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Characterization and expression of the coat protein-coding region of banana bract mosaic potyvirus, development of diagnostic assays and detection of the virus in banana plants from five countries in southeast Asia*

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Summary. We have sequenced the entire coat protein (CP)-coding region and 5′ 162 nucleotides of the 3′ untranslated region (UTR) of nine different isolates of banana bract mosaic virus (BBrMV) from five different countries. Further, we have sequenced the 3′ 621 nucleotides of the NIb-coding region of a Philippines isolate. This is the first report of BBrMV in Thailand, Vietnam and Western Samoa. When the sequences of the CP-coding region and 3′ UTR were compared to each other, variability of between 0.3% and 5.6%, and 0.3% and 4.3%, was observed at the nucleotide and amino acid levels, respectively. Phylogenetic analysis of the BBrMV isolates did not reveal any relationship between the geographic location of the isolates. The BBrMV CP was expressed in *Escherichia coli* as a fusion protein and the purified recombinant protein was used to produce a high titre BBrMV-specific polyclonal antiserum. This antiserum was used to develop a $F(ab')_2$ indirect double antibody sandwich ELISA and compared with immuno-capture PCR (IC-PCR) and reverse transcription PCR (RT-PCR) assays for BBrMV detection. RT-PCR was shown to be the most sensitive test followed by ELISA and IC-PCR.

Introduction

Banana bract mosaic potyvirus (BBrMV) is a recently described virus of bananas which has flexuous filamentous virions that react with a general potyvirus antiserum [5, 9, 20]. BBrMV has been reported in the Philippines [11], India [27]

*Sequence data deposited with GenBank, accession numbers AFO71590 and AFO71582–71590.

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and Sri Lanka [34] and causes yield losses of up to 40% [28]. The virus poses a considerable quarantine risk due to its limited distribution, and its ability to rapidly spread through vegetative plant parts or aphids [20]. With an increase in the international movement of banana germplasm in recent years it is important that the geographical distribution of BBrMV is determined. It is possible that BBrMV occurs in other countries since uncharacterised potyvirus-like particles have been observed in bananas from Costa Rica and several African countries including Uganda, Ghana, Zanzibar and South Africa [1, 24, 37]. The development of a diagnostic test to detect BBrMV will assist to accurately determine the distribution of the virus.

BBrMV has been classified as a member of the genus *Potyvirus* in the family *Potyviridae* [10]. The potyvirus genome consists of a positive-sense ssRNA molecule of approximately 10 kb which is polyadenylated at the 3′ end and is translated as a single polypeptide that is post-translationally cleaved into nine native proteins [17]. Amino acid sequence comparisons of the C-terminal region of the BBrMV CP with other potyviruses revealed that BBrMV was most similar to maize dwarf mosaic virus (MDMV) with 71% homology, while the nucleotide sequence of the 3′ UTR was most similar to ornithogalum mosaic potyvirus (OMV) with 39% homology [5].

There are currently no control strategies for BBrMV. BBrMV -resistant banana cultivars have not yet been identified thus precluding conventional breeding as a strategy for obtaining BBrMV resistance. Pathogen-derived resistance (PDR) provides an attractive alternative control strategy and has been reported for a number of potyvirus/host combinations including papaya ringspot virus (PRSV) [33], potato virus Y (PVY) [32] and tobacco etch virus (TEV) [18]. A number of different viral genes have been targeted with the coat protein and replicase genes most commonly used. In general, PDR tends to be specific for the virus or viral strain from which the transgene was derived [19]. However, there is no means of predicting the degree of sequence variability tolerated between the expressed transgene and the challenge viral gene which will still confer protection [12]. It is important, therefore, to determine the level of virus variability in the field prior to developing potential resistance constructs.

This paper reports the nucleotide sequence of (i) the entire coat protein-coding region and 5′ 162 nucleotides of the 3′ UTR from nine different isolates of BBrMV from five different countries, and (ii) the 3′ 621 nucleotides of the NIb-coding region of a Philippines isolate. The relationship between these isolates and other potyviruses is also examined. Further, we report the expression and purification of a BBrMV recombinant coat protein, the production of a high titre BBrMV-specific antiserum and the use of the antiserum for BBrMV diagnosis.

Materials and methods

Virus isolates

Banana leaves showing a range of symptoms were obtained from the Philippines, India, Western Samoa, Vietnam and Thailand (Table 1) and were stored at −80 °C.

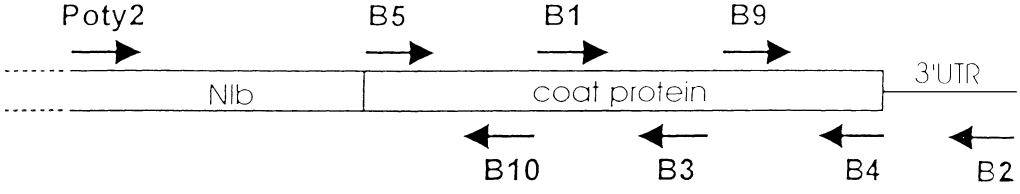
RT-PCR, cloning and sequencing

RNA was extracted either directly from two to three grams of leaf tissue [7] or from partially purified BBrMV [34] using the method of Robertson et al. [25]. For first strand cDNA, 0.2–2 μg of RNA was denatured for 3 min at 65 °C, quenched on ice and cDNA was synthesized using Superscript reverse transcriptase (200 U, Gibco-BRL) and B2 as the primer (Fig. 1). The PCR reaction mix and cycling conditions were as previously described [27]. The location and sequence of the primers used to amplify the NIb, CP and 3′UTR of each isolate is shown in Fig. 1.

The PCR products were ligated directly into pGEM-T (Promega) and transformed into *E. coli* DH5α cells. Transformants were screened by digesting recombinant plasmids with *Not*I and *Nco*I, and some were digested with exonuclease III [29] and subcloned to obtain smaller fragments. Potential recombinants were sequenced using an Applied Biosystems 373A automatic DNA sequencer with universal forward and reverse primers, and BBrMV-specific primers (Fig. 1).

Sequence analysis

The nucleotide sequences were compared using DISCALC (G. Weiller and A. Gibbs, pers. comm.) to produce distance matrices. Pairwise distance matrices of nucleic acid and amino acid differences were compared using DIPLOMO [38]. Nucleotide sequences were aligned using CLUSTALW [35] and phylogenetic trees were prepared using DNADIST, NEIGHBOR then DRAWGRAM programs in PHYLIP (version 3.5c, copyright J. Felsenstein, University of Washington).



B1: 5' GAC ATC ACC AAA TTT GAA TGG CAC ATGG 3'

B2: 5' CCA TTA TCA CTC GAT CAA TAC CTC ACA G 3'

B3: 5' GCC ATA ATT TGC CGA AAA GTA GGC TG 3'

B4: 5' TAA GAA TTC ATT TAT TCA TGT TT 3'

B5: 5' TCT GGA ACG GAG TCA ACC 3'

B9: 5' GCT GCA GCT ATT CGT GGA TC 3'

B10: 5' TTG CGG TTT TTC CTC GAT ACC 3'

Poty2: 5' GG(TCG) AA(CT) AA(CT) AG(CT) GG(GTA) CA(AG) CC 3'

Fig. 1. Schematic representation of the 3′ region of the BBrMV genome. The location and sequence of the primers used for PCR and sequencing are indicated

For nucleotide sequence comparisons, the N-terminal and core regions of the BBrMV coat protein were designated as comprising nucleotides 1–195 and 196–843, respectively [30]. The coat protein core region for other selected potyviruses was also assigned using the same definition.

Construction, expression and purification of BBrMV coat protein from E. coli

The entire coat protein-coding region of BBrMV (isolate P1) was amplified from B2-primed cDNA using *Pfu* DNA polymerase (Boehringer Mannheim) and primers B5 and B4 (Fig. 1). The amplified product was gel purified and cloned into the *Ehe*I site of the pProEX-1 vector (Life Technologies) and transformed into *E. coli* M15 cells containing the pREP4 repressor plasmid. Transformants were screened by digesting recombinant plasmids with *Not*I and *Nco*I and potential recombinants were sequenced using the universal forward and reverse primers and the BBrMV-specific primers B1, B3, B9 and B10 (Fig. 1) to confirm the integrity of the cloned DNA. Fusion proteins were expressed and purified as described in the QIA expressionist manual [2]. Fractions were analysed by electrophoresis in 12% polyacrylamide gels as described by Laemmli [16].

Antisera production and Western analysis

Purified fusion protein (70 μg) was emulsified with an equal volume of Freunds incomplete adjuvant and intramuscularly injected into the rear flank of a 10 week old New Zealand White rabbit. Three booster injections were done on days 11, 19 and 35. The rabbit was bled 25, 35 and 45 days after the first injection and the antiserum analysed by Western blots [36]. Proteins were electroblotted (70 V constant/4 h) onto 0.25 μm nitrocellulose (Schleicher and Scheull) using 10 mM CAPS containing 10% methanol, 0.001% SDS as the transfer buffer. The membrane was blocked overnight in 1% blocking solution (Boehringer Mannheim) and probed with the BBrMV CP- specific antisera. The bound antibodies were detected with goat anti-rabbit IgG horseradish peroxidase conjugate (1:1000) and POD chemiluminescent substrate (Boehringer Mannheim).

Comparison of detection methods

BBrMV isolates and preparation of extracts

Three BBrMV isolates from the Philippines and India (Table 1) were tested for the presence of BBrMV using ELISA, IC-PCR and RT-PCR. Extracts were prepared by grinding tissue with a mortar and pestle in STE buffer (0.1 M Tris-HCl, pH 6.8, 0.5 M NaCl, 20 mM EDTA), 0.02% Tween 20 and 2% polyvinyl pyrrolidone (MW 44,000) at a rate of 1 g/9 ml [26]. The extract was centrifuged at 16 000 **g** for 2 min and the supernatant used for testing.

ELISA

The $F(ab')_2$ indirect double antibody sandwich technique was used essentially as described by Barbara and Clark [4]. A 200 μl aliquot was used at each step, each sample was duplicated, and each ELISA plate contained buffer and sap from healthy and BBrMV-infected plants as controls. Plates were coated with a 1/200 dilution of BBrMV-specific $F(ab')_2$ and BBrMV-specific whole IgG added at 5 μg/ml. The Fc portion of the whole IgG was detected using a 1/10 000 dilution of protein A-horse-radish peroxidase conjugate and 3,3′5,5′ tetramethylbenzidine (TMB) as the substrate. Colour reactions were stopped using 3M H_2SO_4 and the absorbance measured at 450 nm. A sample was considered positive if the optical density was greater than twice the mean of the healthy controls.

Table 1. Geographical origins and GenBank accession numbers of the BBrMV isolates used in this study

Name	Origin	Cultivar	Accession No.
P1	Philippines (LosBanos)	Saba	AFO71590
P2	Philippines (LosBanos)	Tanygang	AFO71585
P3	Philippines (Davao)	?	AFO71586
I1	India (Coimbatore)	*Musa balbisiana*	AFO71582
I2	India (Coimbatore)	Klue Terapod	AFO71583
I3	India (Tiruchchirappalli)	Pisang Awak	AFO71584
WS1	Western Samoa (Nu'u)	SH3362	AFO71587
VT1	Vietnam (Hau)	Cavendish	AFO71588
Th1	Thailand	?	AFO71589

IC-PCR

IC-PCR was done essentially as described by Nolasco et al. [21]. Samples were treated as described for ELISA up to, and including, the three washes with PBS-T following the overnight incubation of the sap extract at 4 °C [4]. First strand cDNA was then synthesized in a 20 μl reaction mix using the BBrMV-specific primer B2 and Superscript reverse transcriptase (200U, Gibco-BRL). A 10 μl aliquot of the cDNA mix was then used for PCR as described previously [27].

RT-PCR

RNA was extracted from 100 μl of sap extract, resuspended in 10 μl of sterile distilled water and was used for reverse transcription using Superscript reverse transcriptase (200U, Gibco-BRL) and primer B2. A 5 μl aliquot of the 20 μl cDNA reaction was then used in a PCR with BBrMV primers B1 and B2 to amplify a 604 bp product including the C-terminus of the coat protein and the 3′ untranslated region of the BBrMV genome [27].

Southern blotting and electron microscopy

Southern blotting and electron microscopy were done as previously described [27].

Results

Symptomatology and confirmation of BBrMV infection in each isolate

Leaves from banana plants exhibiting symptoms typical of BBrMV were collected from the Los Banos (P1 and P2) and Davao regions (P3) in the Philippines and from the Tamil Nadu province of southern India (I1 and I2). Leaves from plants showing CMV-like symptoms were collected from the Tiruchchirappalli region in southern India (I3) and from Vietnam (Vt1) and Thailand (Th1). Banana plants collected from Western Samoa (WS1) showed symptoms characteristic of those induced by banana streak virus (BSV).

The presence of BBrMV in the different isolates was verified by PCR (at least twice for each isolate) and either ELISA, Western blots, Southern blots or electron microscopy (Table 2). Isolates P3 and I3 have been previously characterized

Table 2. Detection of BBrMV isolates

Test	Healthy	P1	P2	P3	I1	I2	I3	WS1	Th1	Vt1
ELISA	–[a]	+[b]	+	NT	+	+	NT	NT	+	+
Western blot	–	+	+	+	+	+	NT	+	NT	NT
Southern blot	–	+	+	+	+	+	+	+	+	+
EM	–	+	+	+	+	+	+	+	NT	NT

[a]Negative result
[b]Positive result
NT Not tested

[5, 27]. Small, bacilliform virions approximately 30 × 130 nm, were also observed in sap dips prepared from the WS1 plant and were presumed to be virions of BSV.

Sequence analysis

The entire CP-coding and 5′ 162 nucleotides of the 3′ UTR of the different BBrMV isolates, as well as an additional 3′ 621 nucleotides of the NIb-coding region of the Philippines P1 isolate, were amplified by RT-PCR and cloned. Three clones from each isolate were sequenced in both directions using BBrMV-specific primers and universal forward and reverse primers. There were no more than 2 base changes between each of the three clones sequenced for each isolate; the consensus sequence between these clones was chosen as the final sequence for each isolate.

Since potyviral mRNA is translated as a single polyprotein, the coat protein-coding region does not have a start codon. Therefore, the CP-coding region was determined by comparison with sequences from related potyviruses including ornithagalum mosaic virus (OMV) and maize dwarf mosaic virus (MDMV). The putative five amino acid protease cleavage site between the NIb and CP-coding regions was VEFQS. The length of the BBrMV CP-coding region of all isolates comprised 900 nucleotides and encoded a putative protein of 300 amino acids with an estimated Mr of 34 kDa. Analysis of the sequence revealed several motifs commonly found in potyviral coat proteins including (i) a DAG amino acid triplet, (ii) a "WCIEN" box in the core region, and (iii) an RQ and AFDF motif. The NIb-coding region contained the motif GDD which has been found in all RNA-dependent RNA polymerases [15].

When the nucleotide sequences of the CP-coding region from all isolates were compared the variability ranged from 0.3% (I3 and Vt1) to 5.6% (I2 and WS1). The amino acid sequence differences were similar, ranging from 0.3% (I3 and Th1) to 4.3% (I2 and WS1; I2 and P2). The majority of non-synonomous nucleotide and amino acid variability occurred at the N-terminus of the CP, a region known to be highly variable [31]. The nucleotide and amino acid sequence differences were also compared to each other using DIPLOMO analysis (results not shown). This analysis showed that the spread of nucleotide and predicted amino acid differences was not greater than that expected for natural random processes. There were no subsets of isolates and most isolates had more nucleotide than amino acid differences.

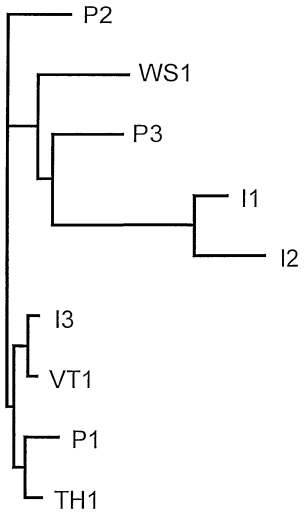


Fig. 2. Phylogenetic neighbor-joining tree of the nucleotide sequences of the coat protein-coding region of nine isolates of BBrMV

At the nucleotide level, the sequence of P3 was most similar to all others over the coat protein-coding region, with variability ranging from 2.4 to 4.7%. At the amino acid level, the P1 isolate was the most similar to all other isolates ranging from 0.7 to 2.3% variability.

A comparison of the 5′ 162 nucleotides of the 3′ UTR showed that the percentage homologies were generally higher for the 3′ UTR than in the coat protein, with P1 and P2, and WS1 and Vt1 having identical nucleotide sequences. The greatest variability was 4.3% between I2 and I3; the P3 isolate had a two nucleotide deletion.

A phylogenetic tree was constructed using the nucleotide sequence of the entire CP-coding region of the nine BBrMV isolates (Fig. 2). The branching pattern revealed that the I1 and I2 isolates grouping closely together as did I3, Th1 and Vt1, and WS1 and P3. There was no clear grouping of the three isolates from the Philippines with P1 being more closely related to Th1 than to P2 and P3, while isolates P2 and P3 were more closely related to Vt1 than to each other.

BBrMV CP expression and antisera analysis

The entire CP-coding region of BBrMV (isolate P1) was expressed as a fusion protein with a 6x histidine tag. Following amplification, the BBrMV coat protein-coding region was subcloned into pProEX-1 vector to produce the recombinant plasmid, pProBBrMVCP. The integrity of the recombinant plasmid was confirmed by sequencing using universal forward and reverse primers as well as BBrMV coat protein-specific primers. Initial attempts to express pProBBrMVCP into *E. coli* strains DH5α and JM109 were unsuccessful. However, the plasmid was successfully transformed into *E. coli* M15 cells containing the pREP4 represser plasmid.

Upon induction with IPTG, pProBBrMVCP expressed low levels of a 39 kDa protein. This protein was not present in extracts from untransformed *E. coli* or

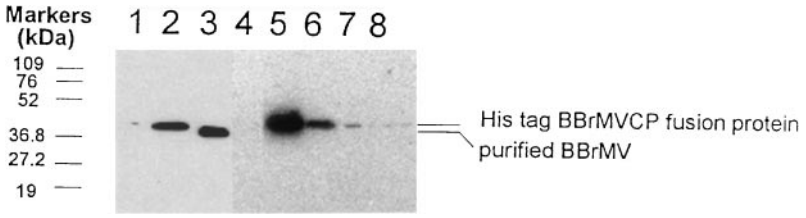


Fig. 3. Immunoblot analysis using antisera produced against purified recombinant BBrMV coat protein. *1* Uninduced *E. coli* cells producing BBrMV CP fusion protein, *2* overnight induction of *E. coli* cells producing BBrMV CP fusion protein, *3* partially purified BBrMV, *4* healthy banana, *5–8* 50 ng of purified recombinant BBrMV coat protein. Antisera used in *1–5* was diluted 10^{-4}, while that used in *6–8* was diluted 10^{-5}–10^{-7}, respectively

those transformed with the native vector. The molecular weight of the protein was in the approximate size range of that expected for the 6xHis-BBrMVCP fusion product (6xHis = 0.84 kDa; BBrMVCP = 34 kDa).

The fusion protein was used directly for immunisation and the specificity of the antiserum was evaluated by Western analysis using purified fusion protein, and healthy and BBrMV-infected banana extracts. The antiserum reacted with a protein of about 40 kDa present in induced cultures of *E. coli* containing pProB-BrMVCP, although several faint, non-specific bands were also detected (Fig. 3). Importantly, the antiserum reacted strongly to a 40 kDa protein present in a partially purified BBrMV preparation and to the purified fusion protein. No reaction was observed in extracts from healthy banana. The sensitivity of the antiserum was evaluated in Western blot analysis by probing 50 ng of purified fusion protein with serially diluted BBrMV CP-specific antiserum. The titre of the antisera was found to be at least 10^{-7} (Fig. 3).

Comparison of ELISA, IC-PCR and RT-PCR

To compare BBrMV detection methods, identical extracts from healthy and six BBrMV-infected banana plants from India and the Philippines (Table 1) were tested for BBrMV by $F(ab')_2$ DAS ELISA, IC-PCR and RT-PCR (Table 3). Using ELISA, all BBrMV-infected banana samples tested strongly positive, except isolate I1 which tested weakly positive. The same result was obtained using IC-PCR, although much weaker products of the expected size were generally amplified (except for isolate P2). The most sensitive of the three tests was RT-PCR with a strong band of the expected size being amplified from all six infected isolates (Table 3). The healthy banana sample tested negative in all three assays.

To test the sensitivity of each assay, serial dilutions of BBrMV-infected sap from isolate I2 were made in healthy banana sap extracts and tested using each assay. The limit for detection for IC-PCR was found to be a 1/20 dilution, while both ELISA and RT-PCR were able to detect BBrMV at all dilutions including the maximum dilution of 1/500 (Table 4).

Table 3. A comparison of ELISA, IC-PCR and RT-PCR to detect six isolates of BBrMV from India and the Philippines

	Healthy[a]	I1	I2	I3	P1	P2	P3
ELISA	–[b]	+[c]	++[d]	++	++	++	++
IC-PCR	–	–	+	+	+	+	++
RT-PCR	–	++	++	++	++	++	++

[a]Healthy banana cv. Williams

[b]Negative result or optical density (OD) less than two times the mean of the healthy control or no band on the gel

[c]OD greater than twice but less than five times the mean of the healthy control or a weak band on a gel

[d]OD greater than 5 times the mean of the healthy control or a strong band on a gel

Table 4. Comparison of the sensitivity of ELISA, IC-PCR and RT-PCR to detect BBrMV in infected sap

	Healthy[a]	1/500[b]	1/100	1/50	1/20	1/10	Undiluted
ELISA	–[c]	++[e]	++	++	++	++	++
IC-PCR	–	–	–	–	+[d]	+	++
RT-PCR	–	++	++	+	+	+	++

[a]Healthy banana cv. Williams

[b]Serial dilutions were prepared by mixing BBrMV-infected banana sap with healthy sap

[c]Negative result or optical density (OD) less than two times the mean of the healthy control or no band on the gel

[d]OD greater than twice but less than five times the mean of the healthy control or a weak band on a gel

[e]OD greater than five times the mean of the healthy control or a strong band on a gel

Discussion

This paper reports the nucleotide sequence of (i) the entire CP-coding region and 162 nucleotides of the 3′UTR of nine isolates of BBrMV from the Philippines, India, Thailand, Vietnam and Western Samoa, and (ii) an additional 5′ 621 nucleotides of the NIb-coding region of a Philippines isolate. This is the first report of BBrMV in Thailand, Western Samoa and Vietnam and was confirmed by PCR and either ELISA, Southern and/or Western blot analysis. The coat protein-coding region of a Philippines isolate of BBrMV was expressed in a bacterial expression system and used to produce a sensitive BBrMV-specific polyclonal antiserum. Further, we have developed and compared three diagnostic assays for BBrMV, namely ELISA, IC-PCR and RT-PCR, and shown that RT-PCR is the most sensitive.

Rodoni et al. [27] reported that symptoms on BBrMV-infected plants were not always characteristic and could easily be confused with the mosaic symptoms

produced by CMV. In this study, BBrMV was shown to be present in plants showing mild mosaic symptoms not unlike those induced by CMV. Further, the BBrMV-infected plant from Western Samoa was shown to be co-infected with a small, bacilliform virus, possibly BSV, and expressed symptoms typical of this virus. These results clearly indicate that BBrMV does not always induce the characteristic mosaic pattern on the bract of the banana inflorescence and that symptoms may vary considerably.

The BBrMV coat protein of all isolates comprised 900 nucleotides and encoded a putative protein of 300 amino acids with a predicted MW of 33.4 kDa which is within the size range reported for other potyviruses [31]. The predicted MW of 34 kDa is slightly smaller than the 39 kDa fusion protein observed in this study as well as the 38 kDa protein reported by Bateson and Dale [5], and the 37 kDa and 39 kDa proteins reported by Thomas et al. [34]. Similar differences in the mobility of several plant viral coat proteins in SDS-PAGE have been observed [13].

Several motifs were present in the amino acid sequence of the BBrMV coat protein which are common in other potyviruses. The amino acid sequence, VE-FQS, located at the predicted cleavage site of the BBrMV NIb- and coat protein-coding regions is consistent with the conserved motif, V-*-*-Q-(A, S, G or V), and is recognised by cysteine type proteinases such as the NIa protease [31]. The highly conserved DAG amino acid triplet located in the N-terminal region of the BBrMV coat protein has been shown to be involved with aphid transmission [3]. A "WCIEN" box was located in the core region of the BBrMV coat protein, the function of which is still unknown [22]. Two further motifs, AFDF and RQ, were present in the C-terminal region of the coat protein and are crucial for potyvirus coat protein structure and assembly [14].

The variability between all the isolates over the coat protein-coding region ranged from 0.3%–5.6%, and 0.3%–4.3% at the nucleotide and amino acid level, respectively. Phylogenetic analysis of the BBrMV isolates did not reveal any relationship between the geographic location of the isolates and the corresponding sequence homology. The variation within India between I2 and I3 was 5.2% compared to the most distantly related isolates I2 and WS1 with 5.6% variability. Interestingly, the greatest variation between the three isolates from the Philippines was only 2.9% suggesting that the movement of BBrMV into the Philippines occurred as one event and that BBrMV has been in India for a longer period of time. A possible explanation for the geographical distribution of BBrMV is that the virus has moved as separate events, perhaps in different cultivars of banana.

No trends were observed between phylogenetic relatedness and symptoms expressed on the host plant. For example, all three Philippines isolates showed characteristic BBrMV symptoms but each of these isolates was more closely related to an isolate associated with non-characteristic BBrMV symptoms than to each other (P1 and TH1, and VT1 to P2 and P3).

Coat protein-mediated resistance has been reported for a large number of plant/virus combinations [12], and, in most instances, the resistance is virus or strain specific [19]. Tennant et al. [33] showed that transgenic papaya plants ex-

pressing the CP gene from a Hawaiian strain of PRSV-P showed varying degrees of resistance to PRSV from 11 other geographical isolates. High levels of resistance were obtained when the transgenic papaya was inoculated with a Hawaiian strain of PRSV (100% homology with transgene), while only low levels of resistance were observed when challenged with an Australian strain (96% similarity with transgene) and were susceptible to a PRSV strain which had 91% homology with the transgene [6, 33]. In this study, the nucleotide sequence of the P3 isolate was most similar to all others, with greater than 95.3% homology over the CP-coding region. At the amino acid level, the P1 isolate was the most similar to all others with greater than 97.7% homology. These sequences will be used to develop pathogen-derived resistance constructs for RNA- and protein-mediated resistance against BBrMV.

A high titre (at least 10^{-7}) BBrMV-specific polyclonal antiserum was produced in this study and was used to develop a BBrMV-specific $F(ab')_2$ indirect DAS ELISA. The sensitivity of this assay was compared directly to IC-PCR and RT-PCR using six BBrMV -infected banana samples and a healthy control. These samples represented a broad range of BBrMV isolates as demonstrated by a comparison of the amino acid sequence of the C-terminal region of the coat protein and 3′ untranslated region [27]. RT-PCR was the most sensitive of the three methods with IC-PCR the least sensitive. PCR-based detection is reportedly several orders of magnitude more sensitive than immunological-based tests [23] and IC-PCR reported to be more sensitive than ELISA [8, 21]. The lack of sensitivity of IC-PCR in this study may be due to the degradation of the BBrMV genome, or due to the activity of endogenous inhibitors from banana sap during the overnight incubation step at 4 °C which may prevent efficient reverse transcription of the BBrMV genome.

The BBrMV-specific $F(ab')_2$ DAS ELISA was able to detect BBrMV-infected sap which had been diluted 1/500 in healthy banana sap. This demonstrates the applicability of this assay for batch testing and for detecting low concentrations of BBrMV. These results indicate the potential of this diagnostic test for routine testing of banana material for the presence of BBrMV.

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