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Association of Selective Deposition of (1→3)-β-Glucan in Floral Tissues with Restricted Movement of Turnip Vein-Clearing Virus in *Arabidopsis*: A Possible Mechanism for Non-Seed Transmission

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Abstract: In this research we used *Turnip vein clearing virus* (TVCV) to study viral non-transmission in *A. thaliana* as a step toward developing a management strategy against seed transmissible viruses. Confocal laser scanning, light, transmission and scanning electron microscopy and immunolabeling techniques were used to study the pattern of the viral movement in reproductive parts of the systemically infected plant. The virus was located in all parts of the flower except ovules and pollen grains. The viral movement was thus confined to the junction of ovule and ovule stalk in the siliques and outside the pollen grains in anther tissue. Subcellular localization of (1→3)-β-glucan (callose) indicated selective deposition of this substance in plasmodesmata at the junction of ovule and ovule stalk as well as exine layer of pollen grains. The callose deposition was not observed in uninoculated and mock inoculated control plants. We propose that the observed (1→3)-β-glucan may be involved in blockage of viral movement into ovules and pollen grains and result possibly in non-seed transmission of TVCV in *A. thaliana*.

Key words: Callose, ovules, pollen, TVCV, microscopy

INTRODUCTION

Virus transmission through seeds was once considered a rare occurrence (Mandahar, 1981; Walker, 1969). Current interest in seed transmission of plant viruses dates back to the middle of the last century when Smith (1951) listed eight viruses as seed transmissible. By 1981, one hundred and thirty two viruses had been identified as seed transmissible. By the early nineties, about 20% of plant viruses were recognized as seed transmissible (Matthews, 1991; Mink, 1993).

Seed transmission is accomplished either by direct invasion of embryo via the ovule or by indirect invasion of the embryo, mediated by infected gametes (Maule and Wang, 1996; Wang and Maule, 1994). Seed transmission via embryo or by way of infected gametes could operate simultaneously in a host as in the case of Barley Stripe Mosaic Virus (BSMV) infection of barley (Mandahar, 1981). A virus such as BSMV may be primarily seed transmissible (Edwards, 1995) while other such as barley mosaic virus may be transmitted by multiple means including seed transmission (Caroll, 1997). Some viruses may be transmitted through seeds in one host, while

others may not be seed transmissible in the same host. For example, *Tobacco ringspot virus* (TRSV) and Soybean mosaic virus (SMV) are seed transmissible in soybean (Athow and Bancroft, 1959; Kendrick and Gardner, 1924; Hartman *et al.*, 1992), while *Bean pod mottle virus* (BPMV) and *Bean yellow mosaic virus* (BYMV) are not considered to be seed transmissible in soybean (Sinclair, 1982). On the other hand, a virus that is seed transmissible in one host may not transmit through seeds in another host. *Peanut mottle virus* (PMV) is seed transmissible in peanut, navy bean and cowpea, but is not seed transmissible in soybean (Kuhn and Demski, 1984). Seed transmission of plant viruses was reviewed extensively by Maule and Wang (1996). These reports provide strong evidence for significant increase in research efforts on seed transmission of viruses since the middle of the last century. However, the majority of those efforts have concentrated on identification of seed-transmissible viruses and much information about mechanism(s) of seed transmission remains to be elucidated (Wang and Maule, 1994).

Viral movement in host plants is an important factor for development of disease. In plants, plasmodesmata

form a symplastic continuum (Ghoshroy *et al.*, 1997; Lucas, 1999) and the plant viruses utilize this continuous, uninterrupted pathway to spread into developing tissues. Following initial infection, plant viruses move through plasmodesmata to spread cell-to-cell until they reach the vasculature (Ghoshroy *et al.*, 1997). Once the viral particles cross the boundary between mesophyll tissue and vascular tissue, they move through the phloem and finally exit into leaves away from the initial site of infection to establish disease.

The tobamovirus, *Turnip vein-clearing virus* (TVCV) has been shown to infect *Arabidopsis thaliana* (Lartey *et al.*, 1997; 1993). Ever since, this virus has emerged as an ideal tool to study host-pathogen interactions in *A. thaliana* (Melcher, 2003). TVCV normally spreads throughout the plant within a week of mechanical inoculation of rosette leaves. The presence of viral coat protein can be detected in the uninoculated cauline leaves using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) or western blotting. A positive detection indicates establishment of systemic infection (Lartey *et al.*, 1997). The virus was detected and observed in all vegetative and reproductive tissues of infected *A. thaliana* plants in this article. The reproductive organs of a systemically infected plant (petals, siliques and seeds) also showed presence of viral coat proteins. However, the seeds collected from an infected plant produced virus-free plants, indicating non-transmission of TVCV through seeds of diseased *Arabidopsis* plants.

As stated before, most research efforts on seed transmission of plant viruses have focused on a relationship in which a virus is transmitted through seeds in plant hosts under consideration. Thus far, available research reports show that both seed-transmissible and non-seed-transmissible viruses have some similarities in their pathogenesis, which results in disease development in their respective plant hosts. From infection site, the virus moves cell-to-cell in both hosts and is followed by systemic spread to other sites, resulting in development of disease. However, the seed-transmissible virus reach the host seeds so as to be transmitted to the next generation, as expected, while non-seed-transmissible virus often succeeds in using a secondary host or vectors such as insects and other organisms for transmission. Understanding the ability of some plant hosts to resist seed transmission may be crucial for developing management strategies (breeding, gene manipulation, etc.) against seed-transmissible viruses. As a first step toward that objective, we present an electron microscopic investigation of movement and localization of TVCV in mature reproductive structures of *A. thaliana*. This study

reveals some possible mechanisms by which some host plants restrict movement of the virus into their reproductive structures to protect their progeny.

MATERIALS AND METHODS

The experiments were conducted in green houses and research facilities at New Mexico State University, Las Cruces NM and The University of South Carolina, Columbia, SC between 2004 and 2007.

Infection of *Arabidopsis* by TVCV: *Arabidopsis thaliana* (ecotype Columbia) plants were grown from seeds. At the onset of bolting, two rosette leaves from each of the eight replicate plants were mechanically inoculated using a viral suspension, which consisted of purified virions in 1% K_2HPO_4 and Celite (Sigma Chemical, MO) at 3 mg mL⁻¹. The inoculation was done using the method as described in Lartey *et al.* (1997). Controls consisted of mock inoculated (suspension solution without virus) or uninoculated *A. thaliana* plants. To evaluate possible seed transmission of TVCV, seeds from previously infected in *A. thaliana* were also planted. All plants were maintained in a growth chamber with an ambient temperature of 20°C and 14 h photoperiod and observed for development of symptoms.

Systemic movement of TVCV by coat protein assay: Eight days after inoculation, two uninoculated lower rosette leaves, upper cauline leaves, flowers and fruit pods were randomly harvested from each of four inoculated, mock inoculated and uninoculated plants and analyzed by SDS-PAGE. The SDS-PAGE was carried out to determine systemic movement of TVCV infection through the detection of viral Coat Protein (CP), an indicator for presence of virus following the method as described in Lartey *et al.* (1997).

Immunofluorescence microscopy: Closed flower buds, four from each of systemically infected, mock inoculated and uninfected control plants, were randomly harvested and fixed in 4% phosphate buffered (pH 7.2) paraformaldehyde for three hours. The samples were then washed several times in phosphate buffer and dehydrated for 10 min each in a graded series of ethanol (25, 50, 75 and 100%). The samples were left in 100% ethanol overnight at 4°C. The samples were then treated with a graded series of xylene (25, 50, 75 and 100%) for 1 h at room temperature. Following the xylene treatment, the samples were infiltrated with a graded series of paraffin (Paraplast Plus; Electron Microscopy Sciences, PA) mixed in xylene (25, 50, 75 and 100%) for 2 h each, at 60°C. The

samples were left in 100% Paraplast Plus overnight at 60°C and then embedded in pure paraffin. The paraffin embedded flower bud samples were sectioned longitudinally at a thickness of 6 µm using a Zeiss Microm HM330 rotary microtome and collected on glass slides. The tissue sections were deparaffinized in xylene and rehydrated for immunolabeling. The sections were then incubated overnight at 4°C in anti-TVCCV CP primary antibody (polyclonal), diluted 1:1500 in phosphate buffer. The sections were then washed in phosphate buffer and incubated for one hour at room temperature in Cy5-conjugated anti-rabbit IgG, diluted 1:50 in phosphate buffer (Jackson ImmunoResearch Lab, PA). The sections were then washed and stored at -20°C in Slowfade-Light antifade (Molecular Probes, OR). The labeled sections were viewed by a Bio-Rad MRC 1024 confocal laser scanning microscope at 647 nm laser line and images were recorded.

Light and Electron microscopy: Closed flower buds from systemically infected plants, mock inoculated and uninfected control plants each, were fixed in 2.5% cacodylate buffered (pH 7.2) glutaraldehyde for three hours at room temperature. The fixed samples were then washed in cacodylate buffer and postfixed in 1% osmium tetroxide for an hour at 4°C. The tissue samples were dehydrated in a graded series of ethanol (50, 70, 80, 90 and 100%) for 10 min each and treated with acetone twice for 5 min each. Following the acetone treatment, the samples were infiltrated with 1:1 (vol/vol) mixture of acetone and Spurr's low viscosity epoxy resin embedding medium (Spurr, 1969) and incubated overnight on a rotator at room temperature. On the following day, the samples were infiltrated further with 1:3 (vol/vol) mixtures of acetone and Spurr's media for 6 h on a rotator at room temperature followed by an overnight incubation in 100% Spurr's at room temperature. Finally, the samples were embedded in 100% Spurr's and cured in a 60°C oven for 24-48 h.

Thick longitudinal sections (0.5-1.0 µm) of resin embedded flower buds were made using a Sorvall MT2B ultramicrotome, stained with epoxy tissue stain (Electron Microscopy Sciences, PA) and photographed in a Zeiss Axioplan microscope. Ultrathin tissue sections (70-80 nm) of the same blocks used for thick sectioning were made using a Microstar (Microstar Technologies Inc, TX) diamond knife. The sections were stained with uranyl acetate (Epstein and Holt, 1963), followed by lead citrate (Reynolds 1963) and examined under a Hitachi H7000 TEM (Hitachi High Technologies, CA).

Ovaries were examined to determine if ovules of infected plants remained attached to the ovary. Young, fresh ovaries from infected and control plants were split

open longitudinally and observed under a Hitachi S3200N variable pressure Scanning Electron Microscope to observe the arrangement and attachment of ovules inside the ovary. The specimens were observed in the variable pressure mode using a backscattered electron detector without any prior chemical fixation and digital images were captured using PCI image acquisition software.

Immunocytochemistry: The flower bud blocks used for thin and thick sections were also used for on-grid immunocytochemistry. Ultrathin tissue sections were collected on formvar coated nickel grids. The sections with the grids were treated with Tris buffered saline containing Triton X100 (TBST, pH 7.6) for 5 min. The sections were then incubated in anti-TVCCV coat protein primary antibody made in TBST, pH 7.6 (dilution 1:1500) overnight at 4°C. The grids were then washed in TBST, pH 7.6 and conditioned in TBST, pH 8.2 for 5 min. The grids were incubated for one hour at room temperature in 12 nm gold conjugated anti rabbit secondary antibody (Jackson ImmunoResearch Lab, PA) at the dilution of 1:50. The grids were washed in TBST pH 7.6 followed by one wash in water. The grids were air dried, stained with uranyl acetate and lead citrate and viewed under the TEM. Three random tissue sections were chosen from each of the three infected and uninfected plants. From each of the above tissue sections, five random areas were examined.

(1→3)-β-glucan localization: Flower bud blocks used above were also used to localize callose in the ovary. Ultrathin tissue sections, collected on formvar coated nickel grids, were labeled with callose specific anti (1→3)-β-glucan monoclonal antibody (Meikle *et al.*, 1991) (Biosupplies Australia Ptv. Ltd., Parkville, Australia). The primary antibody was used at 1:50 dilution, followed by 12 nm gold conjugated anti-mouse secondary antibody (Jackson ImmunoResearch Lab, PA) at the dilution of 1:50. The grids were air dried, stained with uranyl acetate and lead citrate and viewed under the TEM. Three random tissue sections were chosen from each of the three infected and uninfected plants. From each of the above tissue sections, five random areas were examined.

RESULTS

Infection of *Arabidopsis* by TVCCV: Symptoms of TVCCV infection were observed in inoculated *A. thaliana* plants. The symptoms were generally observed between 8-10 days post inoculation and were characterized by drooping of the whole plant when compared to an uninfected plant (Fig. 1). Furthermore, the infected flower

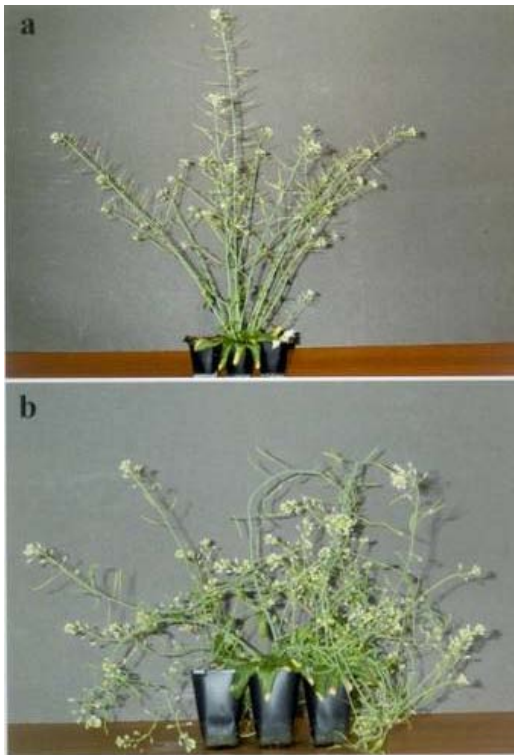


Fig. 1: TVCV infection in *A. thaliana* var Columbia, (a) Uninfected (control) plant showing erect branches with slight curvature and (b) Systemically infected plant showing crawling flowering stalks

stalks failed to grow upright within a few days post inoculation (Fig. 1b), while the mock inoculated (not shown) and uninfected plants (Fig. 1a) had multiple flowering stalks that grew upright with only a slight curvature. The severe drooping of diseased plants generally resulted in the flowering stalks sagging to the ground. Like uninfected controls, plants grown from the seeds of previously infected plants also showed no symptoms of TVCV infection (not shown).

Systemic movement of TVCV by coat protein assay: To corroborate systemic movement of TVCV, eight days after inoculation, uninoculated lower rosette leaves, upper cauline leaves, flowers and seed pods, were assayed for the presence of virus. The virus was detected by the presence of viral coat protein (17.5 kD) in sample plant tissues using SDS-PAGE. The virus was not observed in uninfected control plants, as indicated by the absence of viral coat protein bands in the gels. On the other hand, the infected plants revealed a considerable accumulation of viral coat protein in the sample tissues (Fig. 2a). No viral

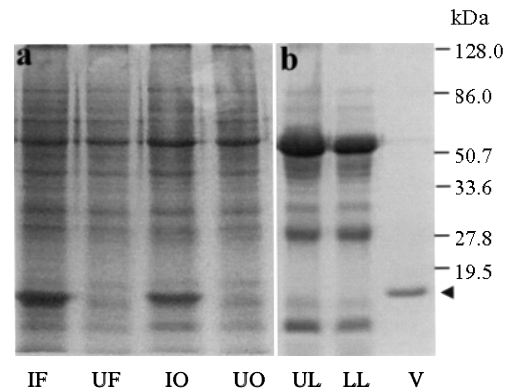


Fig. 2: Presence of TVCV coat protein in leaves and whole seedpods of *A. thaliana*, detected by SDS-PAGE of extracts. Lane V represents purified coat protein and arrowhead indicates position of TVCV coat protein. (a) Leaves and ovaries from an infected plant showed presence of viral coat protein; IF infected flower, IO infected ovary. Leaves and ovaries from an uninfected plant showed absence of viral coat protein; UF uninfected flower, UO uninfected ovaries and (b) Leaves of a plant, germinated from seeds of a systemically infected plant, showed absence of viral coat protein. UL upper cauline leaf, LL lower rosette leaf

coat protein was observed in cauline leaves of plants which were grown from seeds of previously infected plants when assayed three weeks after planting (Fig. 2b).

TVCV movement and localization of TVCV in floral parts: Immunofluorescence microscopy was used for initial determination of viral presence in flower buds. The flower buds from all samples of infected plants showed substantial accumulation of the virus in petals, sepals, exocarp, mesocarp and endocarp-b of the ovary valve, the central partition (septum), between the two joints (replum) of the siliques ovaries (Fig. 3b), anther sacs and anther walls (not shown). However, no fluorescence signal was detected in the space between the innermost cell layer of the septum and endocarp-a of the two ovary valves, indicating absence of virus particles. The flower buds from uninfected (Fig. 3a) and mock inoculated (not shown) plants did not show any strong fluorescence indicating apparent absence of virus.

Light and scanning electron microscopy was then used to determine TVCV movement and localization, within the ovaries, by first examining, longitudinal sections of flower buds. The ovules in the two conjoint ovaries of the siliques were attached to the replum,

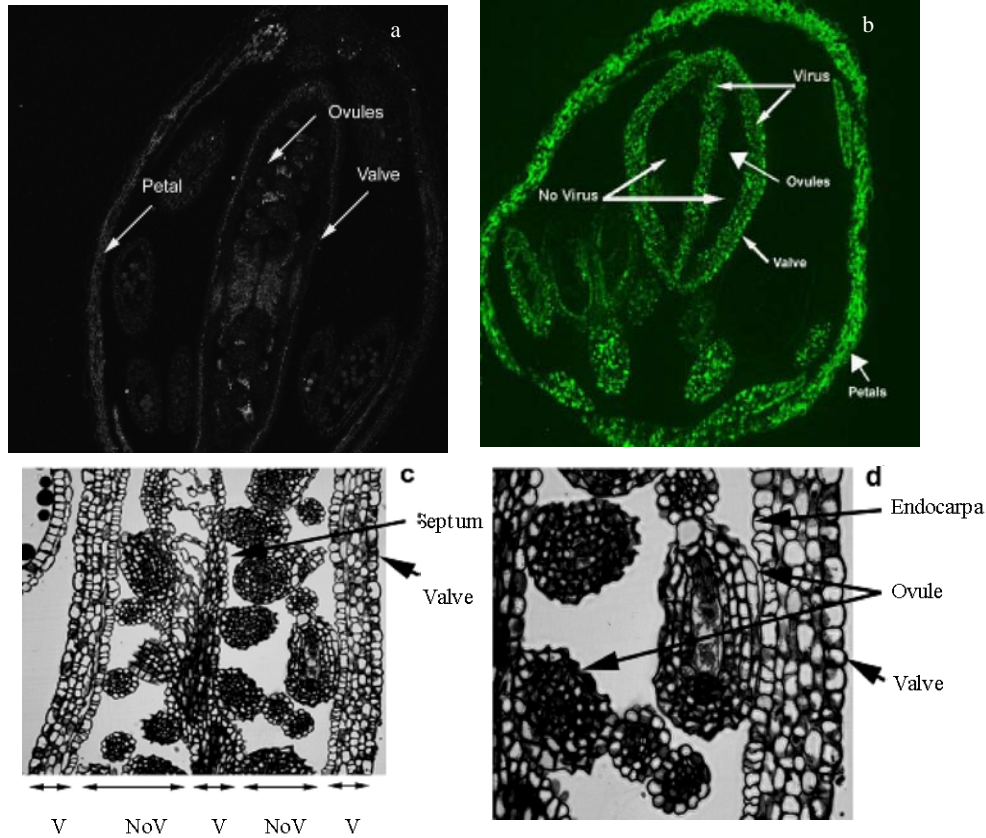


Fig. 3: Fluorescence image of *A. thaliana* flower bud in longitudinal section. The tissue sections were incubated with anti TVCV-CP antibody followed by Cy5 conjugated anti rabbit secondary antibody. Magnification: x300, (a) Uninfected flower bud showing no viral particles. (Few fluorescent spots were visible in the tissue, indicating non-specific background fluorescence). (b) TVCV infected flower bud displaying strong fluorescent signals, which indicated presence of viral particles. The fluorescent signal is visible in many parts of the flower bud (including the ovary), although is absent from the seed and pollen grains. (c) Light microscope images of TVCV infected *A. thaliana* flower bud in longitudinal section showing various compartments with or without virus. V: presence of virus, No V: absence of virus. A low magnification image of *A. thaliana* flower bud section shows the structure of the ovary. The septum and the valves have multiple layers of cells. Magnification: x300 and (d) The high magnification photographic image shows that the ovules are attached to the inner replum wall. Magnification: x500

composed of multiple layers of cells (Fig. 3c). Each ovary had a multi-cell layered valve and joined to each other by a common septum (Fig. 3c, d). A comparison between the immunofluorescence images of infected and uninfected flower buds (Fig. 3a, b) demonstrated presence of the virus at specific locations within the infected flower bud.

Ovaries were further examined for ovule attachment:

Scanning electron microscopy revealed that (Fig. 4a, b) the ovules were connected to the ovary by a funiculus. Examination of the ovary of infected plants, using transmission electron microscopy, showed that the ovule

tissue was filled with electron dense material, whereas the funiculus showed no dense cellular contents (Fig. 5a). Higher magnification images showed the presence of viruses in funicular cells but complete absence of viruses in ovular tissue (Fig. 5b, c). Subcellular localization of TVCV, performed using antibody against viral coat protein, showed the presence of virus particles in the ovary walls, however, no labeled virus particles were observed in ovule tissue (Fig. 6). As with infected plants, ovules in mock inoculated and uninoculated control plants also remained connected to ovary walls and demonstrated no viral particles in any part of the plant (not shown).

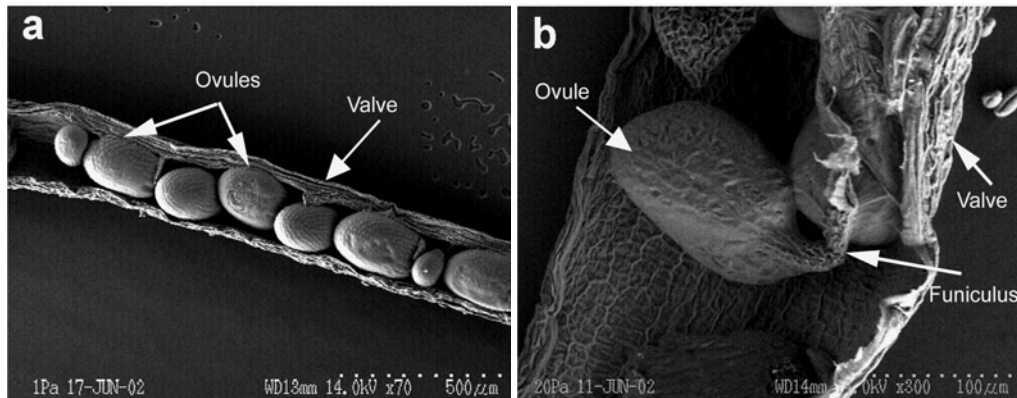


Fig. 4: Scanning electron micrographs of intact ovaries split open longitudinally. The microscope was operated at variable pressure mode to allow introduction of fresh samples without any processing. (a) A low magnification image showing the arrangement of young seeds (ovules) inside the silique and (b) A high magnification image showing the connection of the ovule to the wall of the replum via funiculus (stalk)

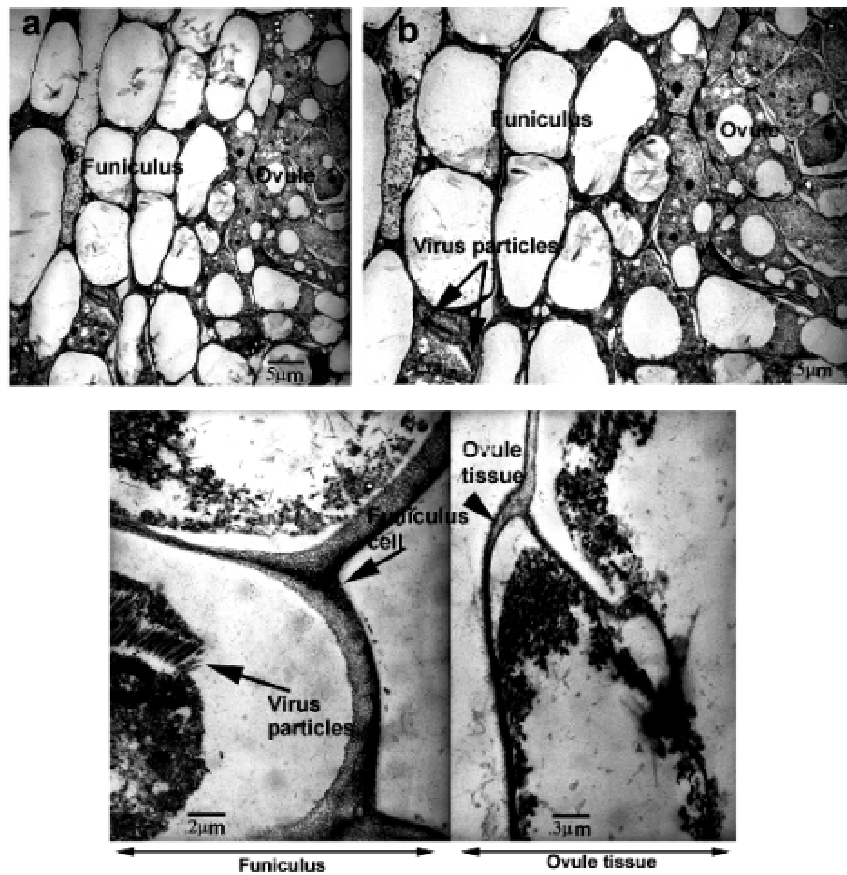


Fig. 5: Transmission electron micrographs of infected ovary in longitudinal section, (a) The low magnification image showing part of the funiculus (stalk) and ovule tissue filled with electron dense material, (b) A more magnified image showing presence of virus particles only in the funicular tissue and (c) A high magnification image of a part of funicular cell showing stacks of viral rods and a part of ovule tissue showing absence of viral particles

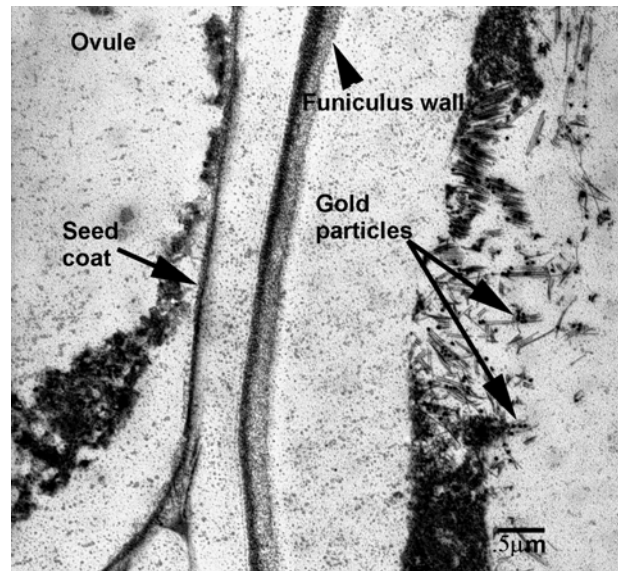


Fig. 6: Immunocytochemistry of ovary tissue. Ultrathin longitudinal sections of ovary were incubated with anti TVCV-CP primary antibody followed by 12 nm gold conjugated secondary antibody. Gold labeled virus particles were present in the funiculus but no labeling was detected in ovule

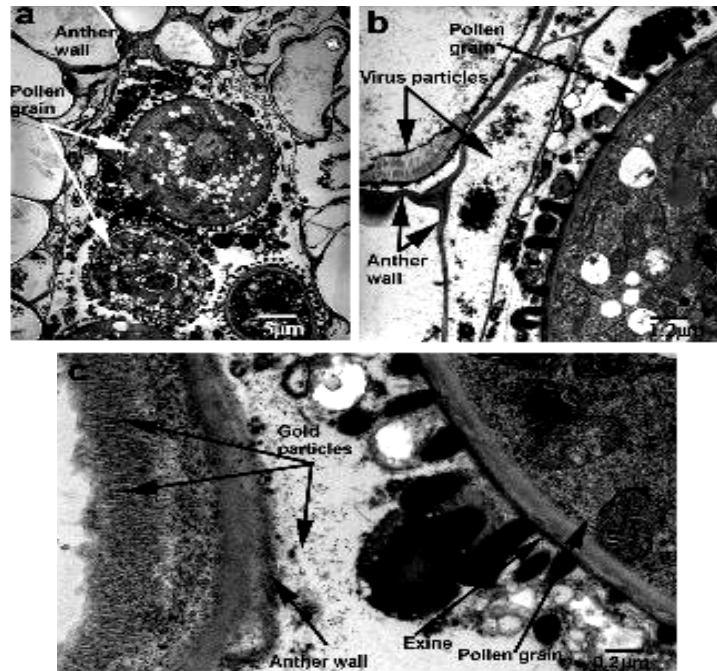


Fig. 7: Transmission electron micrographs and immunocytochemistry of anther tissue from systemically infected *A. thaliana* plant, (a) Longitudinal section of anther showing part of tapetum and pollen grain arrangement. The pollen grains have a thick exine. (b) A higher magnification image of anther tissue from a systemically infected *A. thaliana* plant showing presence of viral particles in the tapetum, while no virus is visible inside the pollen grains and (c) Immunocytochemistry of same tissue (probed with anti TVCV-CP antibody followed by 12 nm conjugated secondary antibody) showing gold labeled virus particles in tapetum. No gold labeling is detected inside the anthers, indicating absence of virus inside pollen grains

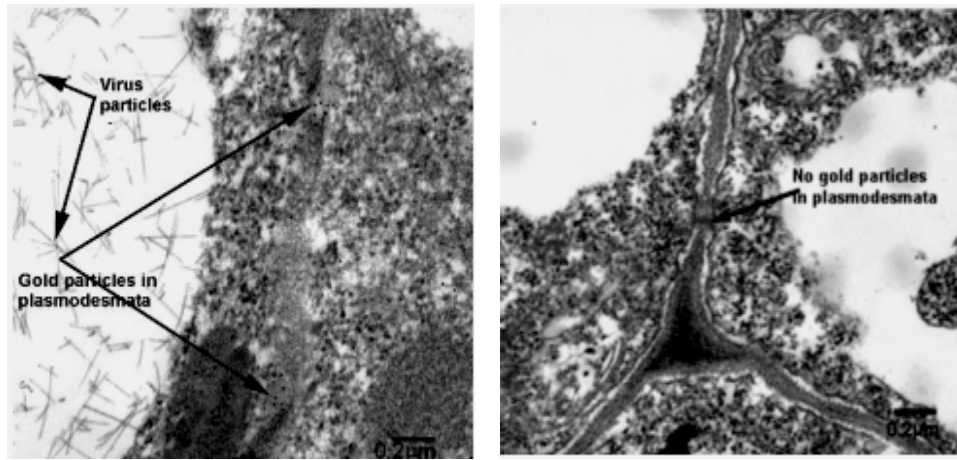


Fig. 8: Callose labeling at the junction of seed and funiculus. Ultrathin longitudinal sections of flower buds from systemically infected plants were incubated with anti-callose antibody followed by 12 nm gold conjugated secondary antibody. (a) Presence of gold particles in the plasmodesmata at the junction of funiculus and ovule indicated deposition of callose. Right side of the image (ovular tissue) shows complete absence of virus, whereas left side of the image (funiculus) shows numerous virus particles and (b) Mock inoculated sample tissues, observed randomly, showed absence of gold particles in the plasmodesmata between funiculus and ovule, thus indicating absence of callose. Viral particles were also absent from funiculus

Examination of pollens for viral particles: Ultrathin sections of anthers revealed the structure of mature pollen grains (Fig. 7a). The pollen grains lie within the tapetum and each had thick exine (Fig. 7a, b). The viral particles are clearly visible in the exine and tapetal cells but absent inside the pollen grains. Subcellular localization of viral coat protein indicated presence of gold labeled viral particles in all parts of pollen sacs except within the pollen grains indicating ability of the virus to penetrate into the exine but is blocked from further entry into the cellular contents of the pollen grain itself (Fig. 7c).

(1→3)-β-glucan localization:

Callose localization in plasmodesmata and funiculus: To understand the restricted movement of virus into ovules, we labeled the longitudinally sectioned TVCV infected flower buds with anti-callose antibody. The plasmodesmata at the junction of ovule and the funiculus, by which the ovule remains attached to the ovary, showed presence of callose deposition (Fig. 8a). The gold particles were only visible at the junction plasmodesmata separating the ovule and the funiculus. Moreover, the virus particles were only visible in the funicular cells and absent in the ovule. Similar labeling in mock inoculated flower buds did not show presence of callose at the junction of the plasmodesmata (Fig. 8b). Flower buds from uninoculated controls also showed no apparent presence of callose (data not shown).

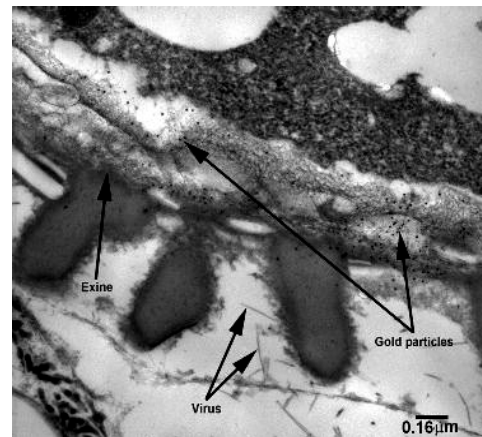


Fig. 9: Callose labeling of anther tissue. Presence of gold particles along the pollen exine, indicating deposition of callose. Virus particles were visible in the tapetum, but not inside pollen grains

Localization of callose in anther tissue: To explore the basis for inhibition of TVCV seed transmission through pollen, anther tissues were examined for potential blockage of viral movement. Longitudinal sections of flower buds labeled with anti-callose antibody were examined under the transmission electron microscope. The exine of the pollen grains demonstrated high level of callose deposition (Fig. 9). Virus particles were only

visible outside the pollen grains, although, the gold particles were restricted to the exine. Pollen grains from mock inoculated and uninoculated flower buds did not show any apparent presence of callose (data not shown).

DISCUSSION

In some plants, seed transmission of plant viruses can play a significant role in spreading the disease from one plant to another. Although TVCV systemically infects *A. thaliana*, yet, it is not seed transmissible. In this study, we observed relatively mild symptoms of TVCV infection in *A. thaliana*, which produced a modest effect on overall fertility and growth of the plant. These observations are consistent with a previous report of TVCV infection in *A. thaliana* (Lartey *et al.*, 1997). In this study, the infection did not appear to be seed transmissible as detected by the SDS-PAGE assays and microscopy (light and electron) of progeny plants grown from seeds of infected plants. We did not observe symptoms, detected viral coat protein or viral particles in progeny plants raised from seeds of infected plants. The results in this study also confirmed a previous finding that TVCV is not transmissible by seeds in *A. thaliana* (Lartey *et al.*, 1997).

To characterize the restriction of viral movement in floral organs of *A. thaliana*, we examined the spread of virus into various compartments of the flower and fruits. We observed that the virus moved relatively easily to sepals, petals and parts of carpels and stamens in infected plants. However, the confocal microscopy and transmission electron microscopy revealed that the virus was unable to move into the developing ovules and the pollen grains of infected plants.

Further examinations showed that the virus had not penetrated beyond the exine of the pollen grain and the ovule-funicular junction. The plasmodesmata at these junctions showed deposition of callose as observed by immunolocalization. The deposition of (1→3)- β -glucan (callose) could explain failure of the viral particles to move beyond these tissues. Callose has been implicated in imparting resistance against viruses by blocking systemic spread of the virus in plants (Collinge and Slusarenko, 1987). There is a consensus that callose, deposited in the neck region of the plasmodesmata, forms plugs which restrict plasmodesmatal movement (Olesen and Robards, 1990). Local deposition of callose may constrict the neck region of plasmodesmata, thus reducing plasmodesmatal trafficking (Botha and Cross, 2000).

Several lines of indirect evidence have also supported the role of callose in a defense mechanism against viruses. It is thought to be deposited between the plasma membrane and the cell wall as part of hypersensitive reaction to viruses. Allison and Shalla

(1974) also proposed that callose acted as a physical barrier that prevented or limited spread of virus. Iglesias and Meins (2000) observed delayed plant viral movement in a Class I β -1, 3-glucanase mutant tobacco plant. They suggested that the absence of callose degrading enzyme in this mutant increased callose deposition and thereby reduced viral movement. Beffa *et al.* (1996) observed decreased susceptibility of viral diseases in β -1, 3-glucanase deficient plants, attributing the resistance to increased deposition of callose in response to infection. On the other hand, local expression of active β -1, 3-glucanase enhanced symptoms of TMV infection in tobacco, which was attributed to increased degradation of callose.

As previously stated, seed transmission is accomplished either by direct invasion of embryo via the seed or by indirect invasion of the embryo, mediated by infected gametes (Wang and Maule, 1994). To gain invasion into the seed of *A. thaliana*, the virus will have to gain entry into one or both gametes. It may have to enter the ovule through the funiculus or via pollen tube during the process of fertilization. Since our plants were mechanically inoculated before emergence of the floral stalk, the infected plants apparently succeeded in blocking the virus from entering either the pollen or the ovule prior to the development of mature flowers.

In *A. thaliana*, TVCV exhibits a rapid systemic movement. The virus is detected in uninoculated leaves within three days after inoculation. This is followed by fast-developing symptoms and systemic infection (Lartey *et al.*, 1997). The virus moves through the vasculature and then unloads into the uninoculated upper leaves to produce systemic infection. In case of flower buds, developing in the presence of the virus within the vegetative tissue, the plant might have selectively blocked the entry of the virus into developing ovules and pollen grains.

A. thaliana is a self fertilizing plant and it is thus critical that the virus be blocked from entry into the seed through the pollen tube. We found considerable amount of virions outside the pollen grains, primarily in the cells of pollen sacs indicating unrestricted movement of viruses through the filament. However, they are unable to penetrate the pollen grains. We also observed what appeared to be selective deposition of callose in the pollen exine indicating its role in blocking viral entry into the pollen. The same phenomenon is visible in the ovary, where selective deposition of callose in the plasmodesmata at the ovule-funicular junction was evident and furthering turn indicated its role in blocking movement of virus. Callose, a (1→3)- β -glucan, has been reported to restrict intercellular transport when deposited between the plasma membrane and the cell wall

(Bucher *et al.*, 2001; Delmer *et al.*, 1993; Iglesias and Meins Jr, 2000). The deposition of callose and inhibition of TVCV movement was recently shown by (Ueki and Citovsky, 2002). In this study, callose accumulation in phloem was indicated to restrict TVCV movement and to restrict systemic infection in tobacco. A β -1, 3-glucanase (callose degrading enzyme) deficient tobacco mutant, showed decreased susceptibility to necrotizing virus infection, due to a substantial increase in callose accumulation in and around necrotic lesions (Beffa *et al.*, 1996). This report demonstrated that increased callose deposition restricted viral movement and resulted in decreased susceptibility. In our study, we also observed a similar phenomenon where selective deposition of callose was associated with inability of TVCV to move into the ovary and the pollen grains. The complete blockage of viral entry into the seed is confirmed by total absence of viruses in the ovule. Furthermore, progeny plants from seeds of infected plants produced virus free plants. Our data provide strong evidence to hypothesize that the inability of TVCV to move into the reproductive structures may be responsible for preventing seed transmission to the seed.

In conclusion, present study presents data to show that TVCV movement in certain reproductive structures of *A. thaliana* is apparently blocked by a selective deposition of callose in the exine of pollen grains and in plasmodesmata at the junction of ovule and its funiculus. This deposition may play a vital role in restricting viral movement into the ovules as well as pollen grains. This blockage is complete and most likely inhibits further spread of viruses into seeds and future plants grown from these seeds. Our results demonstrate a possible host strategy to prevent passage of a pathogenic virus to its progeny. Whether or not, the deposition of the callose to blocks viral movement into these crucial organs is induced, before or during the movement of the viral infection into the vegetative organs, remains to be answered in future research. Induction of callose could also be studied among various gametophytic and sporophytic tissues of the flower bud. Further understanding of a possible cellular signal and its nature of communication to yet to develop gametes remains as a critical question. Future work in this area will shed light on the mechanism of cell-cell interaction in infected plants that impart a protection to the progeny against viruses preexisting in the flower.

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