RNA extracted by RNeasy®Plant Mini Kit with 1.07 and 1.02 nm A260/A280 and A260/A230 absorbance ratios by spectrophotometer, respectively (data not shown).

**Table 1. Yield and purity of RNA isolated from transformed rose petal using magnetic nanoparticles method**

| Transformed rose lines | A260/A280 | A260/A230 | Yeild (ng $\mu l^{-1}$) |
|---|---|---|---|
| L4 | 1.98 ± 0.04 | 2.03 ± 0.07 | 19250 ± 1.72 |
| L7 | 1.98 ± 0.03 | 2.08 ± 0.04 | 19660 ± 0.24 |
| L11 | 2.02 ± 0.02 | 2.14 ± 0.03 | 19360 ± 0.05 |

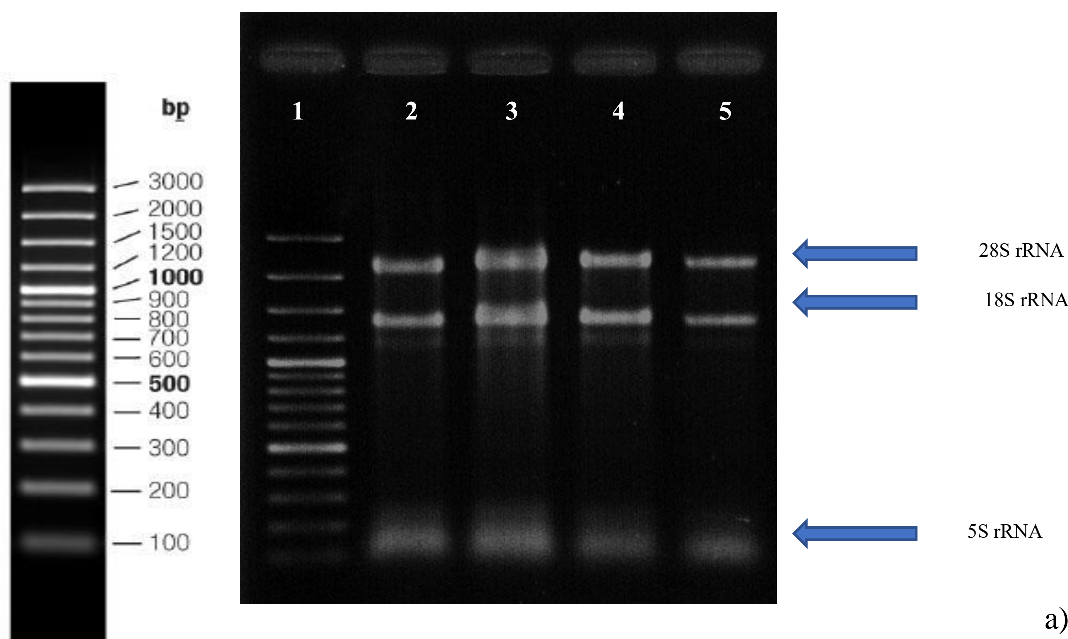
Note: L, Rose Lines. Results are expressed as the means of 3 samples ± standard error (SE).

## Discussion

Extracted RNAs are very sensitive to degradation as may contain inhibitory compounds which can lead to partial or complete inhibition of downstream PCR (8, 24). Although number of RNA isolation protocols were developed across multiple plant species and tissues, extraction of pure, intact, and high-quality RNA is difficult in colorful rose petals which contain large amounts of pigments. Petals have a wide spectrum of polysaccharides and polyphenols including flavonoids and other secondary metabolites which interfere with the extraction of pure RNA (8, 24, 25, 26). Ideally, isolation and purification of nucleic acids from biological samples by simple, fast and non-toxic protocol with good yields of high quality is a critical step in many research fields in life science (27). Magnetic nanoparticles separation technology is simple, low cost, recyclable, fast and without toxic chemicals compared to traditional methods (28).

Agarose gel electrophoresis is employed to determine the yield and purity of a RNA isolation or PCR reaction (29, 30). The ability to distinguish RNAs by their molecular size is critical for the analysis of rRNA transcription and processing. Separation of 3 rRNA bands (28S, 18S and 5S rRNA) with good resolution via agarose gel electrophoresis has been an indispensable technique for representing the best yield and purity of RNA (29, 30). It has been reported that the isolation and separation process of RNA molecules are related to the structure of magnetic nanoparticles which have a specific coating acting as charged solid surfaces to bind RNA fragments. Therefore, nanoparticles existence causes RNA strand absorption invidiously from other

impurities (11). It seems that the high quality and quantity of RNA extracted in our research could be due to this property of the nanoparticles. In addition, separation technology by magnetic nanoparticles starts with low material which decreases the inhibitory substances, and results in better removal of these compounds when RNA is extracted from the specimens (21).



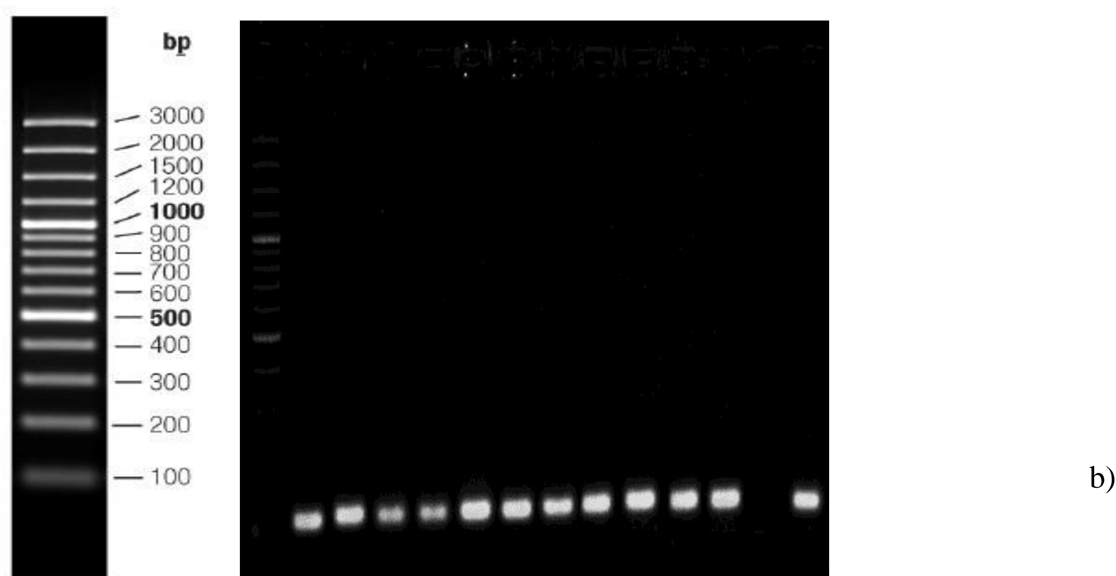




**Figure 1. a. Visualisation of total RNA isolated from petals by magnetic nanoparticles on a 1% agarose gel stained with ethidium bromide. b. Electrophoresis of PCR amplification products using cDNA made from extracted RNA and tubulin primers on a 1% agrose gel and stained with ethidium bromide. Lane 1. 3000 bp DNA ladder; Lane 2-12, tubulin; Lane 13 Negative control (water); Lane14, Positive control (gDNA).**

High efficiency and speed of present method in regard to the absence of toxic chemicals make it an attractive alternative plant RNA extraction protocol. These results show that the RNA produced by this simple, low cost, fast and safe protocol could be introduced as promising starting point in PCR-based techniques on a

wide range of plant organs. Therefore, this method is recommended even in low-equipped laboratories for high-throughput sample preparation suitable for various molecular analytical techniques. Also, the results indicate that, apart from the choice of chemical reagents, the method of magnetic nanoparticles is a critical step during RNA extraction from "difficult" sources.

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