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A RELIABLE METHOD FOR THE RECOVERY OF dsRNA FRAGMENTS OF CITRUS TRISTEZA VIRUS (CTV) FROM AGAROSE AND POLYACRYLAMIDE GELS[1]

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SUMMARY : *A simple and efficient method for the recovery of dsRNA fragments from agarose and polyacrylamide gels was used for four citrus tristeza virus (CTV) Florida isolates. The recovered dsRNAs were run on agarose gel. It was seen that recovered dsRNAS migrated on agarose gels according to their molecular weight.*

AGAROZ VE POLİAKRİLAMİDE JELDEN TURUNÇGİL TRISTEZA VİRUSUNUN dsRNA FRAGMANLARININ YENİDEN ELDE EDİLMESİ İÇİN UYGUN BİR METOT

ÖZET : *Agaroz ve poliakrilamide jelden dsRNA fragmanlarını yeniden elde etmek için basit ve kullanışlı bir method dört turunçgil tristeza virus izolatı için kullanıldı. Yeniden elde edilen dsRNA'ların moleküler ağırlıklarına göre agar jelde yerlerini aldıkları görüldü.*

INTRODUCTION

Single-stranded RNA viruses compose approximately 90 % of all known plant viruses. During their replication in plant cells dsRNA is produced as an intermediate product. This dsRNA is called the replicate form (RF). The method most commoly used for isolation of dsRNA from plant tissue is that of Morris and Dodds (1979). Analysis of dsRNA profiles from plant samples by gel electrophoresis after purification of dsRNA could be of diagnostic value (Valverde et al, 1990). Different groups of plant viruses and isolates of same viruses hava characteristic dsRNA profiles (Dodds et al., 1984). The uniqueness of a profile is based on the numbers

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and molecular weights of the dsRNA fragments. Agarose and polyacrylamide gels electrophoresis is a most efficient method for the rapid separation of RNA fragment on different sizes.

Many methods have been developed to recover DNA from gel. The recovery of RNA fragments from gel can be achieved, using similar methods. But these methods one not reliable and satisfactory.

There is a need for ar rapid and reliable electoelution medhod to recover RNA fragments from gel. The method, developed in the laboratory of R.F. Lee, It is simple end reliable, has worked very vell.

MATERIALS AND METHOD

Four Florida isolates of CTV, T-30, T-55, T-26 and T4 which vary in their biological activity were used in this study; The DsRNAs were extracted and purified from infected citrus tissues by modifying the procedure used to detect citrus viroid (Duran-Vila et al., 1986). Plant samples (5 gr) were phenol-extracted and LİCl partitioned (Semancik et al., 1975). When further purification was performed, nucleic acid samples were chromatographed on CF-11 cellulose (Franklin, 1966).

The dsRNAs (T-30, T-55) were fractioned on 1.2 % agarose gel (Sambrook et al., 1989) in TBE buffer (0.089 Tris borate, 0,089M boric acid, 0, 002 M EDTA) at 4°C for 3 hrs. at 100 V.

The dsRNAs (T - 26; T-4) were fractioned for 16 hrs. at 45 mA on a vertical 5 % polyacrylamide gel (Loening, 1967) (30 % acrylamide, 2.5 % bisacrylamide) containing 10 % TEMED, 10 % ammonium persulfate (APS), in electrophoresis (E) buffer (0.04 M Tris-Hcl, 0.02 M sodium acetate, 0.001 EDTA pH 7.8) at room temperature.

After, both gels were stained in 50 ug/ml ethidium bromide in the appropriate electrophorosis buffer, illuminated with UV light and photographed with poloroid type 55 film.

The ds RNAs were recovered from agar and polyacrylamide gels in electroelution apparatus, using a method developed in the laboratory of R.F. Lee as follows.

Six of the dsRNA bands belong to four CTV isolated (I. band of T-26, III. band of T-4, I., II. bands of T-30 and I., II. bands of T-55) were cut out, using a sharp scalpel. Electroelution apparatus was filled with 500 ml of 1/2 TBE buffer (8 mM Tris, 4 mM Na Acetate, 0-2 mM EDTA pH 7.2). Bubbles were removed. Six gel

slices containing dsRNA bands were placed in the slots. The level of TBE buffer was adjusted (Just below the top of gel slice). 75 ml of high salt buffer (5 M NaCl, 15 % Glycerol, 0.08 BPB-bromophenol blue) was loaded into V- channel.

The dsRNAs were eluted from gels in two runs, each of 60 minutes. After the first run at 125 volts, power supply was turned off and salt buffer together dsRNA was removed from V-channel with a 200 ul pipette. V-channel was refilled with additional salt buffer. The precedure was repeated to recover the remainder of the material from gel slice. The two salt materials containing dsRNA were mixed into a tube containing 600 ul obsolute ethanol and 1 ul glycogen.

Electroeluted materials were run on 1.2 % agarose gel in TBE buffer for 3hr. at. 100 V. After electroforosis the gel was stained in 50 ug/ml ethidium bromide, then photographed with poloroid type 55 film.

RESULS AND DISCUSSION

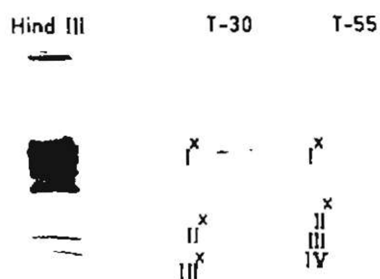
The dsRNAs of two CTV isolates (T-30, T-55) were fractioned on agarose gel and dsRNA profiles were illustrated on Fig 1. It was seen that T-30 isolate has three bands and T-55 has five.

The dsRNAs of two CTV isolates (T-4, T-26) were fractioned on polyacrylamide gel and dsRNA profiles were illustrated on Fig 2. It was seen that T-4 and T-26 isolates have four bands each.

Four CTV isolates which vary in biological activity suggests that the profiles of subgenomic dsRNA for each CTV isolate one unique indeed. The unique profiles for each CTV isolate could be useful in future tests at a molecular level (Lee, 1984). Further study is needed to determine the purpose of subgenomic dsRNAs and if they can be used to obtain probes specific for a CTV isolate.

After electroelution dsRNA bands belonging to T-26, T-4, T-30 CTV isolates were seen on agarose gel. But the bands of T-55 were not seen. This situation may be due to the low quantities of dsRNA. The results for electroeluted T-55 band were the same, although the method was repeated several times (Fig. 3).

Several methods have been developed to recover DNA from gel. These methods involve a variety of steps, used singly or in combination : These methods are electroelution into dialysis bags (Mc Donnell et al., 1977) or dialysis membrane (Girvitz et al, 1980) or into troughs, passage through DEAE-Sephacel, Low -melting-temperature agarose (Weislander, 1979) and DEAE-cellulose paper (Dretzen et al. 1981).



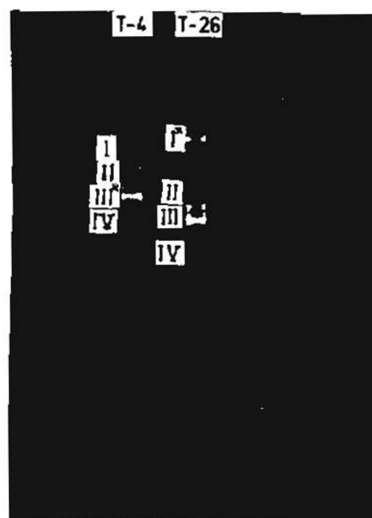


Fig 1. Agrose gel electroforosis of dsRNAs extracted from infected plants with citrus tristeza virus isolates (T-30, T-55).

Fig 2. Polyacrylamide gel electrophorosis of dsRNAs extracted from infected plants with citrus tristeza virus isolates (T-4, T-26) x dsRNA bands which were cut out.

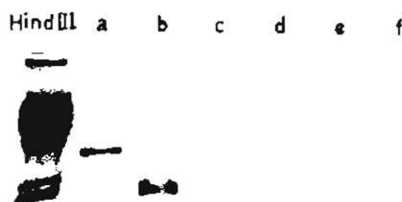


Fig 3. Agarose gel electroforosis of electroeluted dsRNA bands from gels.
a) I. band of T-26 isolate, b) III. band of T-4 isolate, c) I. band of T-30 isolate,
d) II and III. band of T-30 isolate, 3) I. band of T-55 isolate, f) II. band of T-55 isolate.

The recovery of RNA fragments from gel can be achieved, using similar methods. But, none of them is entirely satisfactory (Maniatis et al, 1982). The primary disadvantage of these techniques is that it is often difficult to recover intact RNA fragments. Furthermore, most grades of agarose are contaminated by sulfated polysaccharid, which are extracted from the gel together with the RNA; these substances are potent inhibitors of many of the enzymes. That are commonly used in cloning steps.

Mentioned electroelution method is easy and rapid. It has been determined that electroclution procedure is reliable because electroeluted RNAs were replaced according to molecular weight and took same previous places that had been obtained from agar and polyacrylamide gels.

Same procedure was used for DNA fragment. The results were satisfactory (unbuslished data).

The fragments (T-30 and T-55) have been used for 5- end labeling with T4 polynucleotide kinase. But these subgenomic dsRNAs didn't work as a probe.

For this reason, it is necessary to develop electroelution procedure. Despite this limitation, the described procedure can be used in plant virus diseases laboratory.

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