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ORIGINAL ARTICLE

Isolation of plant RNA for early detection of RNA Viruses

Rakesh Kumar Meena[1*], Prashant Sukla [2] and Mahima Dubey [3]
[1]Aditya Biotech Lab & Research Pvt Ltd, Raipur [2]Devleela Biotechs, Raipur (C.G.),
[3]Department of Plant Molecular Biology and Biotechnology, IGKV, Raipur
*Correspondence author:-Email dbt.rakesh@gmail.com

ABSTRACT
A procedure for isolation of RNA from virus infected plant is described. The method relies on homogenization of infected plant tissue in double distilled water and subsequent extraction of RNA in ethanol. With the method, Allexivirus which is RNA virus infecting garlic was successfully isolated and confirmed by PCR amplification of a conserved region of the virus which is of 200 bp in length, after RT-PCR. On gel electrophoresis of the PCR product of cDNA the band was found at 200 bp which confirmed positive isolation of RNA virus through the method. The isolated RNA was also confirmed through nanodrop spectrophotometer and the readings of nanodrop suggest that method yields as good RNA as the kits available commercially.
Keywords: RNA virus, Allexivirus, garlic, ethanol

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INTRODUCTION

Garlic is infected with multiple viruses of different taxonomic group which are known as garlic viral complex of which belong to *Potyviruses*, *Carlaviruses*, and *Allexiviruses* [1,2]. Identification of these viruses is based on serological or molecular tests. As reported by Melo Filho et al., [1] serology is not a good tool for virus differentiation among *Allexivirus* even though it can be used for detection of the group. Molecular tools are better in both detection as well as differentiation of *Allexiviruses*.

Since the protocol for RNA isolation developed by Chomczynski and Sacchi [3] has come a long way. The steps of RNA isolation varies with the type of organism from which it is being isolated. RNA isolation from plants may result in using different protocols as some protocols do not give adequate RNA for every type of plant [4,5]**.** Jaiprakash et al. [6] from tea and Hu et al. [7], from fruit trees having high content of polyphenol compounds and polysaccharides, have described simple methods for RNA isolation. In every type of organism from which RNA is isolated the number of chemical use is large and sometimes expensive. Even if the numbers of chemicals are kept to the minimum the amount of time taken is large and chemicals sometimes expensive and even hazardous.

The basic need for any diagnostic laboratory is speed combined with accuracy. For diagnosis of RNA virus in laboratory the requirement of speedy protocol is there as the sample load in such laboratories is very high. Keeping this in mind the current work was planned so as to keep the amount of chemicals to bare minimum and time in matter of minutes not hours. The present work details isolation of RNA virus from garlic from established methods and by the method generated in our lab and evaluating the viruses through PCR followed by gel electrophoresis. *Allexivirus* was chosen for the study which is RNA virus infecting garlic.

MATERIALS AND METHODS

Materials

For isolation of *Allexivirus* garlic cloves were used which were purchased from the local vegetable market. Trizol method was used as standard method for isolating RNA virus from garlic cloves. For isolating RNA virus from garlic 100 mg garlic cloves were used. They were homogenized in 1 ml double distilled water using pestle and mortar. The homogenized mixture was then centrifuged at 8000 rpm for 10 min (Spinwin, Tarson).

The supernatant was used for RNA virus isolation in case of garlic. To this equal volume of chilled ethanol was added and the mixture incubated for 30 min in -20 ºC. The supernatant containing ethanol was taken in a fresh microfuge tube and 500 µL of ethanol was added followed by incubation for 30 min at room

temperature. The mixture was then centrifuged at 12000 rpm for 10 min. The supernatant was discarded and the pellet was resuspened in sterile wate. For *Allexivirus* cDNA was made using commercial kit (Thermo Fisher Scientific India Pvt. Ltd.) product code AB1453B using isolated RNA. PCR was set up as usual with two primers (forward and reverse) and the products were visualized in 1.8% agarose gel in UV transilluminator for *Allexivirus*.

RESULTS AND DISCUSSION

Three garlic samples testing positive for Allexivirus by ELISA were used for RNA isolation by our method and quantified by the Nandrop spectrophotometer ND 1000 (Table 1) and tested for *Allexivirus*. All the samples were found positive. Similar results were obtained from the standard methods as well as lab generated protocol. *Allexivirus* primer (Forward sequences CYGCTAAGCTATATGCTGAARGG and Reverse sequence TGTTRCAARGTAAGTTTAGYAATATCAACA) generates a product which is 200 bp long (**Fig1**). The markers were run along with the test samples and authenticated. The primer used in this study was generated by Barnawal et al. (2006).

In our method RNA can be extracted in the least time and with good quality and quantity by using simple materials and equipments. In a workday, one person can complete RNA isolation from more than 100 plant samples using this method. This method has been routinely used to extract RNA from garlic leaves and cloves for PCR based applications in our laboratory but it can be used for other species of different families that contain polyphenols and secondary metabolite.

Table: 1 RNA concentrations of the garlic samples with ratio

S. No	Name of the Sample	RNA Concentration (ng/µl)	Absorbance Ratio (260/280 nm)
1	Garlic 1	400	1.89
2	Garlic 2	380	1.92
3	Garlic 3	430	1.99

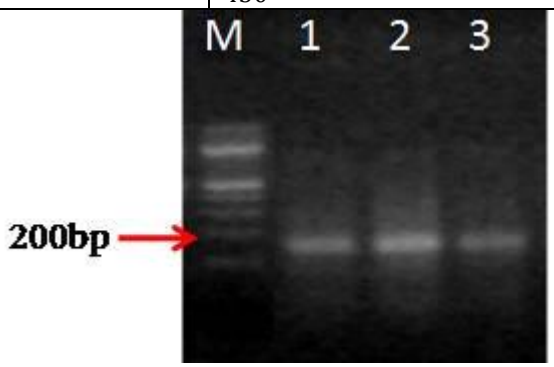

Fig. 1: Amplification of Allexivirus specific bands corresponding to 200 bp fragment in three garlic samples using RNA isolated by lab generated protocol.

Isolation of RNA viruses becomes cumbersome when the isolation is for routine diagnosis which requires speed as well as accuracy. Our method provides speed as well as just two basic chemicals which are available in all the laboratories thus reducing the cost of virus isolation. The RNA which was isolated through our method performed well with PCR and results were obtained as expected and similar to the results of those which were isolated using standard methods. This also proves the accuracy of our method as PCR can be set up without the need of any more purification or removal of PCR inhibitors. Our method also scores on the point that there is no need for even diluting the product of isolation which again minimizes the time to get the final results.

This method has several advantages such as economical spending, no need for the specialized and expensive equipments, spending little time, with no need of the experimented and experienced staff and more important, RNA extraction from plant leaves stored at usual fridges for long time. In this method, RNA with high quality and quantity can be acquired for other species that contain polyphenols and secondary metabolite is cumbersome. Time of extraction of RNA in our method is less than three hours.

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