2.1. Extraction of RNA

Samples from both control and stressed transgenic *A. thaliana* seedlings were harvested after their mentioned time. Crushed in liquid nitrogen by mortar and pistil to homogenize them, the ground sample was used for the extraction of RNA. Extraction was done by using manual protocol using DNase (RQ1 RNase-free DNase; Promega M6101 (45). The extraction of RNA is summarized in the following steps.

1. In step #1, 0.3 mL of solution 1 was supplemented to the ground tissue sample, and the mixture was properly mixed by gentle tapping, inverting, and vertexing the Eppendorf tubes for a few seconds. The tubes were left at room temperature for 5 minutes.
2. Next step was the protein DNA precipitation phase. Solution 2 was added to the lysate of the cells and the tubes were gently overturned. The tube was incubated for 10 minutes at 4°C refrigerator then Eppendorf tubes were centrifuged at 4°C for 30 minutes at 3500 rpm.
3. After centrifugation two layers were formed, the supernatant was lifted to a new Eppendorf tube and again centrifuged for 18 minutes at 4 °C (3500 rpm), and the supernatant was shifted to Eppendorf tubes.
4. Furthermore, 300 µL (0.3 mL) of solution 3 (Isopropanol) was added to new Eppendorf tubes, well over-turned, and tubes were then centrifuged for 15 minutes at 4 °C (3500 rpm).
5. White pellet was observed, and the pellet was washed out very carefully with 70 % ethanol (solution 4) after that air dried the RNA pellet and then dissolved in 25 µL of (Nano pure water) solution 5.
6. That Eppendorf tubes were left for at least 10 minutes at room temperature.
7. Tubes were vortexed and centrifuged for 15 minutes. The resulting supernatant was shifted to another tube.
8. Then supernatant was treated with 3 µL of 10X DNase buffer and 2 µL of DNase I (RQ1 RNase-free DNase; Promega M6101).
9. RNA sample (30 µL) was added with 70 µL of Nano pure water (solution 5), very next to this 50 µL of solution 6 was added, then 0.4 mL (400 µL) of 100 % ethanol (solution 7) was added and were properly mixed.
10. RNA samples were centrifuged at 4 °C (3500 rpm) for 25 minutes. The pellet was washed out with solution #4.
11. Resulted in the pellet was air dried and then 20 µL of Nano pure water (solution 5) was added.

Finally, the Eppendorf tubes containing a pure form of RNA were stored for future use at -80 °C (Table. S. 1).

Table. S1. Reaction components for isolation of RNA.

S/N	Solutions	Formulation	
1.	Solution 1 (cell lysis solution)		50 mL
		2 % SDS	1 g
		69 mM sodium citrate	1 g
		133 mM citric acid	1.26 g
		1.1 mM EDTA	0.1 mL of 0.25 mM stock
2	Sol # 2 (protein DNA precipitation solution)		50 mL
		4.2 M NaCl	11.50 g
		17 mM sodium citrate	0.23 g
		33 mM citric acid	0.31 g
3	Sol # 3	Isopropanol	
4	Sol # 4	70 % ethanol	
5	Sol # 5	Nano pure water	
6	Sol # 6	3.8 M NH_4AC	27.4 g/50 mL
7	Sol # 7	100 % ethanol	

2.2. Confirmation of quality and quantity of RNA

For excellence and quantity confirmation Nanodrop technique (Nanodrop 1000 spectrophotometer, Thermoscientific) was used. For this, the glass disc of Nanodrop was loaded with 1 µL of Nano pure water for blank reading, then loaded 2 µL of each sample to Nanodrop and results were obtained by using ND-1000, Ver. 3.8 soft. For RNA integrity gel electrophoresis of the whole plant was also performed, the clear band shows the veracity of the RNA (figure. 1). Eventually, the total result was saved on an Excel sheet. Finally, the confirmed RNA samples were processed to the next step and stored at -80 °C.

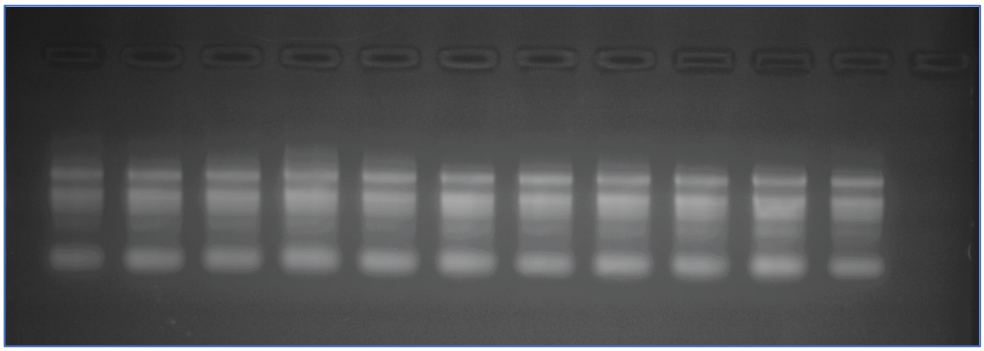

Figure S1. RNA confirmation via gel electrophoresis.

2.3. cDNA Synthesis

After quantification of total RNA obtained from transgenic *Arabidopsis* plant samples, cDNA was prepared. Reverse transcriptase was used for the preparation of cDNA from RNA. The excellence and quantity of cDNA were completed by using a Nanodrop spectrometer. For cDNA synthesis, the following steps were carried out.

1. Samples of confirmed RNA were used to synthesize cDNA, 1 µL of Oligo $(dT)_{18}$ primer 25 pM (Gene Link™: e-oligos), and 2 µL of Nano pure water was added in 1 µg of extracted RNA in small PCR tubes to make total 12 µL volume.
2. The whole process was retained on the ice box. This reaction mixture was spun for a short time and then incubated at 70 °C for at least 7 minutes.
3. Later, different components were added to the reaction mixture.
4. Addition of the prementioned component's volume of the reaction mixture reached 20 µL.
5. Then the reaction mixture was incubated at 42 °C for 1 hour.
6. Later incubation temperature was elevated to 95 °C for 5 minutes. At high-temperature revert Aid, Reverse Transcriptase stopped its activity.
7. Again, the temperature was down to 5 °C for 10 minutes.
8. This process was done in PCR.
9. At the very end, the cDNA was formed and stored at -80 °C.

2.4. Quality and quantity of cDNA

The class and quantity of cDNA were inveterate by using normal PCR. For cDNA confirmation, normal PCR was performed with the same range of annealing temperature (55 - 61 °C) for both gene primers i.e. primers of GUS (reporter gene) and Actin gene (housekeeping gene), the following steps were carried out for confirmation of cDNA.

1. PCR components were used to obtain an amplified product of both Actin and GUS.

2. PCR products load on agarose gel for confirmation.
3. These cDNA samples were directly used for the next steps (RT-PCR) and were stored at -80 °C for future use.

q- PCR (RT-PCR) quantified and viewed the products in real-time. It is an advanced form of normal PCR. Results of qRT were then used for assessing the fold induction. The results of qRT-PCR are highly accurate because "SYBER green" is employed as a double-stranded DNA binding dye. They bind to minor groves of the DNA and produce flash or fluorescence by binding with DNA. The thresh hold cycle is the cycle in RT-PCR when the fluorescence (generated by the binding of "SYBER green" with DNA) becomes detectable in comparison with background fluorescence (generated by Light Cycler® 96 System) and is abbreviated as the CT cycle (Table. S. 2). In the present study, both primers housekeeping (Actin) and reporter gene (GUS), were used to perform RT-PCR. It covers four very important steps. For RT-PCR the required reagent was supplemented in PCR tubes to make the total volume of the reaction mixture 11.16 µL. In the RT-PCR reaction, an evident increase in fluorescence of "SYBER green" was found that was parallel to the rise in the expression level of GUS and actin genes.

Table. S2. Constituents of the reaction mixture for RT-PCR

S.NO	**Reagents**	**Volume**
1.	cDNA (1:5 dilution)	2 µL
2.	SYBR Green dye	6 µL
3.	Primer mix (50 pM) (Forward & Reverse)	0.16 µL
4.	Nuclease free water	3 µL
	Total	11.16 µL

2.6. Data analysis

Characterization of data and elucidation of fold induction were done by using RT-PCR. After performing RT-PCR, the data were evaluated in the form of fold induction using a mathematical CT model. They are summarized by the following steps.

1. RT-PCR was done to check the important fold induction of GUS that was driven by *Os*PPO in seedlings of *A. thaliana* and generated data was collected and analyzed by a mathematical model CT (2-ΔΔCT), via Line Gene K ver. 4 software.

2. To work with CT (2-ΔΔCT), a calibrator and internal control were selected. In RT-PCR results, *Os*PPOGUS samples showed significantly variable induction in comparison with untreated control samples.
3. Results of RT-PCR were stored on an Excel sheet.
4. Fold induction in each reaction was considered and graphically displayed on the Excel sheet.