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Research Paper

RNA Extraction from Sugarcane Leaves

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ABSTRACT

There are many protocols used for RNA extraction from plants, which vary according to the composition of the tissue, the part of the plant, the cultivation area and the atmosphere. Here, in Malaysia, we will focus on RNA extraction from the sugarcane leaves (*Saccharum officinarum)* by using two different protocols and one RNA extraction kit and compare between them to find the best. In both protocols, we used general RNA extraction method, by using TRizol, chloroform and isopropanol, with different amount of TRizol, and leaves to get high yield from RNA with good quality. The samples were checked by using Nano-spectrophotometer to determine the carbohydrate and protein contamination as well as the amount of RNA in (μg/ μl). This is a simple method to extract high quality RNA from Sugarcane leaf that is suitable for several applications. This method takes less time and money for RNA extraction and is effective for other tissues where the quality and quantity of RNA is highly affected by the presence of carbohydrate and ethanol.

Keywords: RNA extraction; RNA extraction kit; Sugarcane leaves; Malaysia.

INTRODUCTION

Sugarcane is considered to be one of the most important plant crops because of its many attributes and uses – produces sugar, bioethanol (used as fuel for vehicles and is widely used in USA and Brazil), and bagasse (the fibers of the remaining raw materials after extracting the syrup from it and which is used in the paper industry) (Nonato, et al., 2001). Furthermore, there are many health benefits of sugarcane, including its capacity to strengthen the organs in the body such as the heart, kidney, stomach and brain. In addition, it is recommend to drink fresh sugarcane after being exposed to hard physical activities as it helps to hydrate the body quickly and has many other benefits for body fitness (Awika and Rooney, 2004; Peterhutch, 2008).

Brazil and India are the main countries for producing sugarcane; studies show that in 2008 the production in Brazil reached more than 700 million tons and more than 300 million tons in India.(Goldemberg, et al., 2008; Plummer, 2006)

In Malaysia, the rapid economic development in recent years has led to a boom in the food industry-confectionery, ice cream, cake, chocolates, and many other sweet foods

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and, in addition, Malaysia is considered as being a pioneer in Halal food production and exports to many Muslim countries (Shafie and Othman, 2006).

If we compare the last two decades of sugar consumption in Malaysia we will find that the demand for this product has increased considerably. In the first five years of the nineties the average sugar consumption was about 800,000 tons, which is significantly more than the first five years of the eighties-500 000 tons. The demand for sugar is increasing at about 14% annually (FAO, 1998).

The National Agricultural Policy Plan (NAP) for 2010, did not give adequate attention to improve sugarcane production, compared to oil palm and other Malaysian crops, and, consequently, depends on sugar imports rather than improving the domestic production(FAO, 1998).

Therefore, in this study we focus on improving the productivity of sugarcane by using molecular work instead of traditional cultivation, which the latter requires more time, money, and large areas for cultivation, and furthermore, does not give accurate results, whereas molecular work needs less space, less money and less time, and gives much better results.

The first and most important step in molecular work is RNA extraction, the success of such studies rely heavily on the quality and quantity of extracted RNA. Thus, we extracted RNA by following two different protocols and compared them with RNA extraction by using the RNA extraction kit from Geneaid (Geneaid Biotech Ltd., 2010).

MATERIALS AND METHODS

There are many protocols for RNA extraction; in this study we extracted RNA from sugarcane leaf by using two different protocols and comparing them to find the best protocol.

***Glass-wares preparation**:* All the glassware was placed in 120°C (Enberg, et al., 2004) overnight after washing with detergent. The most important step in RNA extraction is deactivating the RNase and removing it from all the glass wares and solutions that are used for RNA extraction by baking the glass wares with high temperature more than 100^0C for 4 hours. Then one should add 0.1% DEPC to all the solutions to deactivate this enzyme.

Sample collection: The leaf was collected early morning, and placed directly in liquid N2 to freeze quickly, (Alternatively we can use dry ice to freeze it).

The first protocol is as follows: (Hilario and Mackay, 2007)

1- Collect fresh young leaves of sugarcane, and grind about 0.1g with mortar and pestle to fine powder by mixing with liquid nitrogen.
2- Add 1ml TRIzol reagent and transfer to centrifuge tubes.
3- Centrifuge for 5min/2°C/12000rpm.
4- Transfer supernatant to another centrifuge tube. It should be done carefully by using micropipette to prevent mixing with other layers.
5- Add 200 µL of chloroform and shake gently.
6- Leave at room temperature for 3min
7- Centrifuge for 5min/2°C/12000rpm.
8- Transfer the upper phase to new tube carefully by using micropipette to prevent mixing with other layers.
9- Add0.5 ml of isopropanol
10- Leave for 10min
11- Centrifuge for 5min/2°C/12000rpm.

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12- Discard supernatant carefully by using micropipette to prevent mixing with other layers.
13- 1 ml of 75% ethanol was added
14- Centrifuge for 5min/2°C/12000rpm.
15- Discard the supernatant and let the pellet dry under the fume hood.
16- Add 20µl from DEPC/treated water to the pellet and keep in -80 refrigerator. RNase free water can be added instead of DEPC/treated water and kept in -20 refrigerator.

The second protocol using TRizol reagent (Biology, 2010) (purchased from Invitrogen):

1- Collect fresh young leaves of sugarcane, and grind about 0.5g (instead of 0.1g) with mortar and pestle to fine powder by mixing with liquid nitrogen.
2- Add 5ml TRIzol reagent (carefully under fume hood).
3- Use Vortex for few seconds to mix it well.
4- Add 200µl chloroform and mix gently.
5- Centrifuge 12000rpm at room temperature.
6- Transfer supernatant to new tube carefully by using micropipette.
7- Add 500µl isopropanol
8- Incubate in ice for few minutes
9- Centrifuge 10sec/4°C
10- Add 1ml ethanol
11- Centrifuge 5min/7500rpm at room temperature
12- Dry the pellet by fixing it horizontally, not upside down to prevent the pellet from going out.
13- Add 44µl DEPC/treated water. RNase free water can add instead of DEPC/treated water.

The third protocol: was by using the RNA extraction kit from Geneaid (Geneaid Biotech Ltd., 2010).

RESULTS AND DISCUSSION

By using the Nano-spectrophotometer from IMPLEN, blanked with 2µl drops from DEPC treated water on spectrophotometer lens, then 3µl from the sample was added to the lens. The results for the three methods are shown in Table 1.

Table-1: RNA yield and quality detected with spectrophotometer.

Data	1st protocol	2nd protocol	Kits from geneaid
Total RNA yield	488 µg/ µl	1480 µg/ µl	0.232µg/µl
A260/A280[1]	1.87	2.003	2.033
A260/A230[2]	1.92	1.900	2.00

- [1]The ratio A260/280 should be from 1.8 – 2.0, if it's less that means the RNA sample contaminated with protein.
- [2]The ratio A260/230 should be 1.8 – 2.0, if it's less that means contaminated with carbohydrate.

As the result shows the second protocol achieved better results than the first one; the concentration of RNA is quite high, and the contamination with carbohydrate and protein is quite low. We increased the amount of leaves that were ground, using 0.5g instead of 0.1g of sugarcane leaves. Although the result of the third method also has high purity from protein and carbohydrate, the RNA concentration is quite low when compared with the first two protocols. Although the protocol that we used works well and achieved a good product for many types of plants, for sugarcane a little bit

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modification is needed to obtain a high concentration of RNA to use it in RT-PCR, cDNA library, northern blotting and many other applications.

CONCLUSION

In conclusion, the changed steps described here can be used for extracting high amounts of RNA from many different plants in a short period and it is especially suitable for obtaining RNA from tissues with high polysaccharide contents.
Putting in mind the fact that the high RNA concentration and its quality have a crucial role in the success of applications in molecular sciences, most researchers recommend the first or the second protocol to get good quality and high concentration RNA.

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