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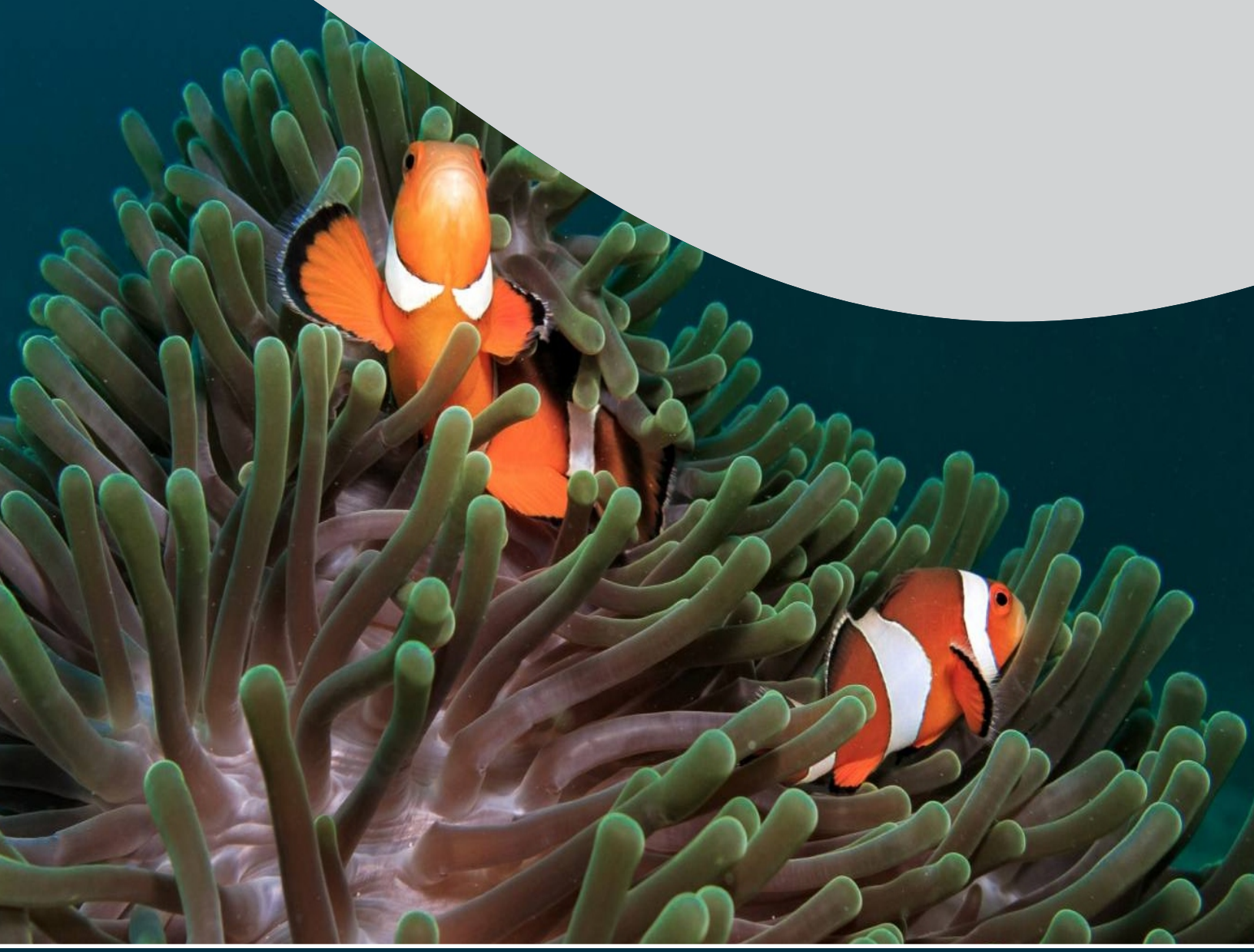

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An improved method for isolation of high-quality RNA from starch-rich wheat grains

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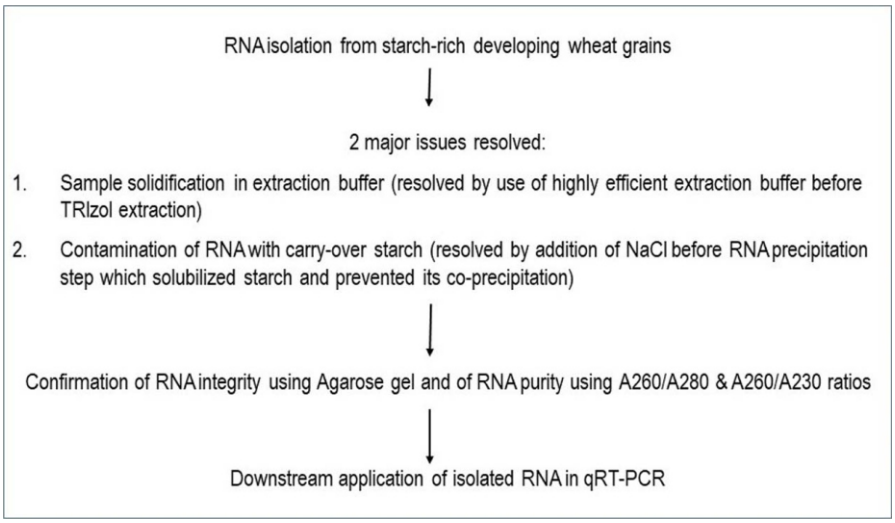
Abstract

Aim : The aim of the present investigation was to develop a quick, easy and reliable method for isolation of RNA from starch rich mature wheat grains; and to check the quality of isolated RNA for downstream applications.

Methodology : In the present protocol, highly efficient modified RNA extraction buffer [100 mM Tris (pH 9.0), 150 mM NaCl, 50 mM EDTA, 1.5% sodium dodecyl sulfate (SDS) and 1.5% 2-mercaptoethanol] was used, subsequently followed by TRIzol extraction. Carryover starch was effectively solubilized by adding NaCl before RNA precipitation step. RNA quality was assured by agarose gel electrophoresis, spectrophotometric analysis and quantitative real-time PCR.



Results : The problem of co-precipitation of starch along with RNA was resolved effectively. Intact sharp bands of 18S and 28S rRNA on agarose gel confirmed the integrity of isolated RNA. The average A260/A280 ratios ranged from 2.06 to 2.11 and A260/A230 ratio was higher than the respective A260/A280 ratio, indicating high purity of isolated RNA. The isolated RNA was found suitable for gene expression analysis through quantitative real-time PCR.

Interpretation : An improved quick, easy and reliable method developed for isolation of high-quality RNA from starch-rich mature wheat grains could be useful for downstream molecular analysis.

Key words: Gene expression studies, Real-time PCR, RNA isolation, Starch-rich grain, Wheat

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Introduction

Wheat (*Triticum aestivum* L.) is a major source of carbohydrate and protein for consumers in developing countries. It is estimated that global cereal production should increase by 56%, with developing countries accounting for 93% of cereal demand growth by 2050 (Rosegrant and Cline, 2003). To develop high yielding wheat varieties, attention needs to be paid to analyse the molecular mechanisms of grain growth and development. Also, molecular studies on developing and mature wheat grains are required for enhancing the grain quality in terms of nutritive value and end-use properties. As the grain development in wheat is adversely affected by various abiotic stresses, therefore, transcriptomic analysis of developing wheat grains is demanded to study stress-induced changes in gene expression profiles. Isolation of high-quality RNA from wheat grains is a prerequisite for the transcriptomic studies on grain-specific genes. Wheat grains are highly rich in starch (65-75%) (Cornell, 2003) and high starch content hinders the extraction of high-quality RNA from wheat grains (Takumi *et al.,* 2017).

As the starch content rises to extreme levels near seed maturity, RNA isolation from seeds during later stages of seed development becomes even more challenging. There are reports on difficulties in extraction of high-quality RNA from starch-rich tissues in other crops as well, for example, *Lens culinaris* (Dash, 2013), *Helianthus tuberosus* (Mornkham *et al.,* 2013), *Dendrobium huoshanense* (Liu *et al.*, 2018).Two main issues during isolation of RNA from starch-rich tissues are solidification of samples in the RNA extraction buffers and co-precipitation of starch with RNA during the precipitation step, which hinders resuspension of precipitated RNA (Wilkins and Smart, 1996; Li and Trick, 2005; Furtado, 2014). Many commercial kits (e.g. Spectrum™ Plant Total RNA Kit, Sigma; RNeasy kit, Qiagen) have been developed for the extraction of total RNA, which are based on specialized solution-based or column-based protocols.

However, most of these commercial kits are unsuitable for RNA isolation from starch-rich wheat grains collected during mid to late stages of grain development as there is problem of column blockage at very first step due to sample solidification. Although, several protocols have been developed to extract RNA from polysaccharide-rich wheat tissues (Salzman *et al.,* 1999; Gao *et al.,* 2001; Li and Trick, 2005; Wang *et al.,* 2012), but most of these existing protocols are time-consuming and result into low yield and/or poor quality of the isolated RNA. Li and Trick (2005) have addressed the key issues of sample solidification and starch co-precipitation in their protocol. But for fully mature grains with very high starch content, we did not find their protocol very satisfactory. Few protocols are reported for isolation of RNA from mature wheat grains (Furtado, 2014; Takumi *et al.,* 2017) but these methods require expensive column-based purification steps and are not cost-effective when sample size is large. Based upon the method reported by Li and Trick (2005), here, we report a modified method to isolate high quality transcriptionally competent total RNA from mature wheat grains.

Materials and Methods

Plant materials: Wheat variety WH 730 (having maturity duration of 149 days) was raised in the field of CCS Haryana Agricultural University, Hisar (India) using standard agronomic practices (Kumar *et al*., 2014). Developing grains were collected at 5, 10, 15, 20, 25 and 30 days after anthesis (DAA) during grain development and stored at -80°C till further use.

Reagents and solutions: RNA extraction buffer used here was modified from extraction buffer-I reported by Li and Trick (2005). Modified RNA extraction buffer included 100 mM Tris (pH 9.0), 150 mM NaCl, 50 mM EDTA, 1.5% sodium dodecyl sulfate (SDS) and 1.5% 2-mercaptoethanol; SDS and 2-mercaptoethanol were added just before use. Other reagents required wereTRIzol® reagent (Invitrogen, Life Technologies, USA), Phenol:chloroform (1:1), chloroform:isoamyl alcohol (24:1), 2 M sodium chloride, chilled isopropanol, 0.1% diethyl pyrocarbonate (DEPC)-treated water and 75% ethanol. All the solutions were prepared in DEPC-treated water and autoclaved. All the glassware, plastic materials, pestles and mortars used in the procedure were treated with 0.1% DEPC before use. Electrophoresis apparatus was dipped in 3% H_2O_2 for 10 h and washed with DEPC-treated autoclaved water. Working bench was cleaned using RNaseZap (Ambion, Life Technologies) solution.

RNA extraction procedure: RNA extraction procedure was adapted from the protocol given by Li and Trick (2005) with modifications at 2 steps, first during dissolving starch-rich sample in extraction buffer and second during starch pre-solubilization before RNA precipitation. The grain tissue was ground to a fine powder in liquid nitrogen using pre-chilled pestle and mortar. The tissue powder (70 mg) was transferred to pre-chilled 1.5 ml microcentrifuge tube, and 500 µl of modified RNA extraction buffer was immediately added. The sample was mixed vigorously using a vortex to form homogenised suspension and incubated on ice for 10 min. Subsequently, a volume of 250 µl of phenol-chloroform mixture (1:1) was added and the sample was mixed well by gentle inversion. The sample was then centrifuged at 13,000× g for 15 min at 4°C. The aqueous supernatant (around 250 µl) was transferred to a fresh 1.5 ml microcentrifuge tube containing 250 µl of the Trizol reagent. The contents of the tube were mixed by gentle inversion and incubated at room temperature for 10 min. After incubation, 250 µl of chloroform-isoamyl alcohol (24:1) was added and the sample was then centrifuged at 13,000×g for 15 min at 4°C. The upper aqueous phase (around 350 µl) was carefully transferred to a new 1.5 ml tube and 100 µl of 2 M sodium chloride was added. After mixing the sample by 4-5 times inversion, the sample was kept at room temperature for 5 min. Then, a volume of 200 µl of chilled isopropanol was added, the sample was mixed by inversion and was incubated at -20°C for 15 min. The sample was centrifuged at 13,000×g for 15 min at 4°C, then the supernatant was discarded, and the RNA pellet was washed carefully with 500 µl of 70% ethanol. The RNA pellet was dried for 10 min at room temperature in laminar airflow hood and was resuspended in a suitable

amount of RNase free water (~60 µl). The isolated RNA was stored at -80°C until further use.

Determination of RNA quantity and quality: The concentration and purity of the total RNA were calculated through the A260/A280 and A260/A230 ratios, through UV spectrometry using the NanoDrop™1000 spectrophotometer (Thermo Scientific). Using the relationship of O.D. unit of 1.0 at 260 nm equivalent to 40 ng μl^{-1} RNA, the quantity of RNA was estimated using NanoDrop. The RNA integrity was assessed by submerged horizontal agarose (1.5%) gel electrophoresis (Green and Sambrook, 2012).

Reverse transcription and cDNA synthesis: Prior to cDNA synthesis, RNA was treated with RNase-free DNase I (Fermentas, Thermo Scientific) according to manufacturer's protocol to remove trace amounts of DNA. To synthesize total cDNA,1 µg of purified RNA was reverse transcribed in 20 µl reactions primed with both Oligo (dT)18 and random hexamer primers, using 'RevertAid First Strand cDNA Synthesis Kit' (Fermentas, Thermo Scientific) as per the manufacturer's instructions. Fifteen-fold dilutions of cDNA stocks were prepared (6µl cDNA+84 µl nuclease-free water) in separate tubes and stored at -80°C for using as template in real-time PCR assays.

Real-time quantitative RT-PCR: The expression profile of soluble starch synthase I (SSSI) gene (GenBank accession number AJ292521.1) was analysed during wheat grain development by real-time quantitative RT-PCR (qRT-PCR) using Thermo Scientific Maxima SYBR Green qPCR Master Mix (2X). The qRT-PCR primers were designed for the target gene and reference gene (Table 1), using Primer Quest tool (http://eu.idtdna.com/PrimerQuest/). The qRT-PCR analyses were performed using iQ5 system (BioRad, USA) in 25 µl reaction volumes containing 3 µl of diluted cDNA template (i.e., cDNA corresponding to 10 µg RNA), 0.6 µM each of forward and reverse gene-specific primers (Table 1) and 12.5 µl of 2X Maxima SYBR Green I master mix. No template and RT-minus controls were run to detect contamination and presence of genomic DNA, respectively. The following amplification program was used: 95°C for 10 min, 40 cycles of 15 s at 95°C followed by 1 min at 60°C (data collection and real-time analysis enabled) and melt curve consisting of 1 min incubation at 95°C followed by 66 cycles of 10 s per cycle, starting at 55°C and increasing 0.6°C after each cycle. For each developmental stage, two biological replicates (resulting from two different RNA extractions) and three technical replicates were set up. Standard curves were run for the gene of interest and the reference gene to determine the amplification efficiency (Table 1).

Results and Discussion

Successful isolation of RNA with high quality and quantity is a prerequisite for gene expression studies. RNA extraction from wheat endosperm is difficult due to the presence of high levels of starch, particularly for samples from the mid-to-late stages of grain development (Singh *et al.,* 2003; Furtado, 2014; Takumi *et al.,* 2017). Initially the standard TRIzol method and commercially available 'Spectrum™ Plant Total RNA Kit' (Sigma-Aldrich, USA) were used for RNA extraction from wheat grains. While using

Table 1: Details of primers used for real-time PCR analysis, corresponding amplification product sizes and efficiencies

Accession number	Forward (F) and reverse (R) primers	Primer sequence (5' to 3')	Tm (°C)	Product size (bp)	Efficiency (%)
AJ292521.1	F/SSSI	GGGAGGATGTTCCTCTGATTG	62	114	110
(*SSSI* gene)	R/SSSI	GCATGACAAACTGCACGTC	62		
Cd879301	F/Ref	CATCCCAAACGGTGAAACTAATG	62	114	100
(reference gene)	R/Ref	GCTGTAAGACCACATCCTCTAC	62		

Table 2: Yield and purity of total RNA isolated by our protocol and Li and Trick (2005) method evaluated by UV light absorption spectra

Grain sample	RNA yield (µg/70 mg grains powder)		Absorbance ratio (260/280)		Absorbance ratio (260/230)	
	Our protocol	Li and Trick (2005)	Our protocol	Li and Trick (2005)	Our protocol	Li and Trick (2005)
5 DAA	69.1± 15.4	65.3± 12.7	2.09± 0.02	1.99± 0.02	2.11± 0.08	1.87± 0.02
10 DAA	78.3± 14.8	76.2± 11.5	2.07± 0.06	1.96± 0.01	2.09± 0.17	1.85± 0.03
15 DAA	96.0± 18.0	63.9± 9.9	2.11± 0.02	1.98± 0.02	2.21± 0.04	1.77± 0.04
20 DAA	62.4± 11.3	42.6± 7.2	2.09±0.02	1.93± 0.03	2.15± 0.03	1.82± 0.04
25 DAA	41.9± 10.5	23.0± 7.8	2.07± 0.04	1.88± 0.03	2.12± 0.12	1.56± 0.06
30 DAA	27.8± 6.2	10.5± 4.3	2.06± 0.04	1.84± 0.04	2.11± 0.16	1.23± 0.05

Data represent mean ± SD of eight replicates; DAA: days after anthesis

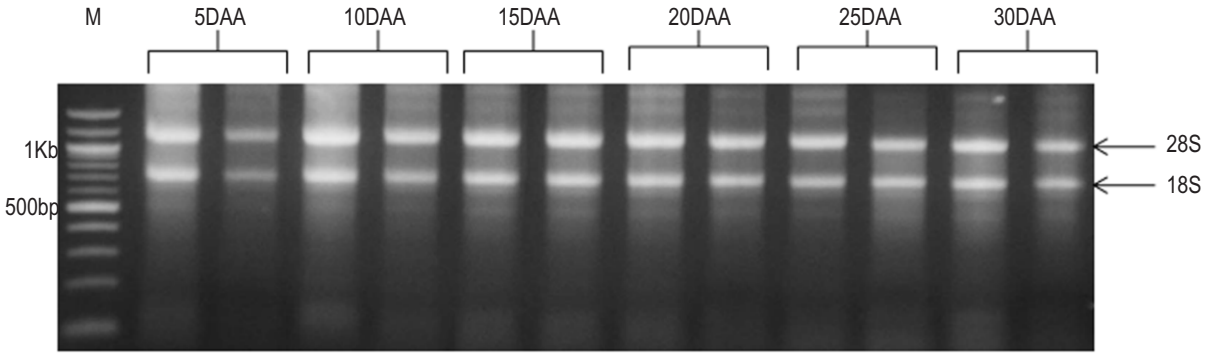

Fig.1: Agarose gel electrophoresis of total RNA isolated from wheat grains at different stages during grain development. Lanes M: 100bp Ladder, 2 lanes each of RNA isolated in replicates from the grains at 5 DAA, 10 DAA, 15 DAA, 20 DAA, 25 DAA and 30 DAA stage.

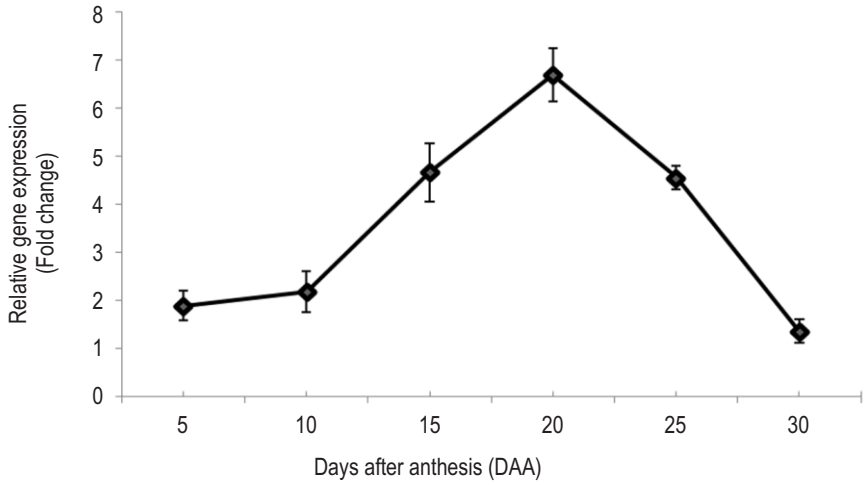

Fig. 2: Expression profiles of *soluble starch synthase I (SSS1)* gene at different stages during wheat grain development.

'Spectrum™ Plant Total RNA Kit', there was problem of column blockage due to sample solidification for the grain samples from mid to late stages (after 10 DAA) of grain development. Hence, this kit could not be used for isolating RNA from wheat grains beyond 10 DAA stage. Standard TRIzol method using wheat grains collected after 10 DAA resulted in sample solidification in extraction buffer causing difficulty in phase separation. Also, carryover starch co-precipitated with RNA during precipitation step making the RNA difficult to resuspend in water and resulting in poor quality RNA. In addition to these methods, attempt was made to isolate RNA using protocol reported by Li and Trick (2005) and the results with grain samples with higher starch content (beyond 20 DAA stage) were not satisfactory (Table 2). In this study, a modified protocol with better yield and quality of isolated RNA was developed based on the method reported by Li and Trick (2005). The new method differed in the composition of RNA extraction buffer, pH of the buffer and to a larger extent in the protocol used. The composition of modified buffer was similar to reported buffer except slightly alkaline pH (9.0) of the buffer and use of NaCl in place of LiCl with an additional step of 10 minutes on ice before phenol-chloroform extraction.

The alkaline pH (9.0) of the buffer inhibited the activity of endogenous ribonucleases in addition to inhibition by 2-mercaptoethanol in the extraction buffer (De Vries *et al.,* 1993; Wang *et al.,* 2012; Rashid *et al.,* 2016). Behnam *et al.* (2019) reported the use of an RNA extraction buffer containing similar constituents like SDS, Tris/HCl, EDTA, sodium chloride, β-mercaptoethanol along with PVP and optimized the concentration of constituents for isolating high-quality RNA from starch-rich tissues of cassava plants. In the next step, phenol-chloroform (1:1) extraction was used to separate RNA from DNA,

protein and the bulk of starch. Subsequently, TRIzol extraction was conducted to further improve the quality of RNA, followed by chloroform-isoamyl alcohol (24:1) extraction. Use of phenol was avoided during chloroform-isoamyl alcohol (24:1) extraction to minimize the contamination of isolated RNA with residual phenol. To prevent starch co-precipitation, an additional step was included for pre-solubilization of carryover starch before the precipitation step. For this, sodium chloride was added at high concentration and 5 min incubation was done to properly dissolve the carryover starch, subsequently followed by precipitation of RNA with isopropanol. Simultaneous addition of sodium chloride and isopropanol during precipitation step (as done by Li and Trick, 2005) does not give enough time to carryover starch for getting properly dissolved and may have resulted in starch co-precipitation. As compared to the present study, the lower A260/A230 ratio (1.85) for wheat RNA extracts as reported by Li and Trick (2005) is an indication of carryover starch. Therefore, in the present protocol, pre-solubilization of starch was done to effectively deal with the problem of starch co-precipitation.

The quantity, purity and integrity of the extracted RNA determine the efficacy of the isolation method used. The average quantity of RNA isolated by our method was sufficiently high for all the grain samples at different developmental stages. Isolation of RNA was also performed following the method of Li and Trick (2005), and a lower RNA yield was obtained for all the grain stages as compared to our protocol (Table 2). Following our protocol, the highest quantity of RNA (123 µg) was obtained from developing grains at 15 DAA using 70 mg of grain powder, while the lowest quantity of RNA (22.9 µg) was obtained from mature grains at 30 DAA. Average RNA yield from mature grains at 20 DAA and 25 DAA (62.4 and 41.9 µg per 70 mg grain powder) was apparently higher than the yield reported in previous studies. Wang *et al.* (2012) isolated RNA from wheat grains at 18 and 24 DAA and reported RNA yield of 27 and 28.9 µg per 100 mg f. wt. of the grain samples, respectively, which was much lower than the RNA yield obtained by following our protocol for corresponding grain stages. We obtained 27.8 µg of total RNA per 70 mg of grain powder from the grains (at 30 DAA stage) maturity. Our method of RNA isolation can, therefore, be of use in extracting a good quantity of RNA even from mature wheat grains.

The average A260/A280 ratios of RNA extracts at different stages of grain development ranged from 2.06 to 2.11, which indicate that there was no significant contamination of proteins or residual phenol. The A260/A230 ratio is a secondary measure of nucleic acid purity. The A260/A230 value for "pure" nucleic acid is often higher than the respective A260/A280 value. Using current protocol, A260/A230 ratios ranged from 2.09 to 2.21, which showed the absence of carryover polysaccharides, phenol and guanidine (contaminants absorbing near 230 nm) in the isolated RNA. The RNA extracts had a higher A260/A230 ratio than the respective A260/A280 ratio for all the samples indicating high purity of isolated RNA. Li and trick (2005) protocol did not produce satisfactory results in terms of quality of isolated RNA as depicted by lower 260/230 absorbance ratios. Using Li and Trick (2005) protocol, lower A260/A230 ratios were obtained as compared to the corresponding A260/A280 ratio, which indicated the presence of contaminants in the isolated RNA. Very low A260/A230 ratios obtained for starch-rich grains at 25 DAA and 30 DAA may be due to the presence of carryover starch. Residual starch has always been an issue of concern in the protocols on RNA isolation from starch-rich samples. Furtado (2014) extracted the RNA from wheat seeds first using Trizol and then purified the isolated RNA using kit-based filter cartridge to get pure RNA without carryover starch, however, the use of filter cartridge for RNA purification is comparatively expensive. The integrity of total RNA samples was validated by sharp 28S and 18S rRNA bands on 1.5% agarose gel (Fig. 1). The electrophoresis results showed the RNA to be of excellent quality with no sign of RNA degradation. The integrity of RNA molecules is of utmost importance for the subsequent downstream applications like gene expression analysis and cDNA cloning.

The RNA extracted by our method was found suitable for many downstream applications. The expression profile of *soluble starch synthase I (SSSI)* gene during wheat grain development was detected by quantitative real-time PCR (Fig. 2). The temporal pattern of *SSSI* gene expression during wheat grain development is highly consistent with the previously reported expression patterns (Hurkman *et al.,* 2003; Ganeshan *et al.,* 2010; Wang *et al.,* 2014). This indicated that the quality of the extracted RNA was good enough for real-time PCR analysis, and this method is not affected by different developmental stages of the grain. We have also successfully used the extracted RNA for other applications like reverse-transcription PCR, cDNA cloning and sequencing (data not shown). This method is very useful for RNA isolation from mature grains containing high levels of starch. This protocol effectively resolved the main issue during RNA isolation from starch-rich samples, *i.e.,* starch co-precipitation with RNA; resulting in extraction of high quality and yield of RNA.

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References

Behnam, B., A. Bohorquez-Chaux, O.F. Castaneda-Mendez, H. Tsuji, M. Ishitani and L.A. Becerra Lopez-Lavalle: An optimized isolation protocol yields high-quality RNA from cassava tissues (*Manihot esculenta* Crantz). *FEBS Open Bio.*, **9**, 814-825 (2019).

Cornell, H.: The chemistry and biochemistry of wheat. In: Bread making: Improving quality (Ed.: S.Cauvain). Woodhead Publishing, Cambridge, United Kingdom, pp. 31-70 (2003).

Dash, P.K.: High quality RNA isolation from ploy phenol-, polysaccharide- and protein-rich tissues of lentil (*Lens culinaris*). *3 Biotech*, **3**, 109–114 (2013).

De Vries, S., H. Hoge and T. Bisseling: Isolation of total and polysomal RNA from plant tissues. In: Plant Molecular Biology Manual (Eds.: S.B.Gelvin, R.A. Schilperoort and D.P.S. Verma). **Vol. B6**, Kluwer

Academic Publishers, Dordrecht, pp. 1-13 (1993).
Furtado, A.: RNA extraction from developing or mature wheat seeds. In: Cereal genomics, methods in molecular biology (Methods and Protocols) (Eds.: R. Henry and A. Furtado). Vol 1099, Humana Press, Totowa, NJ,pp. 23-28 (2014).
Ganeshan, S., J.M. Drinkwater, A. Repellin and R.N. Chibbar: Selected carbohydrate metabolism genes show coincident expression peaks in grains of *in-vitro* cultured immature spikes of wheat (*Triticum aestivum* L.). *J. Agri. Food Chem.,* **58**, 4193-4201 (2010).
Gao, J.W., J.Z. Liu, B.Li and Z.S. Li: Isolation and purification of functional total RNA from blue-grained wheat endosperm tissues containing high levels of starches and flavonoids. *Plant Mol. Biol. Rep.,* **19**,185a-185i (2001).
Green, M.R. and J. Sambrook: Extraction, purification, and analysis of RNA from eukaryotic cells. In: Molecular cloning: A laboratory manual. 4th Edn., **Vol. 1**, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 345-454 (2012).
Hurkman, W.J., K.F. Mccue, S.B. Altenbach, A. Korn, C.K. Tanaka, K.M. Kothari, E.L. Johnson, D.B. Bechtel, J.D. Wilson, O.D. Anderson and F.M. DuPonta: Effect of temperature on expression of genes encoding enzymes for starch biosynthesis in developing wheat endosperm. *Plant Sci.,* **164**, 873-881 (2003).
Kumar, A., R. Singh, G. Singh, R.K. Sharma, M.S. Saharan, R.S. Chhokar, B.S. Tyagi, S. Ramadas, R. Chand and I. Sharma. In: Wheat cultivation in India (Pocket Guide).Vol. Extension Bulletin 52, Directorate of Wheat Research, Karnal, India, pp. 30-34 (2014).
Li, Z. and H.N. Trick: Rapid method for high-quality RNA isolation from seed endosperm containing high levels of starch. *Bio. Techniques,* **38**, 872-876 (2005).
Liu, L., R. Han, N. Yu, W. Zhang, L. Xing, D. Xie and D. Peng: A method for extracting high-quality total RNA from plant rich in polysaccharides and polyphenols using *Dendrobium huoshanense. PLoS ONE,* **13**, e0196592 (2018).
Mornkham, T., P.P. Wangsomnuk, Y.B. Fu, P. Wangsomnuk, S. Jogloy and A. Patanothai: Extractions of high quality RNA from the seeds of *Jerusalem artichoke* and other plant species with high levels of starch and lipid. *Plants,* **2**, 302-316 (2013).
Rashid, A., T. Baldwin, M. Gines, P. Bregitzer and K. EsveltKlos: A high-throughput RNA extraction for sprouted single-seed barley (*Hordeum vulgare* L.) rich in polysaccharides. *Plants (Basel),* **6**, 1–7 (2016).
Rosegrant, M.W. and S.A. Cline: Global food security: Challenges and policies. *Sci.*, **302**, 1917–1919 (2003).
Salzman, R.A., T. Fujita, S.K. Zhu, P.M. Hasegawa and R.A. Bressan: An improved RNA isolation method for plant tissues containing high levels of phenolic compounds or carbohydrates. *Plant Mol. Biol. Rep.,***17**,11-17 (1999).
Singh, G., S. Kumar and P. Singh: A quick method to isolate RNA from wheat and other carbohydrate-rich seeds. *Plant Mol. Biol. Rep.,* **21**, 93a-93f (2003).
Takumi, S., M. Okada, A. Michikawa, Y. Miki, Y. Ryoko, Ohno and K. Yoshida: High-quality RNA isolation from wheat immature grains. *Wheat Inf. Serv.,* **124**, eWIS124.2 (2017).
Wang, G., G. Wang, X. Zhang, F. Wang and R. Song: Isolation of high quality RNA from cereal seeds containing high levels of starch. *Phytochem. Anal.,* **23**,159-163 (2012).
Wang, Z., W. Li, J. Qi, P. Shi and Y. Yin: Starch accumulation, activities of key enzyme and gene expression in starch synthesis of wheat endosperm with different starch contents. *J. Food Sci. Technol.,* **51**, 419–429 (2014).
Wilkins, T.A. and L.B. Smart: Isolation of RNA from plant tissue. In: A laboratory guide to RNA: Isolation, analysis and synthesis (Ed.: P.A. Krieg). Wiley-Liss, New York, pp. 21-41 (1996).

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