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Genetic variants of Banana streak virus in Mauritius

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Abstract

Genetic variations among isolates of Banana streak virus (BSV) were assessed using two sets of primers. The virus, found in banana accessions in Mauritius, was compared to a Nigerian isolate from cultivar Obino l'Ewai (BSOEV). On the basis of the observed size of amplicons, some Mauritius strains were different from l'Ewai BSOEV. Both Southern blot hybridization and the nucleotide sequences of the PCR products confirmed that they were of episomal BSV origin. An isolate of sugarcane bacilliform virus (SCBV) was found to be also very similar to the BSV isolated from banana samples. Nucleotide sequence analysis showed that even the same size PCR products had differing sequences. The dendrogram placed the isolates from Mauritius in a cluster separate from BSV and SCBV from other geographical locations.

Keywords: Badnavirus; Integrated sequences; Genetic diversity

1. Introduction

Banana streak virus (BSV) has been characterized only recently and is now known to be an important disease of *Musa* and affecting the productivity of both bananas and plantains. It has been reported in nearly all countries where this crop is grown including Mauritius, India and many countries of the African continent. BSV is member of the *Badnavirus* genus, in the Caulimoviridae family, which includes all the known plant pararetroviruses. Badnaviruses are bacilliform and do not contain the translational transactivator protein found in other caulimoviruses. All Caulimoviridae have an open-circular, double-stranded DNA genome, whose replication occurs via a reverse transcription step. The genome organization of most badnaviruses consists of three major open reading frames (ORFs). ORF III is translated into a 216 kDa polyprotein, which includes the essential, conserved replication features of reverse transcriptase and RNaseH.

In plant pararetroviruses, unlike true retroviruses, integration into the host genome is not required for replication. However, genome sequences of several such viruses, including BSV (Ndowora et al., 1999; Harper et al., 1999), tobacco vein clearing virus (Lockhart et al., 2000) and petunia vein clearing virus (Richert-Poggeler et al., 1996) are found integrated in their plant host genome and are thought to be capable of activation to cause disease. Other integrations, either partial, incomplete or incapable of causing disease are also known. This is exemplified in *Musa* where two types of integrants have so far been described for BSV. One type, carries the full virus genome interspersed with non-contiguous, inverted sequences and is capable of undergoing recombination and excision to produce episomal form of the virus, now termed Banana streak Obino l'Ewai virus (BSOEV) (Ndowora et al., 1999). There are indications that such an excision can be triggered during in vitro plant regeneration processes. It is further known or suspected that there are at least another three very different integrated BSV sequences, each capable of activation to cause disease Banana streak Goldfinger virus (BSGfV), Banana streak Imove virus (BSImV) and Banana streak Mysore virus (BSMysV) (Geering et al., 2001a,b).

All these types of integrant are associated with the B genome of *Musa*. The second type of integrant does not appear to be related to infectious disease either because the

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integrated sequence is incomplete, e.g. the cocoa swollen shoot-like Calcutta 4 integrant within the A genome of *Musa* (Ndowora et al., 1999), or because it contains mutations rendering it defective. Geering et al. (2005) have reported on a large group of badnavirus integrations in various *Musa* species, termed Banana endogenous viruses (BEVs), some of which may be unique to a particular *Musa* species and some are more dispersed throughout the genus.

The molecular characterization of BSV has been possible following the sequencing of a Nigerian isolate of the virus (Harper and Hull, 1998; BSOEV, EMBL: AJ002234). This sequence shows clear similarities to other badnaviruses but is different enough to be classified as a separate specie. Detection of episomal BSV by PCR must be preceded by an immunocapture step or the reaction can be carried out on purified virus preparations, to prevent amplification of integrated sequences. In this study, several sets of primers for BSV were screened and two pairs were selected as they produced polymorphic bands from the local Mauritius isolates of BSV episomal forms. In all the PCR reactions DNA from the BSOEV isolate, which originates from Obino l'Ewai was used as a control. Since the detection of BSV in Mauritius, this is the first study to determine the molecular variation among the strains of the virus found among the locally grown banana varieties.

2. Materials and methods

2.1. Sample collection and preparation and virus purification

Virus samples were from local banana varieties known as Mamzelle, Mamoule, Ollier, Gingeli, Banane Rouge, Banane La Grain as well as the imported varieties of Grande Naine, Williams and Pisang Mas.

Distinctive and typical yellow streak symptoms characteristic of BSV were observed on the leaves of plants from different parts of the island. Symptomatic leaves were collected and used either for virus purifications according to Harper and Hull (1998) or for making crude extracts as described below. Crude extracts of some samples were tested with ELISA using a polyclonal antibody (AGDIA, Co.) and following the manufacturers protocol (Thottapilly et al., 1998). The crude preparations were used for immunocapture PCR while the pure preparations were used directly for amplification by PCR. SCBV samples were isolated from sugarcane leaves from a collection known to harbour the virus.

Virus purification was performed according to Harper and Hull (1998).

2.2. Immunocapture PCR

This procedure required that the virus particles be first immobilized onto the surface of the PCR tubes before the reaction is done. PCR tubes were coated with anti-BSV antibodies (AGDIA, Co.) at a concentration of 1 μg/ml in 0.05 M sodium carbonate buffer, pH 9.6 and incubated for 2 h. Crude leaf extracts were made by grinding the leaf tissues in 2 ml of 0.05 M Tris–HCl, pH 7.4 containing 5% skim milk and 0.5% sodium sulphite. After centrifugation, 25 μl of the supernatant were added to the coated tubes followed by an overnight incubation at 4 °C. Washing was done twice with phosphate buffered saline + 0.05% Tween 20 (PBS-T). After washing twice with PBS-T, the PCR mix (see below) was added and tubes were placed in a thermal cycler with the appropriate cycling programme. The PCR products were visualized after electrophoresis on a 1.5% agarose gel. A positive control for the PCR was always used with genomic DNA isolated from *Musa* cv. Obino l'Ewai, as described in Harper and Hull (1998).

The primers used to screen the BSV isolates were 3012 (5′-GGAATGAAAGAGCAGGCC-3′) and 1573 (5′-AGTCATTGGGTCAACCTCTGTCCC-3′) (which correspond to BSV4673 and BSVr5317, respectively, as in Harper and Hull, 1998).

The Cavendish primers were Cav-F1 (5′-AGG ATT GGA TGT GAA GTT TGA GC-3′) and Cav-R1 (5′-ACC AAT AAT GCA AGG GAC GC-3′) (Geering et al., 2000). In addition, three other primer pairs for Red Dacca, Mysore and Goldfinger (Geering et al., 2000) were tested.

2.3. PCR amplification and cloning of products

Standard PCR procedures were used. The reactions were done in 30 μl volumes each containing 1× Taq polymerase buffer (1.5 mM $MgCl_2$), 200 μM each dNTPs, 20 pmol of each primer and 1 unit of Taq polymerase. For cloning purposes the selected fragments were purified from the agarose gel using the PCR Purification Kit (Promega, Madison). These were ligated into pGEM vectors and the ligation product transformed into *E. coli* DH5α, using standard procedures. White colonies were selected and putative transformants analysed by PCR with vector primers M13 and T7. Only those showing inserts of the expected size were used for sequencing, carried out at Inqaba Biotechnical Industries, South Africa. The sequence results were analysed using Chromas version 1.2. Sequence alignment was effected using Multalin 5.4.1 (http://www-archbac.u-psud.fr/genomics/multalin.html). Sequences were aligned and neighbor-joining trees calculated and bootstrapped using ClustalX (Thompson et al., 1997).

Sequences have been submitted to Genbank (records DQ115590–DQ115592).

3. Results

3.1. PCR amplification of BSV

The virus preparations were tested with each of the different sets of primers indicated above. Only the *primer pairs*