Virology 462-463 (2014) 71–80

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

The capsid protein p38 of *turnip crinkle virus* is associated with the suppression of *cucumber mosaic virus* in *Arabidopsis thaliana* co-infected with *cucumber mosaic virus* and *turnip crinkle virus*

Ying-Juan Chen [a,b], Jing Zhang [a], Jian Liu [a], Xing-Guang Deng [a], Ping Zhang [a], Tong Zhu [a], Li-Juan Chen [a], Wei-Kai Bao [b], De-Hui Xi [a,*], Hong-Hui Lin [a,*]

[a] *Ministry of Education Key Laboratory for Bio-Resource and Eco-Environment, College of Life Science, State Key Laboratory of Hydraulics and Mountain River Engineering, Sichuan University, Chengdu 610064, China*

[b] *Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China*

ARTICLE INFO

Article history:
Received 28 February 2014
Returned to author for revisions
9 May 2014
Accepted 27 May 2014

Keywords:
Cucumber mosaic virus
Turnip crinkle virus
Arabidopsis thaliana
Antagonism
Capsid protein

ABSTRACT

Infection of plants by multiple viruses is common in nature. *Cucumber mosaic virus* (CMV) and *Turnip crinkle virus* (TCV) belong to different families, but *Arabidopsis thaliana* and *Nicotiana benthamiana* are commonly shared hosts for both viruses. In this study, we found that TCV provides effective resistance to infection by CMV in *Arabidopsis* plants co-infected by both viruses, and this antagonistic effect is much weaker when the two viruses are inoculated into different leaves of the same plant. However, similar antagonism is not observed in *N. benthamiana* plants. We further demonstrate that disrupting the RNA silencing-mediated defense of the *Arabidopsis* host does not affect this antagonism, but capsid protein (CP or p38)-defective mutant TCV loses the ability to repress CMV, suggesting that TCV CP plays an important role in the antagonistic effect of TCV toward CMV in *Arabidopsis* plants co-infected with both viruses.

Introduction

Natural infection of plants by two or more plant viruses is a common phenomenon and can result in various effects, such as antagonism, synergism or coexistence. Synergism is a type of interaction in which co-infection by two or more different plant viruses can induce more severe symptoms than single infection, and this phenomenon is most often observed in interactions between unrelated viruses (Zhang et al., 2001; Choi et al., 2002). In synergistic interactions, in addition to the disease symptoms, the titers, movement, or both may be enhanced for one or both viruses. For instance, *Potato virus Y* (PVY) has been demonstrated to significantly enhance the replication and symptoms of several viruses, including *Potato virus X* (PVX), as well as *Cucumber mosaic virus* (CMV) in the well-studied PVY/PVX or PVY/CMV interactions (Rochow and Ross, 1955; Goodman and Ross, 1974a, 1974b; Vance, 1991; Vance et al., 1995; Pruss et al., 1997; Ryang et al., 2004; Mascia et al., 2010). Mixed infection of CMV and *Turnip mosaic virus* (TuMV) can induce more severe symptoms in *N. benthamiana* than single infection, but local interference between the two viruses can be detected even in the synergism (Takeshita et al., 2012). In contrast with synergism, mixed infection of two or more viruses can cause different degrees of antagonism (Bennett, 1951; Aguilar et al., 2000). In this phenomenon, the activity of a virus in a plant prevents or significantly reduces the expression of a subsequent challenge virus, which has been shown to be a strategy that can be used to control several viral diseases, including protection of crops from potyviral diseases, as well as CMV (Fulton, 1986; Sherwood, 1987; Aguilar et al., 2000). This phenomenon often occurs in unrelated viruses from different families or two closely related viruses belonging to one genus, including both RNA and DNA viruses, but the mechanism remains elusive (Kurihara and Watanabe, 2003; Owor et al., 2004; Kamei et al., 1969; Otsuki and Takebe, 1976).

Several mechanisms have been proposed to explain interactions between viruses. It is well established that in plants, multiple regulatory and defensive reactions are mediated by RNA silencing, which is a sequence-specific host defense mechanism against viral invaders (Brodersen and Voinnet, 2006; Voinnet, 2009). To combat this major line of plant defense, viruses have generally evolved various viral suppressors of RNA silencing (VSRs) that have distinct modes of action in the RNA silencing machinery of host plants (Voinnet et al., 1999). Many VSRs have been demonstrated to disturb the host gene-silencing machinery and induce various

* Corresponding authors. Fax: +86 28 85415389.
E-mail addresses: xidh@scu.edu.cn (D.-H. Xi), hhlin@scu.edu.cn (H.-H. Lin).

http://dx.doi.org/10.1016/j.virol.2014.05.031
0042-6822/

malformed phenotypes and developmental defects when expressed in transgenic plants (Mallory et al., 2002; Chapman et al., 2004; Dunoyer et al., 2004; Zhang et al., 2006; Shiboleth et al., 2007; Lewsey et al., 2007; Siddiqui et al., 2011). In many cases, the interactions between viruses are associated with the function of VSRs. It has been suggested that VSRs have important roles in tissue invasion patterns in mixed virus infections. The class 1 RNase III protein encoded by *Sweet potato chlorotic stunt virus* (SPCSV), which is a VSR, has the ability to break down resistance to *Sweet potato feathery mottle virus* (SPFMV) by eliminating the antiviral defense in sweet potato plants (Cuellar et al., 2009). The strong VSR helper component proteinase (HC-Pro), encoded by PVY, plays a key role in enhancing the accumulation of PVX in mixed infections (Brigneti et al., 1998; Vance, 1991). This synergistic effect also occurs in the interactions between PVX and other unrelated viruses, including *Tobacco vein mottling virus* (TVMV), *Tobacco etch virus* (TEV), and *Plum pox virus* (PPV) (Vance et al.,1995; Sáenz et al., 2001; Yang and Ravelonandro, 2002).

CMV (genus *Cucumovirus*, family *Bromoviridae*) and *Turnip crinkle virus* (TCV) (genus *Carmovirus*, family *Tombusviridae*) belong to different families, and both of them are among the relatively few viruses that are highly virulent on *Arabidopsis* (Van Regenmortel et al., 2000; Cohen et al., 2000). CMV 2b is one of the best characterized VSRs and has complex activities to suppress RNA silencing, control host basal resistances, and operate synergistic interactions with other viruses in both a virus- and a host-specific manner (Palukaitis and García-Arenal, 2003; Ding et al., 1994, 1995; Wang et al., 2004). It is well established that CMV can cause synergistic infections with *Tobacco mosaic virus* (TMV) in tomato and tobacco plants, and 2b protein of a mild strain of CMV (e.g., Kin) alone is sufficient to cause synergistic interaction with TMV, resulting in filiformic leaves which completely lack leaf blades in tobacco (Garces-Orejuela and Pound, 1957; Matthews, 1991; Bazzini et al., 2007; Cillo et al., 2009; Ye et al., 2009; Siddiqui et al., 2011). TCV can cause antagonistic interactions with infection of *Tobacco necrosis virus* (TNV) or CMV (Xi et al., 2010; Yang et al., 2010). In the present study, the interaction between CMV and TCV was investigated. We found that the infection of CMV is strongly suppressed by TCV, and the capsid protein (CP or p38) of TCV plays an important role in the resistance to CMV in *Arabidopsis* plants co-infected with CMV and TCV.

Results

The infection of CMV is strongly suppressed by TCV in Arabidopsis plants

CMV and TCV belong to different families. *A. thaliana* is a commonly shared host for both viruses, in which they can proceed systemic movement and induce markedly different symptoms (Van Regenmortel et al., 2000; Cohen et al., 2000). As shown in Fig. 1(a), in Col-0 plants, CMV infection exhibited moderate stunting with reduced petioles, and the newly emerging leaves were strongly distorted and clustered, whereas TCV induced strong symptoms such as obvious chlorosis and then progressed to severe vascular wilt and plant death. However, when CMV and TCV (T+C) were simultaneously inoculated onto the same leaf of *Arabidopsis* plants, the plants only developed strong chlorosis in the inoculated leaves and upper leaves which were the typical TCV-induced symptoms at 15 days post inoculation (dpi). A similar phenomenon was also observed in the sequential inoculations with CMV 3 days after TCV (T-C), indicating that CMV-induced symptoms are strongly suppressed by TCV. But in the sequential inoculations with TCV 3 days after CMV (C-T), both chlorosis and distorted leaves were observed, showing that symptoms induced by CMV are relatively unaffected in CMV pre-infected plants.

To test the interaction between CMV and TCV at different growth stages in Col-0 plants, viral RNAs extracted from inoculated leaves (IL) and systemic leaves (SL) at 7 and 12 dpi were analyzed by Northern blot. In three repeated experiments, the accumulation of CMV in the T+C or T-C inoculation was much lower as compared with that of single CMV infection at 12 dpi, even was below detection limits of Northern blot analysis at 7 dpi. However, in the C-T inoculation, CMV accumulation was similar to that of the CMV single infection in IL but was slightly lower in SL at 7 or 12 dpi, indicating that the systemic movement and replication of CMV is slightly suppressed by TCV (Fig. 1b–e). Subsequently, we analyzed the accumulation of TCV in CMV and TCV co-infected Col-0 plants. As shown in Fig. 1(b, c), at the early stage of mixed infection, the accumulation of TCV fluctuated somewhat. It may be that the level of antiviral defense against TCV varies during the course of infection. But at 12 dpi, the RNA levels of TCV in various inoculations were enhanced to a similar level in both IL and SL (Fig. 1d, e). These results are consistent with the symptoms induced by the two viruses, showing that the replication of TCV is not negatively affected by CMV, whereas TCV causes strong suppression to the replication and systemic movement of CMV in the T+C or T-C inoculation but mild suppression in the C-T inoculation. It is possible that TCV provides effective resistance against the infection of CMV in *Arabidopsis* plants co-infected with CMV and TCV, but this antagonistic effect can be overcomed by delaying the introduction of TCV for three days.

The relative locations on the plants of CMV and TCV inoculations affect the degree of the antagonistic effect

It has been shown that the relative locations of Fny-CMVΔ2b and Fny-CMV inoculations in tobacco plants affect the degree of cross-protection (Ziebell et al., 2007). Therefore, experiments were conducted in which Col-0 plants were co-inoculated with CMV and TCV on different leaves. Symptom development was monitored for 15 days after the first inoculation. When CMV and TCV were co-inoculated on different leaves of the same plant, the plants developed obviously different disease symptoms compared with co-inoculation on the same leaf. As shown in Fig. 2(a), in the T+C or T-C inoculation, the plants not only developed strong chlorosis, which was similar to the symptoms of TCV alone, but also exhibited mild distorted and clustered phenotypes, comparable to the symptoms of CMV alone. To further confirm this result, the RNA levels of TCV and CMV in SL were analyzed by Northern blot, and the accumulation levels of TCV in doubly infected Col-0 plants were found to be similar to those of plants singly infected with TCV at 7 dpi (Fig. 2b, c) and then increased to higher accumulation levels at 12 dpi (Fig. 2d, e). In contrast, at the early stage of infection, they were detectable but lower CMV accumulation in the T+C inoculation, which was not detected when CMV and TCV were inoculated on the same leaf (Fig. 2b, c). Five days later, although the accumulation levels of CMV in various mixed inoculations were all significantly increased, the titers were still much lower than those of CMV single infection (Fig. 2d, e). These results suggest that the systemic movement and replication of CMV in *Arabidopsis* plants is suppressed by TCV when the two viruses are inoculated on different leaves but the degree of antagonistic effect is less than when they are inoculated on the same leaf.

Host plants affect the interaction between CMV and TCV

The model plants *A. thaliana* and *N. benthamiana* are well-adapted plant hosts of CMV and TCV (Qu and Morris, 1999; Hou et al., 2011).