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Pathogen profile

Bean dwarf mosaic virus: a model system for the study of viral movement

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SUMMARY

Taxonomy: *Bean dwarf mosaic virus*-[Colombia:1987] (BDMV-[CO:87]) is a single-stranded plant DNA virus, a member of the genus *Begomovirus* of the family *Geminiviridae*.

Physical properties: BDMV virions are twinned incomplete isosahedra measuring 18 × 30 nm. The viral particle is composed of 110 subunits of coat protein, organized as 22 pentameric capsomers. Each subunit has a molecular mass of ~29 kDa. BDMV possesses two DNA components (designated DNA-A and DNA-B), each ~2.6 kb in size.

Host range: The natural and most important host of BDMV is the common bean (*Phaseolus vulgaris*). *Nicotiana benthamiana* is often used as an experimental host. Common bean germplasm can be divided into two major gene pools: Andean materials, which are mostly susceptible to BDMV, and Middle American materials, which are mostly resistant to BDMV.

Disease symptoms: The symptom intensity in common bean plants depends on the stage of infection. Early infection of susceptible bean seedlings will result in severe stunting and dwarfing, leaf distortion and mottling or mosaic, as well as chlorotic or yellow spots or blotches. BDMV-infected plants usually abort their flowers or produce severely distorted pods. Late infection of susceptible plants or early infection of moderately resistant genotypes may show a mild mosaic, mottle and crumpling or an irregular distribution of variegated patches.

Biological properties: As a member of the *Begomovirus* group, BDMV is transmitted from plant to plant by the whitefly *Bemisia tabaci*. BDMV is a nonphloem-limited virus and can replicate and move in the epidermal, cortical and phloem cells. As a nonphloem-limited virus, it is sap-transmissible.

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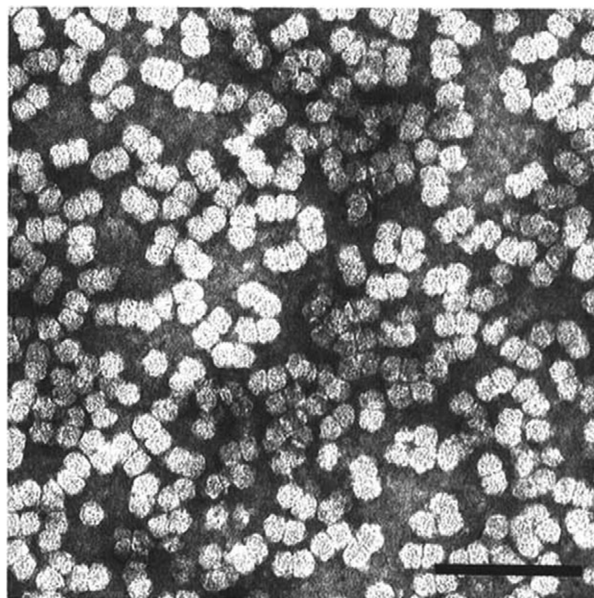

Fig. 1 Electron microscopy image (JEOL 100 SX) of purified *Bean dwarf mosaic virus* (BDMV) particles from bean plants (*Phaseolus vulgaris* cv. Topocrop). Bar, 0.1 μm. (Reproduced from Morales *et al*., 1990, with permission.)

INTRODUCTION

The family *Geminiviridae* contains some of the smallest independently replicating viruses (Rojas *et al*., 2005). This family includes plant-infecting viruses with circular, single-stranded DNA (ssDNA) genomes which are encapsidated in geminate virions (Fig. 1). A rolling-circle mechanism is used by the virus for replication through a double-stranded (ds) replicative form (RF) in the nuclei of infected plant cells. The ds RF also serves as a transcription template for expression of the viral genes (Hanley-Bowdoin *et al*., 2000; Rojas *et al*., 2005; Timmermans *et al*., 1994; Zhang *et al*., 2001b). The family is composed of four genera, *Begomovirus*, *Curtovirus*, *Mastrevirus* and *Topocuvirus*, which are distinguished by their insect vector, host range and genome structure (Fauquet *et al*., 2003, 2008). *Begomovirus* is

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Fig. 2 Disease symptoms induced in common bean plants (*Phaseolus vulgaris* cv. Benton) by *Bean dwarf mosaic virus* (BDMV). Both BDMV DNA-A and its cognate DNA-B were introduced into hypocotyls of bean seedlings 3 days after germination by particle gun bombardment. Photographs were taken 30 days post-inoculation: right, inoculation with cloned BDMV DNA-A and DNA-B; left, control bean plant.

the largest genus, and includes viruses transmitted by the whitefly *Bemisia tabaci* (Brown, 2000; Leshkowitz *et al*., 2006) to various dicotyledonous species (Fauquet *et al*., 2003, 2008). Begomoviruses can be either bipartite, like the *Bean dwarf mosaic virus* (BDMV), which possesses two DNA components (Hidayat *et al*., 1993), or monopartite, like the *Tomato yellow leaf curl virus* (TYLCV), with only one component of DNA but with two additional genes, both encoding proteins involved in the movement of the virus (Rojas *et al*., 2001). BDMV is one of several begomoviruses that infect common bean in nature; others include *Bean calico mosaic virus* (Brown *et al*., 1999), *Bean golden mosaic virus* (Gilbertson *et al*., 1993a) and *Bean golden yellow mosaic virus* (BGYMV) (Garrido-Ramirez *et al*., 2000a).

The natural and most important host of BDMV is the common bean (*Phaseolus vulgaris* L.). Initial disease symptoms in infected susceptible bean plants include severe stunting and dwarfing and leaf distortion and light green to yellow mosaic and mottling (Fig. 2). Bean plants infected with BDMV-like virus were first investigated in Brazil (Sao Paulo) by Costa (1965), who showed that the virus can be transmitted by the whitefly *B. tabaci* from weed reservoirs to bean plants, but not through seeds. In the early 1980s, the disease caused by BDMV became economically important, as spread of the virus devastated over 40 000 hectares of common bean plantings in north-western Argentina (Morales, 2006; Morales *et al*., 1990). Since then, BDMV has also been identified in Colombia (Hidayat *et al*., 1993). It should be noted, however, that although the common bean is one of the most widely cultivated legumes in the world, bean dwarf disease usually occurs at low incidence (Costa, 1976; Morales, 2001, 2006), probably as a result of the limited geographical distribution of the virus and the planting of resistant varieties. In the Americas, where the common bean originated, two major gene pools can be identified, one consisting of the Andean germplasm (from South America) and the other of Middle American germplasm (from Mexico and Central America) (Gepts, 1998). Whereas Andean varieties are susceptible to BDMV, most Middle American varieties are resistant (Gepts, 1998; Seo *et al*., 2004; Zhou *et al*., 2007).

Like many begomoviruses, BDMV possesses two DNA components (termed DNA-A and DNA-B), each approximately 2.6 kb in size (Hidayat *et al*., 1993). Genes on DNA-A encode the coat protein (CP) and proteins involved in replication and gene expression, whereas genes responsible for host range determination and viral movement are located on DNA-B (Garrido-Ramirez *et al*., 2000b; Gilbertson *et al*., 1991a, 2003; Gutierrez, 2000; Hanley-Bowdoin *et al*., 2000; Harrison *et al*., 2002; Hehnle *et al*., 2004; Hou *et al*., 2000; Timmermans *et al*., 1994) (Fig. 3).

Pseudorecombinants or reassortants can be made among bipartite begomoviruses by exchanging DNA components (Harrison and Robinson, 1999). The first viable pseudorecombinants between two bipartite begomovirus species were formed by exchanging components of *Tomato mottle virus* (ToMoV) and BDMV (Gilbertson *et al*., 1993b). One of the two pseudorecombinants (ToMoV DNA-A/BDMV DNA-B) became highly pathogenic following serial passages. Sequencing of BDMV DNA-B of this pseudorecombinant revealed intermolecular recombination between the two components, resulting in the replacement of most of the BDMV common region (CR) with that of ToMoV. This result was among the first evidence for recombination being a driving force in begomovirus evolution.

BDMV is used extensively as a model for studies of geminivirus movement. Because the viral CP is dispensable for BDMV movement in its natural host, a green fluorescent protein (GFP) reporter system was developed by replacing the CP-encoding gene on DNA-A with a GFP-encoding gene. The GFP reporter system has contributed to investigations into the movement patterns of BDMV from cell to cell and throughout the plant (Levy and Czosnek, 2003; Sudarshana *et al*., 1998). In addition, expression of the GFP gene as a chimera with viral genes enhances the ability to identify the latter's roles and locations in the plant cell (Garrido-Ramirez *et al*., 2000b; Gilbertson *et al*., 2003; Levy and Czosnek, 2003, 2006; Seo *et al*., 2004; Sudarshana *et al*., 1998; Wang *et al*., 1999; Zhou *et al*., 2007). In this

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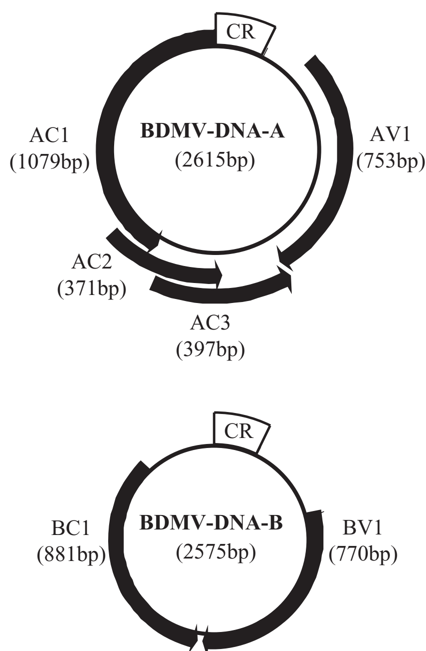

Fig. 3 Genomic organization of *Bean dwarf mosaic virus* (BDMV). Open reading frames (ORFs) are designated V (viral sense orientation) or C (complementary sense orientation); AV1 gene encodes the coat protein, AC1 encodes the replication-associated protein, AC2 encodes the transcription activator protein, AC3 encodes the replication enhancer protein, BV1 encodes the nuclear shuttle protein, BC1 encodes the movement protein; CR (common region). Numbers indicate the lengths of the ORFs. (Reproduced from Levy and Czosnek, 2003, with permission.)

review, we focus mainly on recent developments in our understanding of BDMV movement in plants.

BDMV GENOME ORGANIZATION

The complete sequence of an infectious clone of BDMV was published by Hidayat and coworkers back in 1993 (Hidayat *et al*., 1993). Infectivity studies (Gilbertson *et al*., 1991a) established that the virus is composed of two distinct components, designated DNA-A (2615 nucleotides) and DNA-B (2575 nucleotides) (Fig. 3). A comparison of the nucleotide sequence with other known begomoviruses revealed that BDMV is most closely related to *Abutilon mosaic virus* (AbMV), ToMoV and other viruses in the AbMV phylogenetic cluster. Although BDMV is closely related to AbMV, the two viruses differ in host range, tissue tropism and the capacity to be transmitted by whiteflies. Hofer *et al*. (1997) suggested that AbMV lost its ability to be transmitted during long periods of vegetative propagation, and Hohnle *et al*. (2001) showed that changing only three amino acids in AbMV CP could restore the ability of the virus to be transmitted by whiteflies.

An intergenic region (IR) is found on both DNA components of BDMV. The IR spans the start codons of the leftmost (the first gene on the complementary sense strand) and rightmost (the first gene on the viral sense strand) genes. The CR (187 bp) is located inside the IR. The CRs of both DNA-A and DNA-B are nearly identical, as also observed in other bipartite begomoviruses (Rojas *et al*., 2005). The stem-loop structure, located inside the CR (nucleotides 133–165), serves as the origin of viral DNA replication, and consists of 11 nucleotides forming the stem and 11 nucleotides forming the loop.

As with most of the begomoviruses, four genes are located on DNA-A of BDMV (Hidayat *et al*., 1993) (Fig. 3). AV1 (753 bp) is in the viral sense strand, and encodes for CP. The other three genes, AC1, AC2 and AC3, are located on the complementary sense strand and their coding sequences overlap. AC1 (1079 bp) encodes the replication-associated protein (Rep), AC2 (371 bp) encodes the transcriptional activator protein (TrAP) and AC3 (397 bp) encodes the replication enhancer (REn) protein (Rojas *et al*., 2005). In begomoviruses, promoters for bidirectional transcription are located inside the IR on both DNA-A (for the transcription of AV1 and AC1) and DNA-B (for BV1 and BC1 transcription) (Shivaprasad *et al*., 2005). The promoter for transcribing AC2 and AC3 of BDMV is located upstream of AC2 within the coding region of AC1, as has been established for *Mungbean yellow mosaic virus* (MYMV) (Shivaprasad *et al*., 2005). On DNA-B, there are only two genes: BV1 (770 bp), located on the viral sense strand, which encodes the nuclear shuttle protein (NSP), and BC1 (881 bp), located on the complementary sense strand, which encodes the movement protein (MP).

THE INFECTION PROCESS

Begomoviruses are transmitted from plant to plant by *B. tabaci* in a persistent manner and remain associated with the insect for its entire adult life (Ghanim *et al*., 2001). The virus moves in the whitefly body in a circulative manner. During the feeding process, the viral particles are acquired by the vector, move from its mouthparts into the gut, and from there to the haemolymph and back to the mouthparts through the salivary gland (Sinisterra *et al*., 2005). During this process, the viral DNA is protected inside the whitefly's body by CP. In the haemolymph, CP binds to a GroEL analogue (Morin *et al*., 2000), which is produced by a bacterial endosymbiont and protects the virus particles from degradation (Harrison *et al*., 2002). CP is thus a determinant of insect vector specificity.

CP of BDMV is a karyophilic protein that possesses a nuclear localization signal (NLS) similar to that of TYLCV CP (Kass *et al*., 2006; Kunik *et al*., 1998). However, although CP of TYLCV is necessary for nuclear import of viral DNA in plant cells (Kunik *et al*., 1998), CP of BDMV is not required for cell-to-cell move-

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ment, nor is it necessary for nuclear import of the virus in its adapted host plant (Levy and Czosnek, 2003; Sudarshana *et al*., 1998). This function is performed by NSP, which also possesses an NLS (Kass *et al*., 2006). It should be noted that CP of BDMV can mediate the nuclear import of synthetic peptides in mesophyll cells of *N. benthamiana* (Kass *et al*., 2006), and it is therefore possible that it functions early in the infection process by mediating the nuclear import of the viral DNA in primary cells, where NSP has not yet been expressed (Gafni and Epel, 2002).

It is likely that the replication of BDMV, similar to other geminiviruses, occurs via a rolling-circle mechanism in the nucleus of infected cells. Geminiviruses replicate through a ds RF that also serves as a transcription template for expression of the viral genes (Hanley-Bowdoin *et al*., 2000). Rep is a crucial element in the viral replication process. It binds specifically to dsDNA intermediates during origin recognition and cleaves the conserved stem-loop motif in the IR, the viral origin of replication, to initiate rolling-circle replication (Hanley-Bowdoin *et al*., 1990; Heyraud-Nitschke *et al*., 1995). Rep also has the ability to act as a ligase, joining the ends of the stem-loop at the termination of replication (Fontes *et al*., 1994; Heyraud-Nitschke *et al*., 1995; Orozco and Hanley-Bowdoin, 1996). Whereas the presence of Rep is sufficient for viral replication (Hanley-Bowdoin *et al*., 1990), REn enhances replication dramatically and, consequently, symptom severity as well (Hanley-Bowdoin *et al*., 2000; Settlage *et al*., 2001). Once replicated, expression of the late-expressed viral genes, AV1 and BV1 (Frey *et al*., 2001; Hung and Petty, 2001; Sunter and Bisaro, 1992), is probably controlled by the early and constitutive TrAP (Hung and Petty, 2001). TrAP may also function in suppressing RNA silencing by activating host genes that encode negative regulators of RNA silencing (Trinks *et al*., 2005). Once replicated in the nucleus, BDMV has to export its progeny into the cell cytoplasm where they move from cell to cell via the plasmodesmata, eventually reaching the host phloem and leading to systemic infection of the host. For a more in-depth discussion on *Geminivirus* infection and replication, we refer the reader to several excellent reviews (e.g. Gutierrez, 2000; Gutierrez *et al*., 2004; Hanley-Bowdoin *et al*., 2000).

BDMV MP–DNA INTERACTIONS

The capacity of a virus to infect plants relies mostly on its ability to interact with plasmodesmata for cell-to-cell movement, and particularly with plasmodesmata associated with the vascular tissues for long-distance movement (Gilbertson and Lucas, 1996; Lucas, 2006). For plant-infecting viruses, this is typically achieved by the action of MP. One of the important tasks of the plant virus MP is to dilate the microchannels of the plasmodesmata to increase the molecular size exclusion limit, so that the infectious form(s) of the virus can move to the adjacent cell (Cilia and Jackson, 2004; Lucas, 1999, 2006). To accomplish this, the begomovirus MP binds to the viral DNA in a nonsequence-specific manner, forming nucleoprotein complexes. MP guides the viral DNA with the help of cellular proteins towards the plasmodesmata.

In geminiviruses, three proteins are involved in viral movement: NSP, MP and CP (Lazarowitz and Beachy, 1999; Rojas *et al*., 2005). MP and NSP of BDMV bind ssDNA and dsDNA in a nonsequence-specific manner. In addition, they recognize nucleic acids on the basis of form and size, making them determinants of viral genome size in begomoviruses (Gilbertson *et al*., 2003; Rojas *et al*., 1998). These two proteins have special features that reflect their abilities. Their N terminus is positively charged and located on the protein surface and, in solution, this structure has the potential to interact with DNA. The proteins also possess hydrophobic domains which indicate their capacity for protein–protein interactions (Rojas *et al*., 1998). Both proteins have the ability to create multimers (dimeric, trimeric and tetrameric forms). Although the monomeric MP and NSP bind to DNA through their N terminus, the multimeric proteins condense the binding viral DNA, as observed by electron microscopy, to create a stabilized protein–DNA complex (Rojas *et al*., 1998). This condensed nucleoprotein complex can then be transported by NSP and MP across the nuclear and plasmodesmal boundaries, respectively (Rojas *et al*., 1998).

It has been suggested that only viral DNA above a certain size can be incorporated into a stable complex (Rojas *et al*., 1998). Furthermore, Gilbertson *et al*. (2003) showed that strict size maintenance of revertants originating from a size-increased BDMV DNA-A is imposed by the plant's endogenous RNA trafficking pathway. The BDMV-A revertants, generated from both homologous and nonhomologous recombination and template switching between DNA-A and DNA-B, showed a very narrow size range. They found that the replication mechanism itself is not responsible for the reduction in revertant size because size-increased forms of BDMV DNA-A replicated in protoplasts. NSP was also found not to be the factor responsible for the selection of genome-sized revertants, because it binds greater than genome-sized molecules for nuclear export (Rojas *et al*., 1998). In contrast, MP mediates cell-to-cell movement of only viral genome-sized molecules. Although MP can bind 2.5–5.5-kb DNA molecules (molecules smaller than 2.5 kb will form unstable MP–DNA complexes, whereas molecules larger than this size will form excessively large complexes that cannot pass through the microchannels of the plasmodesmata), it prefers an optimal size of 2.5–3 kb. The selection for this preferred DNA size is established during one to two cycles of replication and movement into adjacent cells (Gilbertson *et al*., 2003).

INTRACELLULAR MOVEMENT

NSP of the *Squash leaf curl virus* (SLCV) has been localized to the microsomal membrane fraction (Pascal *et al*., 1993). The BDMV

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NSP–GFP fusion protein has been localized to the nucleus (Rojas *et al*., 2001). NSP facilitates the export of viral ssDNA and dsDNA from the nucleoplasm to the cytoplasm through the nuclear pore complex (Gafni and Epel, 2002; Rojas *et al*., 1998). It increases the export of viral ssDNA and dsDNA from the nucleus through the nuclear envelope towards the cytoplasm. The enzyme GTPase, which hydrolyses guanosine triphosphate (GTP), plays an important role in protein translocation through the membrane. Carvalho *et al*. (2008) have shown that a cellular protein termed NIG (NSP-interacting GTPase) binds the begomovirus NSP *in vitro* and *in vivo* and is activated as a cofactor for NSP in nucleocytoplasmic trafficking of viral DNA. They also showed that, when co-expressed in tobacco cells, NIG enhances NSP nuclear export and, in transgenic plants, enhances susceptibility to begomoviruses. NIG may function as a molecular switch to facilitate the intracellular transport of viral DNA–NSP complexes from the nucleus to the cortical cytoplasm, where it may be replaced by MP in preparation for cell-to-cell movement (Carvalho *et al*., 2008).

CELL-TO-CELL MOVEMENT

BDMV MP mediates the cell-to-cell movement of viral DNA through the plasmodesmata (Noueiry *et al*., 1994). This protein is located in the cell wall and plasma membrane fractions and also around the nucleus of the infected cell (Pascal *et al*., 1993; Von Arnim *et al*., 1993). BDMV MP–GFP fusion protein has been localized to the cell periphery (Rojas *et al*., 2001). In AbMV (the virus most closely related to BDMV), the central portion of MP encloses a domain responsible for membrane association, whereas the N terminus determines the dichotomy between membrane and perinuclear localization (Zhang *et al*., 2002). Using the microinjection technique, Noueiry *et al*. (1994) showed that MP of BDMV moves extensively from cell to cell and mediates the movement of dsDNA via mesophyll plasmodesmata. Seconds after injection, it increases the selective intercellular transport of dsDNA.

The MPs of ToMoV (Duan *et al*., 1997) and SLCV (Pascal *et al*., 1993) have been found to be symptom-inducing elements. DNA-B of BDMV has also been found to have an influence on symptom severity (Levy and Czosnek, 2003, 2006). Hou *et al*. (2000) have reported that transgenic tomato plants expressing BC1 of BDMV show a viral disease-like phenotype. Introducing BDMV DNA-A with its cognate DNA-B into bean plants produced the typical BDMV symptoms, whereas the pseudorecombinant BDMV DNA-A and AbMV DNA-B showed only very mild symptoms.

The ability of BDMV MP and NSP to bind both ssDNA and dsDNA stands in contrast with their counterparts in SLCV. SLCV NSP preferentially binds ssDNA, whereas its MP binds only weakly to ssDNA and not at all to dsDNA (Pascal *et al*., 1994). Although both ssDNA and dsDNA forms may be involved in cell-to-cell spread of BDMV, open circular dsDNA is probably the main form for BDMV movement within the plant (Rojas *et al*., 1998). As for SLCV, it has been suggested that MP and NSP work cooperatively to facilitate the movement of the viral ssDNA in the plant, although no evidence was presented for ssDNA being the form in which SLCV moves. SLCV NSP and MP bind directly to each other, whereas MP can also relocalize NSP from the nucleus to the cell periphery. Thus, it has been suggested that the movement of SLCV occurs via the formation of an NSP–ssDNA complex which moves from cell to cell by binding to MP (Sanderfoot and Lazarowitz, 1995).

In contrast with the phloem-limited SLCV, the nonphloem-limited BDMV can also move into surrounding mesophyll tissues. Thus, it has been suggested that these viruses may utilize different modes of movement. BDMV NSP facilitates the movement of the viral dsDNA genome from its site of replication in the nucleus to the cytoplasm. Then, the viral DNA is transferred to MP for cell-to-cell movement as an MP–dsDNA complex. It moves across the cell wall to adjacent uninfected cells through the plasmodesmata (Rojas *et al*., 1998). MP of BDMV (Lee, 2008), like those of *Tobacco mosaic virus* (TMV) (Trutnyeva *et al*., 2005; Waigmann *et al*., 2000), AbMV (Kleinow *et al*., 2008) and others, can be phosphorylated at its C terminus by cell wall-associated kinases. This may be necessary for increasing the plasmodesmata size exclusion limit, allowing viral cell-to-cell movement. Waigmann *et al*. (2000) have suggested that the phosphorylation status of MP specifies its set of functions. Lee (2008) identified a host protein kinase, termed plasmodesmal-associated protein kinase (PAPK), which can phosphorylate both TMV and BDMV MPs. It has been proposed (Kleinow *et al*., 2008) that there are multiple phosphorylation sites on AbMV MP and that the virus exploits the cell signal transduction and signalling pathways via protein phosphorylation during plant infection. Furthermore, the phosphorylation status may vary during infection according to different kinase activities in different plant tissues and developmental stages of the plant. Kleinow *et al*. (2008) have suggested a model in which the phosphorylation process switches off MP following successful transfer, leading to the inhibition of its transport capability.

LONG-DISTANCE MOVEMENT

Both of the DNA-B-encoded proteins of BDMV have distinct and independent functions and both are necessary for systemic infection of nonphloem tissues (Levy and Czosnek, 2003; Noueiry *et al*., 1994; Sudarshana *et al*., 1998). It is important to note that the introduction of BDMV DNA-A alone by agroinoculation can result in a symptomless systemic infection, presumably as a result of direct introduction of the virus into the vascular tissues (Hou *et al*., 1998). Thus, MP and NSP, encoded by BDMV DNA-B,

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are not required for long-distance movement of begomovirus DNA in the phloem.

In contrast with the monopartite begomoviruses, the bipartite BDMV does not need CP for either cell-to-cell or long-distance movement through the phloem of its host plant (common bean) (Levy and Czosnek, 2003; Sudarshana *et al*., 1998; Wang *et al*., 1999). It does, however, need it for long-distance movement in nonadapted hosts, such as soybean and certain Middle American common bean cultivars (characterized by very low viral titre and by very mild symptom formation on inoculated plants). This implies a role for CP in virus movement (Seo *et al*., 2004; Wang *et al*., 1999). Further support for CP involvement in viral movement can be found in a report from Ingham *et al*. (1995), who showed that, if NSP of SLCV is mutated, CP can complement this defect and enable movement. CP may also function as a pathogenicity determinant, as infection of plants with the BDMV reporter in which CP has been replaced with a GFP gene induces symptoms that are slightly attenuated compared with those from infection with the wild-type virus (Levy and Czosnek, 2003, 2006; Sudarshana *et al*., 1998).

Sudarshana and coworkers introduced a BDMV–GFP reporter by particle gun bombardment into the epidermal cells of bean hypocotyls (Sudarshana *et al*., 1998). The virus was seen to move from cell to cell from the epidermis of the hypocotyls towards the phloem cells through the cortex (Fig. 4). It took 18–24 h for BDMV–GFP to move from the epidermis into the underlying cortical cells, and 2–4 days to reach the vascular tissues. From the phloem, the virus moved downwards via the long-distance transport system of the phloem to the root. In the root, BDMV–GFP could be detected in companion cells, phloem parenchyma and protophloem cells, although it could not be detected in the surrounding tissues, reflecting its inability to egress from the phloem. In contrast, movement of the virus upwards was in part cell to cell as, after 4 days, it was detected only a few centimetres above the bombardment site in the region of the cotyledon–hypocotyl junction (Sudarshana *et al*., 1998; Wang *et al*., 1999). Six days post-bombardment, the virus had spread towards the protophloem of the shoot apex, probably through long-distance movement in the phloem. The virus was not detected in the apical meristem. It was detected in primary leaf veins of all orders and in both phloem and nonphloem mesophyll cells. In contrast, 18 days post-bombardment, GFP-associated fluorescence was detected in newly emerging trifoliolate leaves, but the virus was confined to the vein cells. Following the flowering phase of the plants, the virus was also detected in nonphloem cells (mesophyll) of trifoliolate leaves. GFP fluorescence was also detected in the phloem of flower petals, in pods and styles and in seed coats, but not embryo. This demonstrated that the virus is restricted to the maternal tissues and never reaches the embryonic tissue (Sudarshana *et al*., 1998). The ability of BDMV to exit the phloem depends on the plant's developmental stage: BDMV is not phloem-limited when it infects *N. benthamiana* (Wang *et al*., 1996) or bean plants (Levy and Czosnek, 2003; Sudarshana *et al*., 1998) during early stages of plant development.

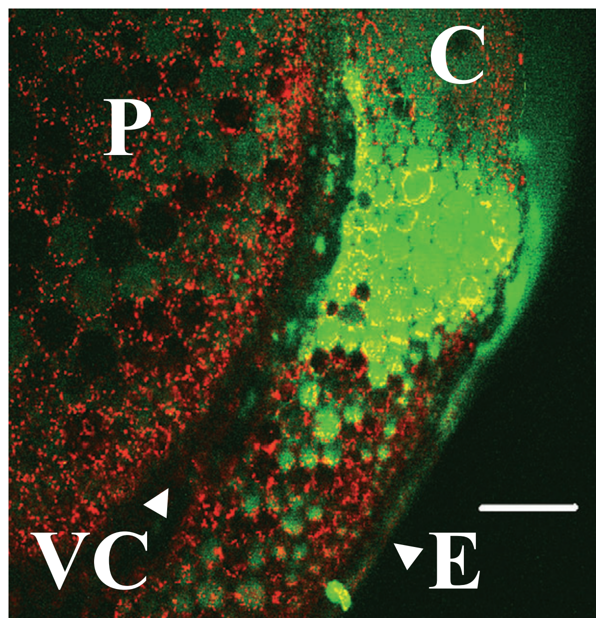

Fig. 4 Cell-to-cell movement of green fluorescent protein (GFP)-tagged *Bean dwarf mosaic virus* (BDMV) in bean plants. Bean seedlings (*Phaseolus vulgaris* cv. Benton), 3 days after germination, were inoculated with BDMV DNA-A in which the coat protein (CP) was replaced by GFP, and with its cognate DNA-B. Viral components were introduced into hypocotyls of bean seedlings by particle gun bombardment. Cross-sections were taken 5 days post-inoculation from the bombarded hypocotyls which were analysed for viral cell-to-cell movement by confocal microscopy. Note the movement of the GFP-associated fluorescence from the epidermis towards the phloem. C, cortex; E, epidermis; P, pith; VC, vascular cylinder. Bar, 200 μm. (Reproduced from Levy and Czosnek, 2003, with permission.)

OTHER VIRAL PROTEINS WHICH MAY FUNCTION IN VIRAL MOVEMENT

Despite the close relatedness of BDMV and AbMV in nucleotide and amino acid sequence comparisons, AbMV is a phloem-limited virus (Wege *et al*., 2000), whereas BDMV is not. Levy and Czosnek (2003) infected bean plants with pseudorecombinants of BDMV and AbMV. In both viruses, CP, which is not essential for systemic infection in beans (Levy and Czosnek, 2003; Sudarshana *et al*., 1998), was replaced by GFP to create BDMV–GFP and AbMV–GFP. On introduction of the pseudorecombinant [BDMV–GFP]-A/AbMV-B into bean plants, the presence of DNA-B from phloem-limited AbMV did not confine BDMV–GFP to the phloem. On the other hand, when the reciprocal pseudorecombinant [AbMV–

GFP]-A/BDMV-B was introduced, DNA-B of the nonphloem-limited virus mediated cell-to-cell movement of AbMV–GFP in nonphloem tissues. This indicates that, in addition to DNA-B, DNA-A of BDMV possesses factors involved in determining the tissue tropism of the virus (Levy and Czosnek, 2003).

Intermolecular replacement of the AC2/AC3 fragment of AbMV–GFP with that of BDMV (Levy and Czosnek, 2006) resulted in the movement of AbMV (and its cognate DNA-B) from cell to cell in nonphloem tissues. When the same AbMV–GFP (with AC2/AC3 taken from BDMV) was inoculated with BDMV DNA-B, extraordinarily strong accumulation of viral DNA was observed, together with very fast viral movement and remarkably strong symptoms. The authors suggested that TrAP (encoded by AC2) and/or REn (encoded by AC3) were involved in viral accumulation and spread in the plant. A possible interaction between BDMV TrAP and the late viral proteins on AbMV DNA-B (MP and NSP), and/or interaction of BDMV REn with the virus replication mechanism of AbMV, would result in rapid infection that could outrun the host defence response, allowing the virus to overcome the phloem limitation and establish cell-to-cell movement of AbMV in nonphloem tissues. Alternatively, as has been suggested for other begomoviruses (Hao *et al*., 2003; Wang *et al*., 2003, 2005; Yang *et al*., 2007), BDMV TrAP might serve as a strong suppressor of the plant defence response, thereby overcoming AbMV's phloem limitation (Levy and Czosnek, 2006).

DIFFERENT MOVEMENT PATTERNS OF BDMV IN HOST AND NONHOST PLANTS AND DEVELOPMENT OF THE HYPERSENSITIVE RESPONSE

The hypersensitive response (HR) is a host plant mechanism involved in resistance to infection by a range of plant pathogens. This phenomenon leads to confinement of the pathogen in a small localized area (Garrido-Ramirez *et al*., 2000b).

As already noted, the common bean originates from the New World of the Americas, where two different gene pools have been identified: Andean (Peru, Bolivia and northern Argentina), characterized by large seeds and a general susceptibility to BDMV, and Middle American (Mexico and Central America), characterized by small to medium seeds and a general resistance to BDMV (Gepts, 1998; Gilbertson *et al*., 1991b; Seo *et al*., 2004; Zhou *et al*., 2007). The BDMV–GFP reporter was used to identify the patterns of BDMV movement in host and nonhost plants (Garrido-Ramirez *et al*., 2000b; Seo *et al*., 2004; Wang *et al*., 1999; Zhou *et al*., 2007). BDMV was unable to establish a systemic infection in the resistant bean cultivar cv. Othello, nor was it successful in other cultivars of the Middle American gene pool. The introduction of the BDMV–GFP reporter into these cultivars by particle gun bombardment confirmed BDMV's ability to replicate and move from cell to cell in the epidermis and cortex of inoculated hypocotyls, but it did not move long distances in the phloem. Almost 2 days after bombardment, the viral infection had activated HR in the host, which was expressed by cell collapse and the onset of necrosis in the cortical and phloem tissues that contained the virus in the initial region of inoculation. Although the formation of HR in cv. Othello was correlated with the inability of the virus to enter the long-distance translocation stream, the notion that the blockage was a result of the formation of HR was not confirmed (Garrido-Ramirez *et al*., 2000b). The blockage was not absolute, as a few fluorescing cells were identified in the root, although no fluorescing cells could be identified in the region above the bombardment site.

The viral determinants for HR formation in cv. Othello were localized to DNA-B of BDMV, because ToMoV introduced into cv. Othello did not induce HR, whereas it was induced in the same cultivar by the pseudorecombinant ToMoV-A/BDMV-B (Wang *et al*., 1999). NSP was found to be responsible for the induction of HR in cv. Othello, as well as being a viral avirulence factor. This finding was established following the induction of HR in cv. Othello by cobombardment of BDMV-A and a hybrid DNA-B composed of BDMV NSP and BGYMV MP (BGYMV systemically infects cv. Othello without inducing HR), whereas the reciprocal DNA-B (i.e. BDMV MP and BGYMV NSP) did not induce HR (Garrido-Ramirez *et al*., 2000b). Later, Zhou *et al*. (2007) mapped the avirulence factor in the Middle American cultivars to the N terminus of NSP between amino acids 1 and 42, the most divergent region on this protein. These findings were established by coinoculating bean plants with BDMV DNA-A and a series of hybrid DNA-Bs expressing chimeric BV1 composed of both BDMV and BGYMV (Zhou *et al*., 2007). HR may not play a direct role in resistance because other BDMV-resistant cultivars, such as Black Turtle Soup (BTS), do not develop HR during the BDMV resistance response (Garrido-Ramirez *et al*., 2000b; Hou *et al*., 1998; Seo *et al*., 2004; Wang *et al*., 1999).

Soybean is a nonadapted host of BDMV and is characterized by a very low viral titre and very mild symptom formation in inoculated plants. It was found that BDMV can systemically infect soybean, whereas BDMV–GFP is able to move from cell to cell in the epidermal, cortical and phloem cells, but is unable to move through the long-distance translocation stream. Again, this implies that CP is required for long-distance movement of BDMV in nonadapted hosts, such as soybean (Wang *et al*., 1999). It has been suggested that, without CP, the cell-to-cell movement of the virus is slower, giving the host's defence responses time to better block the spread of the virus (Seo *et al*., 2004; Zhou *et al*., 2007).

MODELS AND FUTURE OUTLOOK

Two different models have been suggested to explain *Begomovirus* intracellular movement. AbMV movement is explained by the

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'couple-skating model' (Kleinow *et al*., 2008), in which the viral NSP transports viral ssDNA or dsDNA from the nucleus to the cell periphery. Once in the cytoplasm, MP facilitates the cell-to-cell movement of NSP–DNA complexes (Frischmuth *et al*., 2004, 2007; Hehnle *et al*., 2004; Kleinow *et al*., 2008; Pascal *et al*., 1994; Sanderfoot and Lazarowitz, 1995; Zhang *et al*., 2001a, 2002). The validity of this model has been supported by several experiments: using localization studies of AbMV NSP–GFP and AbMV MP–GFP, Zhang *et al*. (2001a, 2002) showed that AbMV NSP is localized in the nucleus, whereas AbMV MP is localized to both the plasma membrane and around the nuclei; Hehnle *et al*. (2004) reported the physical interactions between AbMV dsDNA genomes and AbMV NSP and MP, and Frischmuth *et al*. (2007) demonstrated that AbMV MP can redirect AbMV NSP from the nucleus to the plasma membrane when coexpressed in fission yeast. In addition, Zhang *et al*. (2001a) reported that a neighbour epidermal cell can receive NSP (following its transfer through the plasmodesmata by the MP) only when it has been coinoculated with viral DNA. It should be noted that the number of times this movement was observed was very small and, in addition, AbMV does not naturally infect epidermal cells.

BDMV movement is explained by the 'relay-race model' in which the viral NSP transfers viral dsDNA from the nucleus to the cytoplasm. Once in the cytoplasm, BDMV dsDNA is delivered to BDMV MP for cell-to-cell movement through the plasmodesmata (Noueiry *et al*., 1994; Rojas *et al*., 1998). This model is supported by data from Noueiry *et al*. (1994), who demonstrated the cell-to-cell movement of MP–dsDNA complexes by *in vivo* microinjection. In addition, Rojas *et al*. (1998) used a gel mobility assay to show that BDMV ssDNA and dsDNA can bind to both BDMV NSP and BDMV MP.

Following cell-to-cell movement, the virus enters the plasmodesmata associated with the vascular tissues for long-distance movement. Neither of BDMV's movement proteins (NSP or MP) is required for the long-distance movement of the virus in the phloem (Hou *et al*., 1998). From the phloem parenchyma and companion cells, BDMV is released to the phloem sieve elements. The virus moves via the long-distance transport system downwards to the root and upwards towards the shoot apex. As noted earlier, the movement of the virus upwards is in part via cell-to-cell movement as the virus could be detected in some nonphloem tissues, such as the mesophyll of trifoliolate leaves (Sudarshana *et al*., 1998).

BDMV CP is dispensable for viral movement in its natural host, but is needed for long-distance movement of the virus in nonadapted hosts (Seo *et al*., 2004; Wang *et al*., 1999). CP also functions as a pathogenicity determinant and has been shown to be very important for efficient and rapid viral movement (Levy and Czosnek, 2003, 2006; Sudarshana *et al*., 1998). Likewise, CP can complement a defective NSP and maintain viral movement (Ingham *et al*., 1995), all of which imply strong involvement of CP in this movement. Thus, in order to understand the natural cell-to-cell movement of the virus, it is important to also take into consideration the function of CP in relation to MP, NSP and cell-to-cell movement. This also holds true for investigations of BDMV long-distance movement in the whole plant, because most of the work conducted to date has utilized reporters that lack CP.

Despite the two different models proposed to explain the cell-to-cell movement of begomoviruses, it is also possible that the closely related BDMV and AbMV have not evolved different strategies for their movement. Additional studies are needed to resolve this situation.

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