Abstract

Return-polyacrylamide gel electrophoresis (R-PAGE) is used to separate and detect viroids and viroid strains. Separation is achieved by two separate electrophoresis runs which require 5 liters of "high salt" buffer followed by 5 liters of "low salt" buffer. Both of these buffers with a chemical content of 96.83 g and a total cost of $14.57 Can. (1987 prices) are discarded after each run.

Experiments with reused buffers led to the following conclusions: (A) Repeated use of high salt buffer for 8 times had no adverse effect on viroid detection provided the return electrophoresis was conducted by fresh low salt buffer; (B) Repeated use of both high and low salt buffers produced acceptable results for 4 runs, however, the low salt buffer should then be replaced to prevent darkened electrophorograms; and (C) Reducing buffer volumes to 2 liters each caused inadequate separation of viroid strains. The reuse of buffers reduces test costs, buffer preparation time and water pollution.

Compendio

La electroforesis bidireccional en poliacrilamida (R-PAGE) es utilizada para separar y detectar viroides y variantes de viroides. La separación se logra por dos electroforesis separadas que requieren cinco litros de solución tampón de "alta concentración de sales" y a continuación cinco litros de una solución tampón de "baja concentración de sales." Ambas soluciones tampón, con un contenido químico de 96,83 g y un costo total de $14,57 Can. (precios de 1987) son descartadas después de su utilización. Los experimentos con soluciones tampón utilizadas repetidamente condujeron a las siguientes conclusiones: (A) El uso repetido de una solución tampón de alta concentración de sales por ocho veces no tuvo efecto adverso sobre la detección de viroides siempre y cuando la electroforesis bidireccional fuera llevada a cabo con una solución tampón fresca de baja concentración de sales; (B) El uso repetido por cuatro veces de ambas soluciones produce resultados aceptables; sin embargo, la solución tampón de baja concentra-
ción de sales debe entonces ser reemplazada para prevenir el oscurecimiento de los electrotorogramas; y (C) La reducción de los volúmenes de las soluciones tampón a dos litros cada una causó la separación inadecuada de las variantes de viroides. La múltiple utilización de las soluciones tampón reduce los costos de las pruebas, el tiempo de preparación de las soluciones y la contaminación del agua.

Introduction

Potato spindle tuber viroid (PSTV), the cause of spindle tuber disease of potatoes, is of quarantine significance in many countries. Large scale monitoring for PSTV in seed potato stocks intended for export is, therefore, carried out routinely by many exporting countries (15). To ensure clean propagating plant materials about 73% of the potato tissue culture programs in North America and 50% in Europe are also regularly monitored for this viroid (5). Materials are tested by bioassays (1, 2, 9, 14), nucleic acid hybridization (1, 3, 7, 8, 11) or polyacrylamide gel electrophoresis (1, 6, 8).

In the last 3 years a number of laboratories have been using return polyacrylamide gel electrophoresis (R-PAGE) (12, 13) for the detection of PSTV (4, 15) and other viroids (4, 10). In the R-PAGE procedure viroid separation is obtained by two independent electrophoresis runs. During the first electrophoresis, which is conducted for approximately 2.5 hr with 5 liters of “high salt” buffer, viroids move from the top towards the bottom of the polyacrylamide gel. At the completion of first electrophoresis, the high salt buffer is discarded and the emptied buffer chambers are filled with 5 liters of “low salt” buffer for the second (return) electrophoresis. Second, electrophoresis (2.2 hr approximately) is conducted with reversed current polarity, therefore, viroids migrate or return from the bottom towards the top of the gel. Five liters each of high and low salt buffers containing a total of 96.83 g chemicals and costing $14.57 Can. (1987 prices; Fisher Scientific Catalogue) are discarded after one test run.

Considerable cost savings could be realized by laboratories using this method for large-scale monitoring of PSTV if buffers could be used repeatedly. These savings would be of significant importance for developing countries where chemicals are in short supply and costly. The objective of this study was to determine if the buffers could be reused without affecting detection of PSTV and its strains.

Materials and Methods

Russet Burbank potato plants were manually inoculated with partially purified nucleic acid extracts containing a mild (MF) and a severe (S-PSTV) strain of PSTV. After five weeks of inoculation nucleic acids from 30 g leaf samples infected with MF and S-PSTV were isolated, as described previ-