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Easy Method for RNA Isolation from the Ripe Banana (Trizol method) for Expression Profiling

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Abstract: ***RNA is a nucleic acid which is most unstable than DNA and very complicated to isolate from the plant material which is rich in polyphenols and secondary metabolites. Banana which have high level of polysaccharides, polyphenols and secondary metabolites due to which the isolation of RNA from banana is a difficult task. There is so much work has been done for the isolation RNA from the ripe banana. Hence there is a method through which we can isolate the high quality amount of the RNA for the further molecular biology aspects.***

Keywords: Banana (Musa accuminata), Trizol.

1. Introduction

Isolation of RNA from plant tissues having levels of polysaccharides, polyphenols and secondary metabolites is a very difficult task. Banana which is a fleshy fruit and is an edible fruit and used as a common fruit in daily life. Several methods has been used for the isolation of the RNA from the banana over many centuries (Logeman etal, 1987, Levi etal,1992, Gomez –Lim ,1992 andC-tab method by Chang(1993) and modified procedure of the C-tab by MeharA. Asif etal , 2000). We have interested in the overexpression of the Ethylene gene (EIN3 gene) which helps in the induction of the ethylene signalling pathway and as well as in the other procedure like abscission and secession etc. During this study we need the high quality and quantity of the total RNAfor further studies. We isolated the RNA form the Trizol method which is a very reliable method for the isolation of the RNA in good yield. It is a very easy and fast method to isolate the RNA form the fruit materials which have higher amount of the polysaccharides and the polyphenols.

2. Literature Review

RNA (Ribose nucleic acid) which is an unstable nucleic acid and very difficult to isolate from the sample sources such as plant tissues. Over centuries the protocols for the isolation of the RNA have been describe but the time to time new methods are added to get a good quantity of the Pure RNA. The first protocol used for isolation of the RNA from plant (Mangosteen tissues) were described by Rochester et al. and Matsumura et al., isolated RNA was partially degraded. There so many methods are used for the isolation of the RNA from the plant fleshy tissues i.e. C-Tab method, GT method and SDS METHOD etc. The requirements for isolation is also differ according to the types of plant tissues.

Problem Definition

We used so many methods for the isolation of RNA but it gives not good outcome during the expression profiling. We used this Trizol method to isolate the RNA which gives a good result and also good result during expression profiling.

Materials and Methods

Plant Material: Mature Banana

Reagents

Trizol Extraction buffer: Saturated phenol buffer, 0.8M Gudininum thiocyanate, 0.4M Ammonium thiocyanate,3M Sodium acetate, pH 5.0, Glycerol, Distilled water, Chloroform,70% ethanol

Gel electrophoresis solution: 1X TBE agarose gel, 1X TBE running buffer, Ethidium bromide, 6Xloading dye.

RNA Extraction protocol:

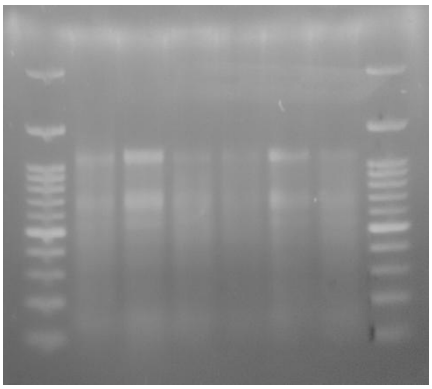
1. Take a small portion of a full ripped banana in the 1.5 ml epenndrof tubes.
2. Chop the banana pieces in the epeendrof in the tube with help of tips or needle like equipment.
3. Add 500microlitre extraction buffer(TRIZOL REAGENT) into the tube containing sample
4. Incubate the sample for 10 minutes in the prepared ice box i.e. at 4°C
5. Centrifuge the sample at 10,000 rpm for 10 min at 4°C
6. Collect the supernatant into fresh vial and add 1000µl chloroform into it
7. Gentle shake the mixture
8. Incubate the mixture for 10 minute at 4°C.
9. Again centrifuge at 10,000 rpm for 10 min at 4°C.
10. Two layers are form after centrifugation; collect the upper aqueous phase into new vial.
11. Add sodium acetate half of the amount of the aqueous layer.
12. Mix the mixture in the invert position.
13. Incubate the mixture for 10-15 min at 4°C.
14. Centrifuge the mixture at 10,000 rpm for 10 min at 4°C.
15. Collect the pellet, discard the supernatant.
16. Wash the pellet with 70% ethanol by dissolving pellet into it.
17. Centrifuge the sample once again at 5,000rpm for 5min at 4°C.
18. Discard the supernatant. Dry the pellet.
19. Suspend the pellet into the 50µl TE buffer (may be into ddH_2O).

20. Observe the RNA bands under UV transiiuimiator by performing gel electrophoresis.

Electrophoresis of the sample:
For the running of the RNA sample the TBE Buffer(Tris-Borate-EDTA) is used , the 1%agarose gel is also prepared in the TBE buffer. The loading sample is used in the ratio of 3:7 (loading dye: RNA sample).The RNA sample was run at the 40-50V. The sample was electrophoresis until the dye travel to the half of agarose gel.
After the completion of the electrophoresis ,the agarose gel is observed under the UV-trans illuminator.

3. Result

Qualitative Analysis result
The clear band of the RNA appears during gel electrophoresis.



Quantative Analysis result

Sample no.	Abosrbance @260	Absorbance @280	Ratio (OD)	Concentration of RNA (µg/µl)
S1.	0.388	0.205	1.892	1.940
S2.	0.325	0.159	2.044	1.625
S3.	0.182	0.092	1.915	0.910
S4.	0.325	0.159	2.044	1.625
S5.	0.355	0.175	2.028	1.775
S6.	0.288	0.153	1.882	1.440

4. Conclusion

The electrophoresis result from the Trizol method in banana is more clear and observable as comparison to the result of C-Tab method.

5. Future Scope

There is no method described for the isolation of the RNA from the fleshy fruit tissues yet. Hence the use of Trizol in the isolation of RNA from the fleshy fruit tissues will help in to isolate a good quality and quantity of the RNA, which further can be used for the other research prospects.There are so many methods can also be used for the isolation of RNA but the result of the Trizol method give a good outcome which can be used for the study of the expression profiling.

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