THE USE OF THE POLYMERASE CHAIN REACTION FOR THE CHARACTERISATION AND DIAGNOSIS OF YAM POTYVIRUSES

R. A. MUMFORD, D. L. JAYARATNE & S. E. SEAL
Natural Resources Institute
Central Avenue, Chatham Maritime, Kent, ME4 4TB. UK

1. Introduction

Yams (Dioscorea spp.) are an important tuber crop in many tropical and sub-tropical regions (Degras, 1993), especially West Africa which accounts for over 90% of world yam production (FAO, 1994). Yams, in common with other vegetatively propagated crops, are susceptible to a wide variety of different viruses (Porth & Nienhaus, 1983). Of all these viruses, the potyviruses (family Potyviridae; genus Potyvirus) are the most diverse (Porth & Nienhaus, 1983; Porth et al., 1987) and can cause significant reductions in crop yield (Thouvenel & Dumont, 1990). Of the yam-infecting potyviruses, yam mosaic virus (YMV) and yam mild mosaic virus (YMMV) are especially important, because of their high incidence and wide distribution amongst the two most widely cultivated yams; Dioscorea rotundata-cayenensis and D. alata. However, despite the importance of YMV and YMMV, very little information is available on the true level of variation found amongst these two viruses and the other potyviruses that have been reported (Porth & Nienhaus, 1983; Porth et al., 1987). This lack of knowledge on variability, coupled with the recalcitrant nature of yam tissue, has resulted in a dearth of reliable diagnostic tests. This current situation is severely hampering efforts to improve the yam crop, especially by preventing the international exchange of breeding materials. In an effort to rectify this situation, an approach using PCR-based techniques has been employed and has resulted in an increased understanding of yam potyvirus variability and the development of reliable diagnostics.

2. Materials and Methods

2.1. VIRUS ISOLATES

Virus-infected yam tubers were obtained from a variety of yam-growing regions and grown in peat-based compost, in an insect-free glasshouse at a temperature of 23°C.
2.2. IC-RT-PCR

Immunocapture-reverse transcription-polymerase chain reaction (IC-RT-PCR) was performed as described in Mumford & Seal (submitted), using 0.5 ml microfuge tubes coated with polyclonal antisera (raised against YMV or YMMV), that was kindly provided by Alan Brunt (Horticulture Research International, UK).

2.3. POTYVIRUS PCR, CLONING AND SEQUENCING

Total RNA was obtained from yam leaf tissue using a method adapted from Chang et al. (1993). RT-PCR was then performed using degenerate potyvirus primers, essentially as described by Pappu et al. (1993) and using Promega reagents. Resulting PCR products were purified using QIA-quick columns (Qiagen) before cloning using the pGEM-T vector system (Promega). Plasmids were obtained from selected clones using the Wizard Plus mini-prep system (Promega) and were sequenced automatically (SequiServe, Germany).

3. Results

3.1. COMPARISON OF YAM POTYVIRUS SEQUENCES

Sequences were obtained from 3'end of the viral RNA of thirteen different yam potyvirus isolates, from a variety of different host species and locations. The results indicate the existence of four distinct yam potyviruses groups (Figure 1). These include isolates known to be YMV (isolates Dr-Nig-N5/N20/8, Dr-Tog-3 and Dr-Cam-2) and YMMV (isolates Da-Van-L1, Da-SL-SLK and Da-Nig-316/2), in addition to two uncharacterised viruses identified from the Indian sub-continent from D. esculenta (DeV; isolate De-SL-SL15) and D. dumentorum (DdV; isolates Dd-SL-SL19/SL28 and Dd-Bang-Y60).

3.2. DETECTION BY IC-RT-PCR

Using sequences obtained for yam potyviruses, two virus-specific pairs of primers were designed. One pair, YMV CP F and YMV UTR R, were designed to be specific for YMV and the second, YMMV CP F and YMMV UTR R, were designed to detect YMMV. Both of these primer pairs, when used in IC-RT-PCR, were specific for their target virus, giving products of 586 and 259 base pairs, for YMV and YMMV respectively (Figure 2). Neither primer pair gave amplification with uninfected yam material, yam tissue infected with the alternative virus or buffer (no sap added) controls (Figure 2).