Virology 460-461 (2014) 91–99

Contents lists available at ScienceDirect

Virology

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

Differential tropism in roots and shoots infected by *Citrus tristeza virus*

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ARTICLE INFO

Article history:
Received 17 March 2014
Returned to author for revisions
6 April 2014
Accepted 19 April 2014
Available online 29 May 2014

Keywords:
Citrus tristeza virus
Tropism
Movement
Resistance

ABSTRACT

Virus tropism is a result of interactions between virus, host and vector species, and determines the fate of an infection. In this study, we examined the infection process of *Citrus tristeza virus* (CTV) in susceptible and resistant species, and found that the tropism of CTV is not simply phloem limited, but tissue specific. In resistant species, virus infection was not prevented, but mostly restricted to the roots. This phenomenon was also observed after partial replacement of genes of one CTV strain from another, despite both parental strains being capable of systemic infection. Finally, the roots remained susceptible in the absence of viral gene products needed for systemic infection of shoots. Our results suggest that all phloem cells within a plant are not equally susceptible and that changes in host or virus may produce a novel tropism: restriction by the host to a location where further virus spread is prevented.

Introduction

The fate of a virus infection relies on two interrelated processes, viral tropism, the cell types within a host that become infected, and viral movement, the means by which the virus moves between infected and uninfected cells. Both processes rely on the precise interaction of virus- and host-specific factors (Johnson and Huber, 2002; Marsh and Helenius, 2006), the absence of which prevents or limits the progress of an infection.

In animals, tropism is determined by recognition between the virus and specific cell-surface receptors for entry (Dimitrov, 2004) and the availability of host enyzmes and proteins to allow replication and assembly (Yuh and Ting, 1993). These viruses may be pantropic (Tashiro et al., 1988), infecting many cell types or, more commonly, limited to specific cell types (Yuh and Ting, 1993). In contrast, plant viruses are confronted with arguably fewer cell types than animal viruses, as all plant organs are comprised of three basic tissues: dermal tissue, vascular xylem and phloem tissue, and ground tissue which includes photosynthetic parenchyma, supporting collenchyma, and structural sclerenchyma cells. Tropism is a term that is rarely applied to plant viruses as most, if the criteria for animal viruses were applied, would be classified as pantropic; capable of infecting a combination of mesophyll and vascular parenchyma, cambium, vascular phloem, and epidermal cells, the latter being the point of entry for many insect transmitted viruses (Carrington et al., 1996). Less frequently infected are seed embryos, pollen, or cells of the apical meristem (Esau, 1967). More rarely, plant viruses infect few cell types, such as only epidermal and meristem tissue with no systemic movement (Bastianel et al., 2010), or are limited to phloem-associated cells. The latter tropism is generally understood to be a restriction of virus exit from the vascular system (Leisner et al., 1993; Carrington et al., 1996; Morra and Petty, 2000) rather than limitation of permissiveness, the ability to replicate in a cell, because even though they do not infect mesophyll tissue of intact plants, and indeed have no need to do so as they are transmitted by phloem-feeding Hemipterans (Peter et al., 2009), many phloem limited viruses have been shown to replicate in mesophyll protoplasts (Barker and Harrison, 1982; Albiach-Marti et al., 2004). Additionally, some have been shown to spread beyond the phloem into mesophyll cells when complemented with mesophyll infecting viruses (Carr and Kim, 1983).

Inextricably linked with tropism is virus movement, the ability to move from infected to uninfected cells. In plants, this occurs by two distinct mechanisms: cell-to-cell and long-distance movement. In the former, virally encoded movement proteins interact with host structures to transit intercellular channels, such as plasmodesmata, to effect the transfer of viral RNA, replication complexes or intact virions (Oparka and Turgeon, 1999; Morra and Petty, 2000; Kawakami et al., 2004). Whereas animal viruses use a myriad of mechanisms to move between cells, including exocytosis of enveloped virions, transit of adherens junctions, fusion of infected and uninfected cells, or transfer via actin microvilli (Johnson and Huber, 2002), plant virus cell-to-cell movement mechanisms focus solely on transit through plasmodesmata (Schoelz et al., 2011) as plant viruses have easier, though not unimpeded, access to neighboring cells due to symplastic continuity between neighboring cells of different function (Oparka and Turgeon, 1999; Morra and Petty, 2000), and thus rarely need

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http://dx.doi.org/10.1016/j.virol.2014.04.035
0042-6822/

to cross the membrane. Requirements for long-distance movement in plants on the other hand, are less well understood. In long distance movement, viruses enter the phloem sieve elements, which, despite being discrete cells, essentially form a continuous pathway for the movement of carbohydrates throughout the plant. The virus is then translocated some distance, which may be anywhere between a few cells to several meters, followed by the virus exiting the sieve element into a companion or phloem parenchyma cell; this may be considered analogous to animal virus movement through the nervous or circulatory system. Exit from the phloem can in turn be followed by limited or extensive cell-to-cell movement (Leisner et al., 1993; Carrington et al., 1996; Peter et al., 2009). In the case of phloem-limited viruses, there is limited cell-to-cell movement following long-distance movement (Carrington et al., 1996); the virus rarely moves beyond a small cluster of cells making up the phloem parenchyma (Folimonova et al., 2008).

Systemic infection of a host requires that cells be both susceptible, in that the virus is able to transit any physical barriers present and enter the cell, and permissive, supporting virus replication. Plants can restrict or prevent one or both processes, resulting in resistance. Long-distance movement mechanisms are a frequent target of host resistance, preventing the systemic spread of the virus, though not cell-to-cell (Fuentes and Hamilton, 1993; Derrick and Barker, 1997); even in non-host species, it is common to find that viruses can infect and move within inoculated leaves (Holmes, 1938). In contrast, virus replication is rarely prohibited despite RNA silencing being ubiquitous as a defense mechanism in plants (Waterhouse et al., 2001), and it has often been observed that viruses can replicate and assemble in protoplasts of resistant species in which they cannot systemically infect (Sulzinski and Zaitlin, 1982; Albiach-Marti et al., 2004).

One virus–host system in which the infection process is poorly understood is *Citrus tristeza virus* (CTV) in citrus and citrus relatives of the family *Rutaceae* (Moreno et al., 2008). This large positive-sense ssRNA virus exhibits a phloem-limited tropism, that unlike more well-examined viruses, moves primarily by long-distance movement with only limited cell-to-cell movement, infecting only a portion of phloem-associated cells. Host species, however, have considerable influence on the extent of CTV infection. In the more susceptible hosts, long-distance movement with limited cell-to-cell movement is observed, resulting in infection clusters consisting of 10–15 phloem cells, whilst in less susceptible hosts, the efficacy of long-distance movement is reduced and almost no cell-to-cell movement is observed, resulting in the infection of only scattered single cells (Folimonova et al., 2008). To infect a host, CTV requires the quintuple gene block common to all closteroviruses (Satyanarayana et al., 2000), and the presence of the three virally-encoded suppressors of silencing, p25, p20 and p23 (Lu et al., 2004; Tatineni et al., 2011), the latter suggesting that host RNAi processes restrict virus movement. In addition, CTV possesses three genes, p33, p13 and p18, which are not needed for infection of more susceptible hosts, but were proposed to have been acquired by the virus to extend its host range as they are required for systemic infection of select species (Tatineni et al., 2011), and by inference appeared to be additional gene products necessary for virus movement.

Within the *Rutaceae*, there are numerous species that have been reported to be resistant to CTV, including *Poncirus trifoliata*, *Severinia buxifolia*, *Atalantia ceylanica*, and hybrids thereof (Garnsey et al., 1987; Yoshida, 1996). Additionally, some commercial citrus cultivar and rootstock species have been shown to be differential, supporting the infection of specific CTV strains whilst excluding others (Garnsey et al., 1996; Harper et al., 2010). Most of these species were assessed as resistant on the basis of failure to detect infection in flush tissue of shoots after experimental inoculation (Garnsey et al., 1987; Yoshida, 1996). Yet, this assumes that the ability of a virus to infect is uniform throughout all host tissues. While the phloem tissue network extends throughout all the major organs of a plant, individual cells are likely responding to positional clues from their neighbors and have different gene expression profiles, and potentially, physical properties (Sjolund, 1997; Oparka and Santa, 2000).

In this study we discovered that the tropism of CTV in citrus is not simply phloem limited, but tissue or organ specific, and that the same biological phenomena, differential infection of roots and shoots, may be achieved under markedly different circumstances. We found that roots of what were considered resistant hosts could be infected at a level comparable to that of known susceptible species, despite an inability to infect shoot tissue. This was found to be strain-specific, as some isolates were capable of systemic infection of a given host, whilst others were limited to the roots, suggesting host-specific adaptation of CTV strains and hence, genes. Infection of roots but not shoots was also observed in this study in hosts that, while not known to be resistant, had previously been shown to be differentially susceptible to different strains (Garnsey et al., 1996; Weng et al., 2010). Partial replacement of genes of one strain from another was not found to overcome tropism limitations but instead reduced infectivity, limiting one hybrid to the roots whereas both parental strains were capable of systemic infection, suggesting that the infection process requires the interaction of co-evolved proteins. Finally, we found that the roots of citrus hosts remained susceptible to infection in the absence of viral gene products needed for infection of shoots (Tatineni et al., 2011). These data cumulatively indicate that the roots of citrus are more susceptible to infection than shoots, and each provides a window into the infection process of CTV and the interaction between virus and host.

Table 1
Primers used for the real-time RT-qPCR amplification and quantification of CTV isolates in this study. CTV primer locations are as per the T36 and T68 references sequences given in Harper (2013).

Primer/probe	Orientation	Sequence (5′-3′)	Location	Notes
Common RT Reverse	−	GCAAACATCTCGACTCAACTACC	10885–10907	Antisense primer for all strains
T36-RT-F	+	ACCTCGGACAAGCGGGTGAATT	10817–10838	T36 sense primer
T36-RT-Probe	+	6-FAM-AGCAACCGGCTGATCGATTGATT-BHQ1	10839–10861	T36 strain-specific probe
T68-RT-F	+	CGATGGTCAAGCGGACGACTT	10780–10800	T68 sense primer
T68-RT-Probe	+	6-FAM-AGCGACAGGCTGATGGTTTGTTCA-BHQ1	10839–10862	T68 strain-specific probe
ACTB-F	+	GTTGCCATTGGTTGGTATTTGATAC	N/A	ACTB reference gene
ACTB-R	−	CGTCGACTGCCATTCCAGAT	N/A	
ACTB-Probe	+	6-FAM-TGGTCGATGATTTGTCCGATTCACA-BHQ1	N/A	
GAPDH-F	+	TGGCGACCAAAGGCTACTC	N/A	GAPDH reference gene
GAPDH-R	−	TTGCCGCACCAGTTGATG	N/A	
GAPDH-Probe	+	6-FAM-TGCTAGCCACCGTGACCTCAGG-BHQ1	N/A	