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Arabidopsis thaliana as a Model Host for Studying Symptom Development During Geminivirus Infection

Sukchan Lee*, Jongbum Park[1] and Keith R. Davis[2]
Department of Genetic Engineering, Sungkyunkwan University, Suwon 440-746, Korea
[1]Department of Biology, Silla University, Pusan 617-736, Korea
[2]Department of Plant Biology and Plant Biotechnology Center, The Ohio State University, Columbus, OH 43210, USA

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Viruses represent an unique class of pathogenic agents because of the extremely intimate associations formed between the virus and its host. Host factors play critical roles throughout the virus life cycle, and are important for controlling the expression of viral genes, viral genome replication and systemic spread of the virus throughout the plant. Currently, very little is known about the specific host factors that are involved in supporting virus infection. To approach these interesting but difficult questions, several groups have characterized a number of Arabidopsis-virus interactions that will prove useful in the identification of plant host factors important for viral pathogenesis and defining how these host factors interact with viral proteins. The results obtained using these systems clearly indicate that Arabidopsis is an excellent host plant for studying plant-virus interactions. The early development of Arabidopsis-virus interactions has been previously reviewed (Dangl, 1993; Davis, 1992, 1998). The following sections describe recent results concerning several of the more developed Arabidopsis-geminivirus pathosystem and our current understanding of the molecular basis of symptom development.

Arabidopsis as a Model Pathosystem

Arabidopsis thaliana is a small weed in the mustard family and has been a convenient subject for studies in classical plant genetics for over forty years (Langridge, 1994; Meyerowitz, 1987; Pyke, 1994; Redei, 1975). Recently, investigators have recognized that this small flowering plant also has many experimental advantages for studying plant molecular genetics, development, physiology, and biochemistry. Arabidopsis is a true diploid, has a rapid generation time (6 to 8 weeks), is self-compatible, produces large numbers of small seeds, and has sufficient variation within ecotypes of the species to allow for screening for natural variability in specific attributes (Redei, 1975). Arabidopsis also has one of the smallest genomes known in higher plants (approximately 100 Mb/haploid genome) and can be genetically engineered easily using standard *Agrobacterium tumefaciens*-mediated transformation protocols (Bechtold, et al., 1993; Feldmann, 1991; Feldmann and Mark, 1987; Kertbundit, et al., 1991). In addition, detailed information concerning the *A. thaliana* genome is available. Genetic maps based on restriction fragment length polymorphism (RFLP; Chang et al., 1988; Nam et al., 1989), random amplified polymorphic DNAs (RAPDs; Reiter et al., 1992), cleaved amplified polymorphic sequences (CAPS; Konieczny and Ausubel, 1993) and simple sequence length polymorphism (SSLP; Bell and Ecker, 1994) have been constructed by several groups. These maps combined with genetic maps based on morphological mutants make it possible to clone specific genes which have been identified by mutation via chromosome walking methods. More recently, an international consortium of research groups have begun sequencing the entire Arabidopsis genome and the entire sequence will be available within 1-2 years. Having the entire genome sequence and the associated BAC and YAC contigs will greatly facilitate isolation of genes corresponding to specific mutations. Because of these many advantages, Arabidopsis has become a prominent model system for experimental plant biology (Davis, 1992; Langridge, 1994; Meyerowitz, 1987; Meyerowitz, 1989; Meyerowitz and Pruitt, 1985).

In recent years, a number of studies have reported the interactions between Arabidopsis and phytopathogenic

*Corresponding author.
Phone) +82-331-290-7866, Fax) +82-331-290-7870
E-mail) sukchan@yurim.skku.ac.kr

microbes (Dangl, et al., 1992; Davis, 1992). Although Arabidopsis has been shown to be an excellent model host plant for phytopathogenic microbes, there are not many reports known about the interactions between Arabidopsis and plant viruses. Cauliflower mosaic virus (CaMV) was first tested on Arabidopsis by Balazs and Lebeurier (1981) and Melcher (1989). These investigators showed that Arabidopsis was a host for CaMV and the responses of several ecotypes to CaMV were different from each other with respect to symptom severity and infectivity. More recent studies by Leisner and Howell (1992) showed that the ecotype En-2 was resistant to CaMV. This resistance appears to be controlled by a single dominant locus. Other studies have been shown that a different Arabidopsis ecotype, Di-O, was resistant to an RNA virus, turnip crinkle virus, (TCV, Dempsey et al., 1993; Simon et al., 1992). Simon et al. (1992) found that restricted virus spread, not a block in virus replication, was responsible for the resistance of Di-O to TCV.

All the studies completed to date demonstrated that resistant and susceptible ecotype could be identified for many of the viruses that infect *A. thaliana*. In addition, susceptible ecotypes showed different degrees of responses to the virus with regard to symptom development, replication level and/or movement efficiency (Davis, 1998). Thus natural genetic variation within *A. thaliana* should be useful for studying the interactions between this model host and other important plant viruses.

Beet Curly Top Virus as an Unique Member of Geminiviridae

Geminiviruses have small single stranded circular genomes of 2.5~3.0 kb and a unique geminated capsid morphology (Davis, 1987; Davis and Stanley, 1989; Lazarowitz, 1987, 1992; Timmermans, et al., 1994). Geminiviruses are agronomically important and are known to infect many important crop plants including both monocots and dicots. Geminiviruses have been classified into three major groups, based on genome organization, host range, and insect vector. Beet curly top virus (BCTV) is sole member of the hybrigeminiviruses. BCTV, which has monopartite genome, is leafhopper-transmitted and infects over 40 different families of dicot plants. The BCTV genome has characteristics of both monopartite and bipartite geminiviruses (Fig. 1). The left half of BCTV genome resembles DNA A of bipartite geminiviruses, while the right half resembles that of a typical monopartite virus. The single intergenic region resembles bipartite viruses and contains the conserved TAATATTAC motif. The BCTV genome contains at least 7 functional ORFs. (Hormuzdi and Bisaro, 1993; Stanley and Latham, 1992; Stenger et al., 1994).

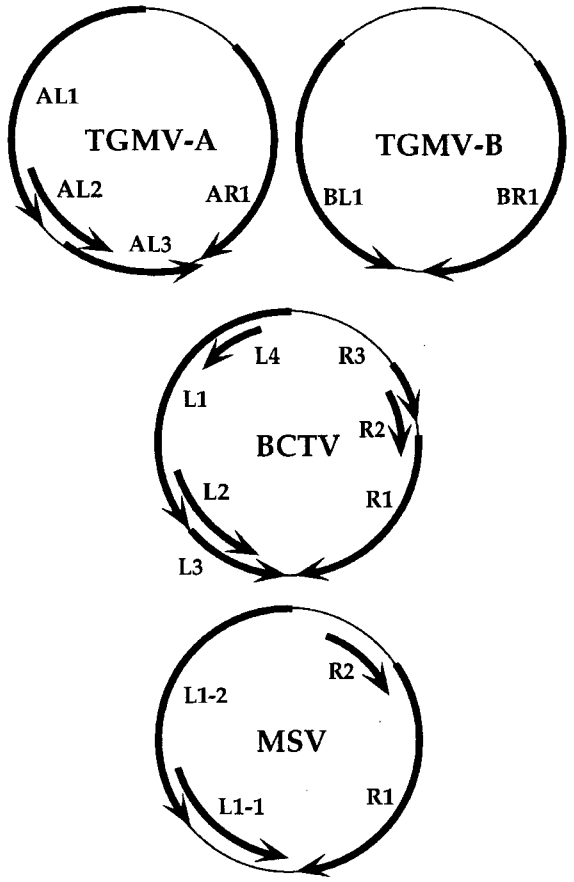

Fig. 1. Genomic maps of bipartite and monopartite geminiviruses. Shown are the limits and direction of transcription of each individual gene which these functions have been determined.

Numerous studies have been done to investigate the potential function of geminivirus ORFs in virus life cycle (Fig. 2). Most of these studies have involved the generation of specific mutations in viral ORFs and examining the effects on infection. Complementation studies have also been done using ORFs from different virus strains and by ORF expression in transgenic plants.

Besides these somewhat conserved viral functions, BCTV contains common seven ORFs (Fig. 3). Among them, there are two unique ORFs. The R2 ORF has been identified on the virion sense strand (Hormuzdi and Bisaro, 1993). Mutations on R2 ORF does not affect the infectivity of BCTV on *Nicotiana benthamiana*, but infections are asymptomatic. These mutants accumulate about 8 fold more dsDNA and 9 fold less ssDNA compared to wild type BCTV. The reduction in ssDNA and thus in viral titer, may account for the lack of symptom development. The altered DNA accumulation pattern implicates the role of R2 in controlling the production of the ss- and ds- forms, possibly by stabilizing ssDNA, or by controlling virion assembly processes. Another apparently unique BCTV ORF is L4. L4

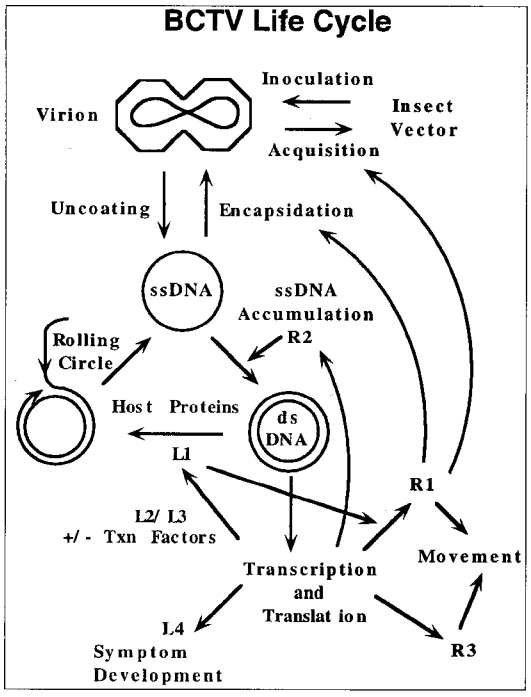

Fig. 2. BCTV life cycle. This figure summarizes the numerous genetic and biochemical studies on BCTV.

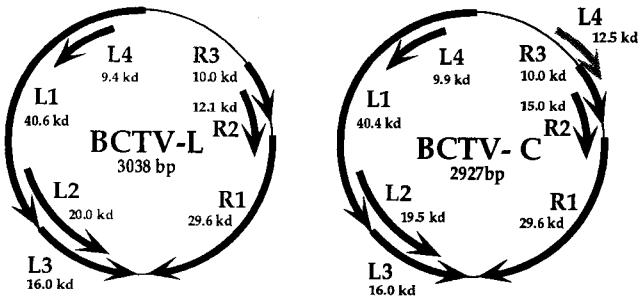

Fig. 3. Physical maps of the BCTV-Logan and BCTV-CFH genomes. Solid arrows denote locations and polarity of conserved ORFs; stripped arrow indicates a putative ORF unique to CFH.

mutants (Stanley and Latham, 1992) do not produce the typical hyperplasia on the stem of *N. benthamiana* after virus inoculation and thus, cause less dramatic symptom development. Transient replication assays indicated that L4 is not required for viral DNA replication.

Novel Symptom Development in BCTV-infected Arabidopsis

The development of callus structures in hypersusceptible ecotype Sei-O in response to BCTV infection is unique to Arabidopsis and has not been reported in any other host plant infected with a geminivirus (Lee, et al., 1994).

To investigate the biology of callus formation on *A. thaliana* induced by geminivirus infection, as a first step, the kinetics of virus accumulation during BCTV infection were examined in different organ systems. These studies showed that viral DNA was detectable in every organ including the callus structures of infected Sei-O plants. Virus DNA was present at higher levels in the inflorescence shoot tips, inflorescence stems and roots as compared to that observed in siliques, rosette leaves and cauline leaves. This distribution suggests that BCTV may actively multiply in phloem-rich tissues and get transported through the vascular tissues. The kinetics of viral DNA accumulation were also consistent with this model.

The callus cells induced in BCTV-infected Sei-O inflorescence stems and shoot tips contained the same high levels of viral DNA found in root and inflorescence tissues. The presence of virus DNA in these callus cells, which are clearly not derived from phloem tissues, suggests that BCTV can move from phloem cells into other tissues. This is very interesting, since BCTV is generally considered to be a phloem limited virus in most host plants.

A possible explanation for the novel pattern of virus movement in Sei-O is that this ecotype interacts more efficiently with the BCTV movement proteins, thus potentiating movement out of the phloem. Comparisons of Col-O and Sei-O infected with BCTV-Logan showed clear differences in the ability of the virus to move through the phloem and eventually move out of the phloem cells. There has been no proceeding report showing same results since the ability and efficiency of BCTV movement from phloem cells to adjacent cells seem to be very host-specific. BCTV appears to be phloem limited in sugar beet and spinach, whereas BCTV moves out of phloem cells into a number of different cell types in *N. benthamiana*. Although it is not surprising that differences in the ability of BCTV to move out of the phloem are observed in different plant host species, it is intriguing that a similar phenomenon was observed in very closely related ecotypes of Arabidopsis.

The virus localization studies demonstrated a correlation among the presence of BCTV, the activation of host cell division and phloem necrosis. Microscopic studies demonstrated that the activation of cell division is preceded by the disruption of the phloem. Phloem is the major conducting tissue of nutrients and other organic metabolites in plants, and disruption of phloem tissue by BCTV infection is likely to cause changes in the balance of some metabolites and hormones. There was a strong correlation between the amount of phloem disruption and the severity of symptoms observed at the whole plant level. BCTV infection of Sei-O resulted in the complete disruption of phloem bundles whereas in Col-O, phloem cell necrosis was much more limited. The mechanism of phloem disruption is not clear yet, but it is likely that virus multiplication and accumulation in nuclei may disrupt cellular functions, resulting in

cell death.

An important aspect of phloem disruption observed in BCTV-infected Arabidopsis was the induction of cell division within the phloem, and in the case of ecotype Sei-O, the induction of cell division in the phloem and surrounding cortex cells. Analysis of GUS reporter gene activity in transgenic plants containing constructs with promoters of the cell cycle genes, *cdc2* and *cyc1* and the auxin-induced *saur* promoter showed that *saur* promoter activity was induced concomitantly with cell cycle gene promoter activities during BCTV infection. Histochemical staining for GUS activity showed that cells in the symptomatic tissues at the inflorescence shoot tip of the three transgenic lines were heavily stained blue. This strongly suggests that changes in auxin concentration are involved in the induction of cell division in BCTV-infected plants. The kinetics of induction of *saur*, *cdc2*, and *cyc1* promoter activities after virus inoculation did not show any clear differences. Thus, the activation of these promoter activities are tightly linked in symptomatic tissues.

RNA blot analyses of *cdc2*, *cyc1* and *saur* transcript accumulation were for the most part consistent with the expression patterns observed in transgenic plants expressing the *cdc2*, *cyc1* and *saur* reporter genes. Accumulation of both transcripts induced by BCTV infection was similar with respect to both the timing and magnitude of induction. These studies taken together clearly demonstrate that there is a strong correlation between auxin-induced gene expression and the activation of cell cycle genes after BCTV infection on Arabidopsis. These results are consistent with several potential models for callus formation in BCTV-infected Arabidopsis (Fig. 4): One possibility is that BCTV infection cause the disruption of phloem tissues, which causes changes in hormone distribution in local infected tissues that promote additional cell divisions and other altered growth and development. A second possibility is that a BCTV gene product may somehow directly control host cell divisions by initiating the expression of host genes required for DNA replication. Finally, it is also possible that both local hormone level changes and the direct activation of the cell cycle by a viral gene product result in the development of the callus-like structures.

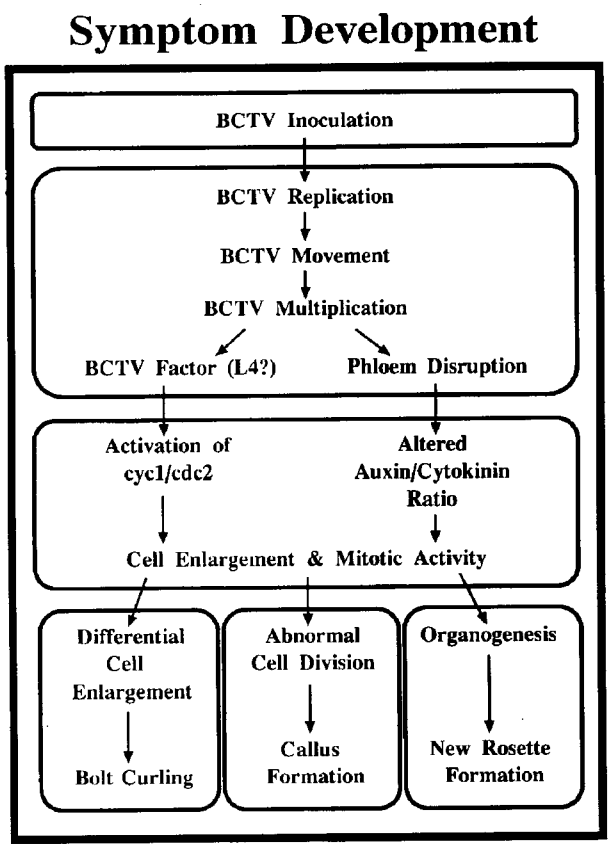

Fig. 4. Schematic representation of a callus formation and other symptom development induced by BCTV infection to Arabidopsis.

Geminivirus Is Able to Induce Host Cell Divisions Directly

Just as small tumor viruses have been proved to be invaluable for the analysis of host gene regulation and the control of cell divisions in animal system (Conzen and Cole, 1994; Moran, 1994), it is anticipated that plant DNA viruses will similarly help to unravel the complexities of cell cycle control in plants. Members of the geminiviruses has been particularly suited to this purpose as they have small circular DNA genomes that replicate in the nucleus by a rolling circle mechanism (Saunders et al., 1991). Because geminiviruses multiply in cells that appear to be fully matured and differentiated (Horns and Jeske, 1991; Rushing et al., 1987), it is likely that they are able to adapt the cellular environment by initiating the expression of host genes involved in regulating the cell cycle machinery. There are two lines of evidence reported recently in support of this idea. Firstly, a plant homologue of human proliferating cell nuclear antigen (PCNA), a processivity factor for DNA polymerase, was shown to accumulate in differentiated *N. benthamiana* cells infected with tomato golden mosaic virus (TGMV) (Nagar et al., 1995). PCNA also accumulated in differentiated cells of transgenic plants expressing TGMV replication-associated protein (L1), implicating the role of L1 gene in the process of cell adaptation. Secondly, wheat dwarf virus (WDV) Rep protein formed a stable complex with $p130^{Rbr2}$, a member of the retinoblastoma (Rb) family of proteins that controls the cell cycle by sequestering transcription factors required for entry into S-phase (Xie et al., 1995).

As mentioned previously, BCTV causes vein swelling as well as dramatic enation on the surface of systemically infected leaves and stems only on *A. thaliana*, resulting in characteristic callus development symptoms. Subsequently, it was shown that Arabidopsis failed to develop vein swelling when infected with BCTV L4 mutants, suggesting that L4 protein may affect host cell division (Stanley and Latham, 1992). To address these possibilities, Arabidopsis was transformed with and expression cassette containing the core coding region of the L4 gene that is conserved between geminiviruses and that has been shown to be important for symptom development. Transgenic plants exhibited a remarkable and novel phenotype and the severity was correlated with the steady state level of the transgene transcript. Severity of tissue distortion, the development of vein swelling and enations in the transgenic plants were compatible with the expression of a L4 gene that participates in the induction of cell division. Indeed, sections through symptomatic tissues revealed large clusters of disorganized small cells, implying rapid unregulated cell division in these tissues.

These observation demonstrates a role of L4 in cell cycle control although the relevance of this activity to virus infection remains unclear. Whether L4 acts by perturbing the balance of plant hormones or has a more direct effect on cell division marker genes or other cell signaling mechanism awaits biochemical analysis of the protein and identification of viral and host factors with which it interacts (Fig. 4).

Conclusion

The establishment of Arabidopsis as an excellent model host for studying plant-virus interactions has advanced rapidly. The application of genetic and molecular genetic approaches that are feasible with this model plant to studies of plant-virus interactions have been largely successful and the data currently available suggest that studies using Arabidopsis as a host plant will continue to be pertinent. The establishment of numerous Arabidopsis-virus pathosystems and the increasing amount of information becoming available about the basic biology of Arabidopsis will insure that this model host will continue play a major role in elucidating the complex processes that underlie the resistance and susceptibility of plant-virus interactions and the biology of symptom development.

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