An optimized method to obtain high-quality RNA from different tissues in *Lilium davidii* var. *unicolor*

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Methodology

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Abstract

Background: The high quality, high yield and purity RNA samples are essential for subsequent molecular experiments such as RT-PCR, qPCR and RNA-seq. However, harvest high quality RNA samples from different tissues in *Lilium davidii* var. *unicolor* is a great challenge due to its polysaccharides, polyphenols and other secondary metabolites. In this study, three RNA extraction methods, namely modified TRIzol method, Kit and CTAB methods were reported to obtain the total RNA from *Lilium davidii* var. *unicolor*, and the efficient RNA extraction protocol (modified TRIzol method) was described.

Results: A Nano drop spectrophotometer and 1% gel electrophoresis were used to detect the RNA quality and integrity. Compared with Kit and CTAB methods, the higher RNA concentrations from different tissues were obtained and the A260/280 ratios of RNA samples were ranged from 1.97 to 2.27 when using the modified TRIzol method. None of intact RNA bands were gained from the modified CTAB method, indicating that the RNA samples isolation were degraded. As a result of the modified TRIzol method, the RNA samples in the different tissues from *Lilium* presented clear 28S and 18S bands.

Conclusions: Here, modified TRIzol method is an easy, efficient, and low-cost method for RNA isolation from *Lilium davidii* var. *unicolor*. Thus, modified TRIzol method is sufficient to gain eligible RNA to support further molecular experiments in *Lilium davidii* var. *unicolor*.

Background

Lilium davidii var. *unicolor*, an important economic crop, is the only sweet lily and only distributed in Lanzhou, Gansu province in China. Bulbs of the lily *Lilium davidii* var. *unicolor* are one of the main traditional Chinese medicines for several centuries, and its flowers also has ornamental value [1, 2]. In recent years, more and more studies focused on bulbs of *Lilium davidii* var. *unicolor*, such as bulblets formation and development. High levels of starch, protein, fat, cellulose, saponin, colchicine, and polysaccharide in scales of the bulb can be used as potential biomarkers for bulb quality [3, 4]. The starch and sucrose metabolism is the major pathway in scales of *Lilium* through KEGG pathway enrichment analysis [5, 6]. In addition, the content of polysaccharides in roots and scales of *Lilium* has been reported almost same [7]. During the growth and development of *Lilium*, despite works on the physiological and molecular aspects is necessary, it is difficult to obtain high quality RNA to support molecular biology techniques. Polysaccharides and polyphenols limit nucleotide purification and precipitation due to that the RNA could be co-precipitated with polysaccharides and polyphenols [8, 9]. Thus, obtaining high-quality RNA for many molecular analyses from scales and other tissues of *Lilium* is difficult due to the high level of other substances. Therefore, the optimized method to isolate high quality RNA from different tissues of *Lilium* should be explored.

Over the past several decades, commercial plant kits play an important role in plant molecular studies, but extracting high-quality RNA from polysaccharides of plants remains a challenge [10]. Moreover, Kit is used to extract high quality and integrity RNA, which is not only depending on the plant species, genotype, tissue type, but also related to the content of polyphenols, polysaccharides, proteins, etc. [10, 11]. Removing polyphenols, polysaccharides, proteins and lipids are a vital procedure during RNA isolation, as RNA with high quality is the key factor to subsequent molecular experiments [12, 13]. Although several RNA extraction methods have been reported available for *Pistacia vera* L. [14], *Brassica* plants [12], oil seeds [15] and cassava tubers [16] fewer protocols are suitable for isolating total RNA from different tissues of *Lilium*, especially for different parts of scales. Despite rapid advances in genetic engineering and transgenic technology of model plants, the genomes database of *Lilium* need to be further improved. Therefore, obtaining integrity, high quality and yield RNA from *Lilium* is an essential condition for functional genomics research.

In this study, three RNA extraction methods, modified TRIzol method, Kit and CTAB method were used to extract RNA with high quantity and quality from different tissues of *Lilium davidii* var. *unicolor* (root, stem, leaf, top-inner scales, middle-inner scales, basal-inner scales, top-middle scales, middle-middle scales, basal-middle scales, top-external scales, middle-

external scales, basal-external scales). The 1% agarose gel electrophoresis was used to assess the quality of extracted RNA samples. Here, we described the modified TRIzol method that could be applied in different tissues containing polyphenols, polysaccharides, proteins and lipids. Therefore, this study might provide an efficient, economical, fast and simple RNA extraction method for different tissues of *Lilium davidii* var. *unicolor* by comparing Kit and CTAB method.

Materials And Methods

Plant materials

Lily plants '*Lilium davidii* var. *unicolor*' were cultivated in the greenhouse (the average temperature was 18.5°C and the average degree of humidity was 79.4%) of Gansu agricultural university, Lanzhou, Gansu province, China (Fig. 1A). Firstly, fresh plants were collected and washed with deionized sterile water and dissected leaves, stems, roots and bulbs separately. Secondly, scales were removed carefully from mother bulbs and sorted into three groups: external scales (1–3 layers), middle scales (4–6 layers) and inner scales (7–9 layers). Then different layers scales were sorted into three equal parts, named as top, middle and basal part (Fig. 1B). Thus, the different tissues from *Lilium* were named successively as top-inner scales, middle-inner scales, basal-inner scales, top-middle scales, middle-middle scales, basal-middle scales, top-external scales, middle-external scales, basal-external scales. The above samples were immediately frozen in liquid nitrogen and then preserved at -80 °C.

Reagents And Solutions

Plastic materials (tubes and pipette tips), mortars, pestles and irons were autoclaved at 121°C for a period of 1 h.

Plastic materials used in CTAB methods were soaked overnight in 0.01% DEPC-treated distilled water and then autoclaved. In other methods, TRIzol® reagent (Invitrogen, USA) and TaKaRa MiniBEST Plant RNA Extraction Kit (TaKaRa, Japan) were applied. Buffers and solutions were prepared as follows. CTAB extraction buffer: 2% CTAB (w/v); 2% soluble polyvinylpyrrolidone (PVP, w/v); 100 mM Tris-HCl (pH 8.0); 2 M NaCl; 25 mM ethylenediamine tetraacetic acid (EDTA); 0.5 g L⁻¹ spermidine and 3% β-mercaptoethanol (added to pre-heated autoclaved buffer prior to extraction). Other reagents include chloroform-isoamylalcohol (C: I) at a ratio of 24:1, 8 M LiCl and 75% ethanol.

RNA Extraction Protocols

Method 1: Modified TRIzol method

TRIzol-based RNA extraction was done according to Ahmad et al. (2017) [17] with some modifications. (1) About 300 mg of each tissue samples was ground into a fine powder using liquid nitrogen and transferred into a 1.5 mL centrifuge tubes. Then 1 mL of TRIzol reagent was added, and mixed by a micropipettor, then incubated for 10 min at 4°C, and centrifuged at 12,000 *g* for 5 min at 4°C. (2) The supernatant was transferred to a 1.5 mL Eppendorf tube. 200 µL of cold chloroform was added and the mixture was hand-shaken to mix and incubated at room temperature for 5 min. Then centrifuged at 12,000 *g* for 15 min at 4°C. (3) The supernatant (~ 700 µL) was transferred to a new 1.5 mL chilled Eppendorf tube and then an equal volume of chilled isopropanol was added and mixed by inverting the tube for 6–10 times. The final mixture was incubated at -20°C overnight to precipitate RNA. (4) The obtained RNA pellet was added to the RNA purification adsorption column followed by a centrifugation of 15 min at 12,000 *g* on 4°C to obtain a visible precipitate, and the supernatant was discarded. (5) 700 µL pre-cooled 75% ethanol were added, and centrifuged at 12,000 *g* for 1 min at 4°C, and then the supernatant were discarded. The ethanol washing was repeated twice centrifuged at 12,000 *g* for 2 min at 4°C and the redundant ethanol was discarded. To eliminate ethanol as much as possible, the procedure was repeated twice. (6) After centrifugation, the tubes were carefully transferred to the ice, and the washed RNA pellet was air-dried for 10 min in super clean bench. (7) 40 µL RNase free H₂O was added to dissolve RNA. RNA samples were incubated at 4°C for 3 min, and then it was centrifuged at 12,000 *g* for 2 min at 4°C to elute RNA. To obtain high concentration of RNA, the first eluent was added back into the RNA purification adsorption column, incubated and centrifuged again as above.

Method 2: Modified CTAB method

According to White et al. (2008) [18] with some modifications, the RNase-free DNase I and RNA purification adsorption column were used in Steps 10 and 11, respectively. Our modified procedure is described as follows: (1) For each sample, 1.2 mL of extraction buffer was preheated in a 50 mL Eppendorf tube (with 3% β -mercaptoethanol added) and bathed at 65°C for 30 min. (2) About 300 mg of each tissue samples was grounded into a fine powder by using liquid nitrogen, samples were transferred to 1.5mL chilled Eppendorf tube and 1.2 mL pre-heated extraction buffer were added. (3) The tubes were vortexed immediately and then incubated at 65°C in water bath for at least 30 min with vortexing once every 5 min. (4) The tubes were centrifuged at 12,000 g for 10 min at 4°C and transferred the supernatant to a new 1.5 mL Eppendorf tube. (5) An equal volume (650–850 μ L) of C: I (24:1) was added to the tube, with vortexing for 30 s and centrifuged at 12,000 g for 15 min at 4°C. (6) The aqueous phase was transferred to a new 1.5 mL Eppendorf tube without disruption of the white interphase. (7) Repetition C: I abstraction as in the previous step (step 5 and 6). (8) 1/3 volumes of 8 M pre-cooled LiCl were added to the supernatant in 1.5 mL Eppendorf tube with mixing upside down, then stored at 4°C overnight for nucleic acid precipitation. (9) The obtained RNA pellet was added to the RNA purification adsorption column followed by a centrifugation of 15 min at 12, 000 g at 4°C, discarded the supernatant. (10) 5 μ L 10 $\times$ DNase I Buffer, 4 μ L Recombinant DNase I (TaKaRa, Tokyo, Japan, RNase free, 5 U μ L⁻¹) and 41 μ L RNase free H₂O were pipetted onto the membrane of the RNA purification adsorption column, followed by an incubation of 15 min at room temperature (25°C) to eliminate possible genomic DNA contamination. (11) The RNA was washed, air-dried and eluted as described in the TRIzol protocol steps 5–7.

Method 3: TaKaRa MiniBEST Plant RNA Extraction Kit

Plant RNA Extraction Kit was extracted according to the manufacturer's instructions (Code: 9769). Importantly, all of the operating steps were performed at 4°C to prevent RNA degradation during the experiment process.

Results

RNA samples with higher quality and yield were extracted by modified TRIzol method from root, stem and leaves in *Lilium davidii* var. *unicolor*

Three methods were used for extracting RNA samples from different tissues of *Lilium davidii* var. *unicolor*, including modified TRIzol method, TaKaRa MiniBEST Plant RNA Extraction Kit (Kit) and CTAB method. At the root of the *Lilium*, RNA samples obtained from modified TRIzol method showed that the A260/230 and A260/A280 ratios were closed to 1.5 and 2.1, and CTAB method showed higher ratio (ratios of A260/230 and A260/280: 1.9, 2.2) (Table 1). However, RNA prepared from root with Kit showed low absorbance ratios (ratios of A260/230 and A260/280: 1.0, 2.0). Among these methods, the higher RNA concentration was obtained when using the modified TRIzol method compared with other methods (565.39 ng μ L⁻¹) (Table 1). For stem tissue of *Lilium*, as shown in Table 1, the A260/A280 and A260/A230 ratios for stem of *Lilium* evaluated were less than 1.0 and 1.8, respectively, indicating that the extracted RNA was of low purity. The modified TRIzol method resulted in higher A260/230 (1.0) ratios, A260/280 ratios (1.97) and yields (concentration; 165.69 ng μ L⁻¹) than Kit and CTAB method. Therefore, compared with Kit and CTAB method, using modified TRIzol method resulted in high-quality RNA from stem in *Lilium*. When RNA was extracted from leaves of *Lilium* using the Kit and CTAB method, spectrophotometric results showed that the A260/230 ratios of RNA from leaves ranged between 0.91 and 1.07, and the A260/280 ratios ranged between 1.69 and 2.01, indicating that the RNA samples were contaminated by proteins, polyphenols and polysaccharides. However, by using modified TRIzol method, the A260/230, A260/280 ratios and RNA concentration increased significantly. Taken together, the modified TRIzol method is the most efficient and reproducible methods to extract high-quality RNA from different tissues of *Lilium* for subsequent molecular experiments.

The integrity of isolated RNA samples was then tested by using 1% agarose gel electrophoresis. The RNA samples from modified TRIzol and Kit methods showed legible and integrated bands of 28S rRNA and 18S rRNA from different tissues of *Lilium davidii* var. *unicolor*, without obvious DNA contamination. However, the RNA samples obtained by CTAB method showed visible chromosomal DNA bands of root, stem and leaves. In addition, RNA extraction from different tissues of the CTAB method resulted in serious degradation (Fig. 2). To this end, the obtained total RNA by modified TRIzol method showed high yield and quality as revealed by a high RNA integrity.

The Kit is the effective method in isolating RNA from inner scales of *Lilium davidii* var. *unicolor*

In inner scales of *Lilium*, the absorbance spectra revealed the A260/230 ratios (ranging from 0.27 to 0.4) of RNA samples isolated by modified TRIzol method, which were lower than that by other methods (Table 2). Compare with the other methods, the purity (A260/280; ranging from 2.10 to 2.27) and concentration of RNA samples segregated by modified TRIzol method from different parts of inner scales were higher, but the A260/230 ratios were lower. The absorbance ratios A260/230 of the produced RNA samples of inner scales by Kit and CTAB method ranged from 1.96 to 2.06 and ranging from 1.74 to 1.92, respectively. Using the Kit and CTAB method, the absorbance spectra of extracted RNA ratios of A260/A280 was close to 2.2 (Table 2) from different parts of inner scales. This result suggested that the RNA samples isolated by Kit contained lower protein, organic solvents, and other contaminants compared to modified TRIzol method. Moreover, the Kit could obtain a higher RNA yield compared to the other methods. Interestingly, analysis of total RNA extracted by modified TRIzol method and Kit from different scales by 1% agarose gel electrophoresis revealed that bands were clear and intact, testifying that these RNA samples were not degraded (Fig. 3). However, on account of the lengthy procedure of CTAB method, the corresponding separated RNA was easily degraded compared to that of the Kit and modified TRIzol method. Thus, the Kit is an optimized method to obtain high-quality RNA from inner scales of *Lilium davidii* var. *unicolor* (Table 1, Fig. 3). In comparison with CTAB method, modified TRIzol method could also extract high quality RNA from inner scales.

The RNA was obtained with high quantities and integrity through modified TRIzol method from the middle and external scales

While isolating RNA from the middle and external scales, modified TRIzol method is considered suitable for extraction in this study. For middle and external scales of *Lilium davidii* var. *unicolor*, A260/230 ratios of RNA samples ranged from 0.29 to 1.39, from 1.85 to 1.99, and from 1.55 to 1.89 for modified TRIzol method, Kit method, CTAB method, respectively (Table 2), indicating that the RNA samples extracted by modified TRIzol and CTAB method were contaminated by polyphenols and polysaccharides except for that by Kit. There was no significant difference in A260/280 ratios among these three methods, demonstrating that all of RNA were free of protein and DNA contamination (Table 2). When compared with CTAB method, modified TRIzol method and Kit could obtain high concentration RNA. Meanwhile, the 1% agarose gel electrophoresis showed that RNA bands were clear and complete by using modified TRIzol method and Kit. Nevertheless, the total RNA obtained by use of CTAB method was degraded seriously (Fig. 4; Fig. 5). Taken together, the concentration of total RNA samples obtained from middle and external scales by using modified TRIzol method were higher than the other methods, while its bands were clear and intact (Table 2; Fig. 4; Fig. 5). What is noteworthy is that the extraction RNA of the basal part of scales showed higher purity and concentration than other parts of scales separated by all three methods (Table 2).

Discussion

Lilium davidii var. *unicolor* has been regarded as both the vegetable and the herbal medicine for several centuries in China because their bulbs could provide polysaccharides, phenolic compounds to treat bronchitis, pneumonia and provide nutrition [19, 20]. Amounts of polyphenols and polysaccharides were accumulated during the bulbs formation and development stages of *Lilium*. However, extracting high-quality RNA samples from *Lilium* bulbs is a tough process as

polysaccharides and polyphenols tend to co-precipitate with RNA [21, 22]. The composition of plant tissues varies greatly, which requires different approaches to obtain high-quality RNA [23, 24]. Several RNA extraction methods have been reported in Pistachio[25], Cassava[23], Sunflower [26], sugar beet [27] and Jerusalem artichoke seeds [21], but it may not be suitable for *Lilium davidii* var. *unicolor*. In this study, several methods were researched to obtain high-quality and integrity RNA from different tissues in *Lilium*, and the optimal RNA isolation method from different tissues of *Lilium* was selected and optimized.

The reported traditional TRIzol method is not suitable for obtaining high-quality RNA from cassava storage roots [23]. Meanwhile, we initially tried to extract RNA samples from different tissues of *Lilium* by applying modified CTAB method and Kit approaches, but the results remained unsatisfactory. However, compared with the modified CTAB method and Kit, modified TRIzol method could extract high-quality RNA from different tissues of *Lilium*. During the operation of modified TRIzol method, after RNA was precipitated by isopropanol, changing the extraction environment to 4 °C, using the RNA purification adsorption column and washing RNA pellet with ethanol repeatedly are critical for obtaining high quality and purity RNA samples. Furthermore, large amount of TRIzol reagents should be used to effectively dissolve tissue samples and gain supernatant. Among these three methods, when using the modified TRIzol method, the highest RNA concentration was obtained from roots, stems, leaves and different parts of scales in *Lilium*, with an A260/280 ratio ranging from 1.97 to 2.27. Although the A260/230 ratios for different tissues were still low, the 28S and 18S ribosomal RNA bands could be clearly observed, confirming the integrity of the RNA samples. Nevertheless, RNA extraction from different tissues by the CTAB method resulted in serious degradation (Fig. 2). In addition, for different scales of *Lilium*, the basal parts are easier to obtain the high yield and quality RNA. This might be because that the basal parts have the higher transcriptional activity and less content of polysaccharide and other secondary metabolites than other scale parts.

Previous studies have reported that CTAB method could extract high purity and yield of total RNA samples from different species which enriched high content of polyphenols and polysaccharides [28, 29]. In this study, while isolating the RNA samples with CTAB method from roots, stems, and leaves, one of the main obstacles in the separation of RNA samples by CTAB method is the existence of DNA contamination (Fig. 2). To further eliminate the interference of DNA from RNA samples, we added the RNA-free DNase I digestion step in CTAB method. Following the treatment with DNase I, RNA samples obtained from tissues with modified CTAB method showed low DNA contamination. In the scales of *Lilium*, as table 2 showed that A260/230 ratios of RNA samples ranged from 1.55 to 1.89 (using CTAB method), indicating that the RNA samples extracted by modified CTAB method were contaminated by polyphenols and polysaccharides. When using the modified CTAB method, the ratio of A260/280 is above 2.1 except for RNA samples of stems and leaves. Unexpectedly, the bands in 1% agarose gel electrophoresis gained from modified CTAB method were incomplete and diffused (Fig. 2), indicating that the RNA samples isolated by the modified CTAB method were degraded. So we speculated that this might be due to the long time in the process of RNA extraction by CTAB method, which led to the degradation of RNA. Meanwhile, compare with modified TRIzol method, modified CTAB method could obtain low concentration and low yield RNA (Table 1). The yield of RNA extracted by CTAB is reduced due to the high affinity of CTAB with nucleic acids and other biopolymers [30]. Thus, modified CTAB method might not be well suitable for acquiring RNA samples from *Lilium davidii* var. *unicolor*.

Compared with the modified CTAB method, Kit resulted in higher yields and purity RNA than modified CTAB method for bulb scales that contain high levels of polysaccharides and polyphenols. However, Kit protocol is not suitable for extracting RNA from roots, stems and leaves of *Lilium*. Meanwhile, Kit protocol is the highest cost method among several methods for extracting high quality and purity RNA from *Lilium davidii* var. *unicolor*. So, we suggested that modified TRIzol protocol is the efficient, simple and economical method to acquire high-quality and integrity RNA from different tissues in *Lilium*.

Conclusion

The modified TRIzol protocol, modified CTAB method and Kit were used to extract RNA from tissues in *Lilium davidii* var. *unicolor*, and the modified TRIzol protocol obtained the highest quality and yield RNA. The intensity and intact RNA bands

on 1% gel electrophoresis and absorbance ratio in the modified TRIzol method showed a distinct advantage among three methods. Thus, this method is an easy, efficient, and low-cost method for RNA isolation from *Lilium*. In all, the modified TRIzol method is sufficient to gain eligible RNA in *Lilium* to support further molecular experiments.

Abbreviations

C: I, chloroform-isoamylalcohol; CTAB, cetyltrimethylammonium bromide; Kit, TaKaRa MiniBEST Plant RNA Extraction Kit; DEPC, diethylpyrocarbonate; PVP, polyvinylpyrrolidone; EDTA, ethylenediamine tetraacetic acid; DNase, deoxyribonuclease

Declarations

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Authors' contributions

The authors WBL and CLW contributed to the study conception and design. Material preparation, data collection and analysis were performed by XMH, NNQ, CXL, YYL, DLH and YHL. The first draft of the manuscript was written by CLW and XMH. All authors read and approved the manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Tables

Table 1 Average purity and yield of total RNA extracted from root, stem and leaf of *Lilium* using the three methods

	260/230			260/280			Concentration (ng μ L ⁻¹)		
	TRIzol	Kit	CTAB	TRIzol	Kit	CTAB	TRIzol	Kit	CTAB
Root	1.51±0.28ab	1.05±0.15b	1.92±0.03a	2.16±0.01a	2.03±0.04b	2.23±0.00a	565.39±9.67a	56.73±8.77c	259.49±13.80b
Stem	1.00±0.24a	0.43±0.25b	0.98±0.25a	1.97±0.02a	1.75±0.10ab	1.68±0.07b	165.69±27.65a	25.35±7.92b	79.69±3.80b
Leaf	1.28±0.23a	1.07±0.03a	0.91±0.01a	2.02±0.02a	2.01±0.10ab	1.69±0.07b	227.85±14.90a	69.45±4.81b	50.27±13.03b

Table 2 Average purity and yield of total RNA extracted from different scales using the three methods

260/230			260/280			Concentration (ng μL^{-1})			
Parts	TRizol	Kit	CTAB	TRizol	Kit	CTAB	TRizol	Kit	CTAB
T-inner	0.27±0.03b	2.06±0.00a	1.74±0.22a	2.10±0.01b	2.28±0.01a	2.20±0.07ab	293.23±47.43b	586.04±45.06a	418.80±16.49b
M-inner	0.3±0.03b	2.01±0.04a	1.92±0.06a	2.27±0.05a	2.25±0.02a	2.26±0.02a	506.23±122.91b	781.20±29.16a	382.29±31.59b
B- inner	0.4±0.04b	1.96±0.02a	1.81±0.17a	2.21±0.02a	2.26±0.01a	2.19±0.04a	609.70±132.36b	1025.19±44.44a	450.56±62.34b
T-middle	0.48±0.06c	1.96±0.01a	1.64±0.11b	2.26±0.01a	2.23±0.00a	2.16±0.04b	1019.69±22.38a	458.83±3.81b	505.67±44.09b
M-middle	0.29±0.01c	1.93±0.02a	1.55±0.013b	2.25±0.01a	2.20±0.01a	2.11±0.03b	748.71±13.24a	414.52±19.48b	344.76±58.54b
B-middle	1.39±0.42a	1.99±0.01a	1.81±0.15a	2.26±0.01a	2.25±0.00a	2.20±0.04a	1581.90±54.03a	688.25±65.19b	448.49±24.13c
T-external	0.45±0.22b	1.85±0.02a	1.89±0.02a	2.18±0.01b	2.19±0.01b	2.25±0.02a	460.92±44.47a	374.31±50.34a	180.32±20.86b
M-external	0.58±0.27b	1.86±0.02a	1.57±0.10a	2.19±0.01a	2.20±0.01a	2.16±0.02a	519.83±107.83a	339.20±29.01ab	156.53±17.00b
B-external	1.04±0.24b	1.97±0.01a	1.55±0.2ab	2.21±0.01ab	2.24±0.00a	2.12±0.06b	590.75±85.80a	763.99±16.73a	203.44±10.93b

Figures

Figure 1

the plant of *Lilium davidii* var. *unicolor* used in this experiment (A). Different layers of bulb scales were sorted into three equal parts, named as top, middle and basal part (B).

Figure 2

1% agarose gel of 5 μg total RNA extracted from 300 mg fresh weight of root, stem and leaf tissue per sample. The different tissues from *Lilium* were named successively as top-inner scales, middle-inner scales, basal-inner scales, top-middle scales, middle-middle scales, basal-middle scales, top-external scales, middle-external scales, basal-external scales.

Figure 3

1% agarose gel of 5 μ g total RNA extracted from 300 mg fresh weight of inner scales per sample. The different tissues from *Lilium* were named successively as top-inner scales, middle-inner scales, basal-inner scales, top-middle scales, middle-middle scales, basal-middle scales, top-external scales, middle-external scales, basal-external scales.

Figure 4

1% agarose gel of 5 μ g total RNA extracted from 300 mg fresh weight of middle scales per sample. The different tissues from *Lilium* were named successively as top-inner scales, middle-inner scales, basal-inner scales, top-middle scales, middle-middle scales, basal-middle scales, top-external scales, middle-external scales, basal-external scales.

Figure 5

1% agarose gel of 5 μ g total RNA extracted from 300 mg fresh weight of external scales per sample. The different tissues from *Lilium* were named successively as top-inner scales, middle-inner scales, basal-inner scales, top-middle scales, middle-middle scales, basal-middle scales, top-external scales, middle-external scales, basal-external scales.