Vertical transmission of tomato viruses

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Abstract

The vertical transmission of tomato viruses through seeds and pollen is a significant yet often overlooked pathway for the persistence and global spread of these pathogens. This review provides a comprehensive synthesis of current knowledge on the mechanisms, epidemiological implications, and management strategies of vertically transmitted tomato viruses. While recent advances in diagnostic techniques such as high-throughput sequencing (HTS), have improved virus detection, key research gaps remain in understanding the molecular and ecological dynamics of seed and pollen transmission.

The interaction between vertical and horizontal transmission modes complicates virus epidemiology, necessitating an integrated management approach that includes rigorous seed health testing, genetic resistance breeding, and biosecurity measures. Emerging threats, such as resistance-breaking virus strains and the impact of climate change on vector distribution, underscore the need for enhanced surveillance and stronger international regulatory cooperation.

This review highlights the need for interdisciplinary research and collaboration to develop sustainable virus mitigation strategies. Future research priorities include optimizing detection methods, exploring next-generation breeding technologies, and strengthening international biosecurity frameworks to safeguard global tomato production against the growing threat of vertically transmitted viruses.

1. Introduction

Tomatoes (*Solanum lycopersicum*) are one of the most widely cultivated and economically significant crops globally, with an annual production exceeding 180 million tons, according to recent Food and Agriculture Organization (FAO) estimates (FAOSTAT, 2024). They are vital for global food security, valued for their economic importance, and recognized for their nutritional benefits, including being rich sources of vitamins and antioxidants (Akotowanou et al., 2022; Jerca and Smedescu, 2023). Unfortunately, tomato production is increasingly threatened by environmental stresses and plant diseases, which can lead to significant yield losses both during cultivation and after harvest (Chavan et al., 2024; Conti et al., 2023; Kitinoja et al., 2019; Panno et al., 2021; Suleiman et al., 2024). Within this range of challenges, viruses represent one of the most severe threats to tomato production.

Tomato plants are highly susceptible to viral infections, with over 300 known viruses capable of infecting them, many of which can significantly reduce yield and compromise fruit quality (Hančinský et al., 2020; Hanssen and Thomma, 2010; Ong et al., 2020; Rivarez et al., 2021). Recently, high-throughput sequencing (HTS) technologies have expanded the understanding of tomato virology by uncovering a complex virome within tomato crops.

This includes the discovery of novel viruses and viruses not previously associated with tomato, which could impact tomato plant health (Rivarez et al., 2021; Rivarez et al., 2023). HTS has identified not only known RNA and DNA viruses affecting tomatoes but also cryptic viruses whose roles in crop health are not yet fully understood (Rivarez et al., 2021; Temple et al., 2023). This rapid increase in virus detection highlights the expanding diversity of viruses capable of infecting tomatoes and the challenge of managing these infections in a sustainable manner (Ong et al., 2020; Rivarez et al., 2021).

The economic consequences of viral diseases on tomato crops are profound, influencing not only immediate yield but also disrupting production processes and placing a heavy financial burden on growers (Sánchez-Sánchez et al., 2024). For example, mechanically transmitted viruses like tomato brown rugose fruit virus (ToBRFV) and pepino mosaic virus (PepMV) are particularly challenging due to their ability to spread through contaminated tools, seeds, and irrigation water (EPPO, 2024b, 2024c; Mehle et al., 2014; Mehle et al., 2023). ToBRFV can, under certain conditions, cause yield losses of up to 70 % in severely affected greenhouses (EPPO, 2024c), and PepMV, depending on the strain, management strategy, and environmental conditions, leads to visible symptoms such as blotchy ripening and fruit marbling, reducing marketable yields by 50–60 % despite minimal impact on total yield (Agüero et al., 2018; Klap et al., 2020; Spence et al., 2006). Similarly, insect-transmitted viruses such as tomato yellow leaf curl virus (TYLCV) and criniviruses like tomato chlorosis virus (ToCV) and tomato infectious chlorosis virus (TICV), transmitted by whiteflies, cause severe symptoms, with TYLCV capable of causing near-total yield losses in regions with high vector pressure (Li et al., 2022; Tzanetakis et al., 2013). Other viruses, such as the aphid-transmitted cucumber mosaic virus (CMV), which can cause lethal necrosis in the presence of specific satellite RNAs, and the thrips-transmitted tomato spotted wilt virus (TSWV), which can inflict yield losses exceeding 80 % particularly in dense greenhouse plantings, further complicate disease management (Chaisuekul et al., 2003; Díaz-Pérez et al., 2003; Gillespie, 2009; Gitaitis et al., 1998; Li et al., 2020; Masuta, 2014). Viruses like potato virus Y (PVY), CMV, TSWV, and tobacco mosaic virus (TMV) pose additional risks due to their ability to infect multiple hosts, which facilitates their spread in mixed-crop systems (Hančinský et al., 2020). Climate change exacerbates these issues by creating conditions favorable for both viruses and their vectors, leading to more frequent and severe outbreaks (Canto et al., 2009; Jones, 2021; Jones and Naidu, 2019; Regassa, 2021; Tsai et al., 2022; van Munster, 2020).

Managing these challenges necessitates the adoption of integrated strategies that include effective vector management, rigorous sanitation protocols, and the development of virus-resistant tomato cultivars (Hugo et al., 2019; Pasquali

et al., 2015; Sánchez-Sánchez et al., 2024; Tatineni and Hein, 2023). Such measures are crucial for mitigating the immediate impacts of viral infections while also ensuring long-term sustainability in tomato production systems. Moreover, the global trade in contaminated seeds and plant material continues to introduce pathogens into new regions, often triggering localized outbreaks, compounding the difficulties in managing these diseases (Pagán, 2022).

This review focuses on vertical transmission as a unique mechanism of virus spread, whereby viruses are passed from one generation to the next via seeds or pollen, allowing infections to persist throughout the plant's life cycle (Fetters and Ashman, 2023; Pagán, 2022). This mode of transmission enables infected plants to serve as long-term reservoirs for the virus from early developmental stages, facilitating unnoticed spread until symptoms become apparent. Furthermore, vertical transmission interacts synergistically with horizontal transmission mechanisms, amplifying outbreak dynamics and complicating disease management (García-Ordóñez and Pagán, 2024). These factors underscore the critical importance of seed and pollen hygiene in controlling virus spread and preventing the introduction of virus contaminated planting material into new regions.

The review delves into the impact of seed and pollen transmitted tomato viruses on global production, highlighting their dissemination through the international seed trade and their detrimental effects on crop health and yield. It explores how viruses associated with seeds and pollen impact plant vigor and productivity, emphasizing the importance of effective strategies for mitigation, including biosecurity, best practices, and advancements in virus detection and control. The interplay between vertical and horizontal transmission routes is analyzed, revealing their combined influence on the epidemiology of tomato viruses and the challenges they present for integrated management strategies.

By offering a comprehensive overview of vertical transmission, this review aims to inform breeding programs, enhance biosecurity protocols, and support sustainable agricultural practices. Through improved understanding and management of tomato viruses, the goal is to reduce their economic and agricultural impacts on global production systems.

2. Tomato viruses transmitted by seeds and pollen

Seed and pollen transmission of plant viruses are key factors in the spread of agricultural crop diseases, facilitating long-distance dispersal and persistent infections through plant propagation. In tomatoes, virus transmission via seeds has been documented for certain taxa, while evidence for pollen-mediated transmission remains scarce and largely speculative (Table 1). Understanding

Table 1 Viruses known to be transmitted by seeds and/or pollen of tomato.

Family	Genus	Species	Virus name	Abbreviation	Seed transmission (virus localization)	Pollen transmission	Other confirmed important mechanisms of horizontal transmission	Source
Alphaflexiviridae	*Potexvirus*	*Potexvirus pepini*	Pepino mosaic virus	PepMV	Yes (seed coat)	Only horizontal	Mechanical, water	EPPO, (2024b), Fetters and Ashman (2023), Hanssen et al. (2010), Hanssen, Mumford et al., (2010), Ling, (2008), Mehle et al. (2014), Schwarz et al. (2010), Shipp et al. (2008)
Amalgaviridae	*Amalgavirus*	*Amalgavirus lycopersici*	Southern tomato virus	STV	Yes (seed coat, endosperm, embryo)	Not reported	Not reported	Hao et al. (2023), Sabanadzovic et al. (2009)

(continued)

Table 1 Viruses known to be transmitted by seeds and/or pollen of tomato. (*cont'd*)

Family	Genus	Species	Virus name	Abbreviation	Seed transmission (virus localization)	Pollen transmission	Other confirmed important mechanisms of horizontal transmission	Source
Bromoviridae	*Anulavirus*	*Anulavirus PZSV*	Pelargonium zonate spot virus	PZSV	Yes (probably internally localized)	Yes (vertical)	Mechanical	Giolitti et al. (2014), Zinger et al. (2021)
Bromoviridae	*Cucumovirus*	*Cucumovirus CMV*	Cucumber mosaic virus	CMV	Yes (localization unknown)	Likely horizontal, but not proven	Aphids, mechanical	Joshi et al. (2023), Li et al. (2020), Longe et al. (2022)
Bromoviridae	*Ilarvirus*	*Ilarvirus TSV*	Tobacco streak virus	TSV	Yes (endosperm, embryo)	Yes (horizontal and vertical)	Thrips	Card et al. (2007), Sdoodee and Teakle (1987). Sharman et al. (2015)
Bromoviridae	*Ilarvirus*	*Ilarvirus TomNSV*	Tomato necrotic streak virus	TomNSV	Yes (localization unknown)	Not reported	Mechanical	Adkins et al. (2015), Badillo-Vargas et al. (2016)

Geminiviridae	*Begomovirus*	*Begomovirus coheni*	Tomato yellow leaf curl virus	TYLCV	Yes (localization unknown)	Not reported	Whitefly	Kil et al. (2016), Li et al. (2022)
Geminiviridae	*Begomovirus*	*Begomovirus solanumtaiwanense*	Tomato leaf curl Taiwan virus	ToLCTV	Yes (seed coat, probably also internally)	Yes (vertical)	Whitefly	Chang et al. (2022), Weng et al. (2015)
Geminiviridae	*Begomovirus*	*Begomovirus solanumflavusthailandense*	Tomato yellow leaf curl Thailand virus	TYLCTHV	Yes (seed coat, probably also internally)	Yes (vertical)	Whitefly	Chang et al. (2022), Weng et al. (2015)
Secoviridae	*Nepovirus*	*Nepovirus nigranuli*	Tomato black ring virus	TBRV	Yes (probably internally localized)	Not reported	Nematodes	Harrison (1964), Pospieszny Hasiów-Jaroszewska et al. (2020)
Secoviridae	*Torradovirus*	*Torradovirus lycopersici*	Tomato torrado virus	ToTV	Yes (probably internally localized)	Not reported	Whitefly	Amari et al. (2017), Gambley et al. (2010), Pospieszny et al. (2019)

(continued)

Table 1 Viruses known to be transmitted by seeds and/or pollen of tomato. (*cont'd*)

Family	Genus	Species	Virus name	Abbreviation	Seed transmission (virus localization)	Pollen transmission	Other confirmed important mechanisms of horizontal transmission	Source
Tombusviridae	*Tombusvirus*	*Tombusvirus lycopersici*	Tomato bushy stunt virus	TBSV	Yes (localization unknown)	Not reported	Mechanical, soil, probably also water	Nawaz et al. (2014), Tomlinson and Faithfull (1984), Yamamura and Scholthof (2005)
Virgaviridae	*Tobamovirus*	*Tobamovirus fructirugosum*	Tomato brown rugose fruit virus	ToBRFV	Yes (seed coat, rarely endosperm)	Only horizontal	Mechanical, water	Avni et al. (2022), Davino et al. (2020), EPPO (2024c), Fidan et al. (2024), Levitzky et al. (2019), Mehle et al. (2023), Samarah et al. (2021)

Virgaviridae	*Tobamovirus*	*Tobamovirus tomatotessellati*	Tomato mosaic virus	ToMV	Yes (seed coat, rarely endosperm)	Not reported	Mechanical, soil	Broadbent et al. (1965), Ishibashi et al. (2023), Kassim et al. (2019)
Virgaviridae	*Tobamovirus*	*Tobamovirus tabaci*	Tobacco mosaic virus	TMV	Yes (seed coat, rarely endosperm)	Only horizontal	Mechanical	Fetters and Ashman (2023), Ishibashi et al. (2023), Okada et al. (2000), Taylor et al. (1961)
Virgaviridae	*Tobamovirus*	*Tobamovirus maculatessel-lati*	Tomato mottle mosaic virus	ToMMV	Likely, but not proven	Not reported	Mechanical	Fowkes et al. (2022), Schoen et al. (2023), Sui et al. (2017), Tiberini et al. (2022)
Virgaviridae	*Tobravirus*	*Tobravirus tabaci*	Tobacco rattle virus	TRV	Not reported	Yes (vertical)	Nematodes	Card et al. (2007), Cooper and Harrison (1973), Lister and Murant (1967), Visser et al. (1999)
Virgaviridae	*Tobravirus*	*Tobravirus capsici*	Pepper ringspot virus	PepRSV	Likely, but not proven	Likely vertical, but not proven	Nematodes	Camargo et al. (1969), Macfarlane (2010)

the biology of seed and pollen transmission, alongside the specific viruses exploiting these mechanisms, is critical for designing effective virus management strategies and ensuring 'virus-free' seed stock. Moreover, mixed infections in tomato seeds have been observed (Atik and Paylan, 2023), adding another layer of complexity to disease management and emphasizing the need for stringent diagnostic and control measures. It is important to note that most reported seed transmission studies do not follow a standardized process. These can vary from many thousands of seed to fewer than 100 seeds, and from artificially infected seed batches with a high incidence of contamination to those intercepted in trade with lower prevalence of contaminated seeds.

2.1 Seed-mediated transmission

Viruses can persist in various parts of seeds, including the seed coat (testa and tegmen), endosperm, and embryo, with significant implications for their transmission mechanisms (Escalante et al., 2024). External contamination of the seed coat facilitates mechanical spread during germination or handling and can often be mitigated through effective decontamination methods (Escalante et al., 2024). In contrast, systemic infections occur when viruses invade internal seed tissues, such as the endosperm or embryo, often via vascular connections during seed development, enabling systemic transmission to seedlings (Escalante et al., 2024; Pagán, 2022). Only viruses infecting the embryo enable true vertical transmission, as they become directly incorporated into the developing seedling. Although rare among tomato viruses, true vertical transmission has been documented in certain viroids, such as potato spindle tuber viroid (PSTVd). This viroid invades embryos through the ovule or pollen during the development of reproductive tissues prior to embryogenesis, leading to transmission rates of up to approximately 90 % in some tomato cultivars (Matsushita et al., 2018). Unlike surface-localized viruses, those embedded within the endosperm or embryo cannot be eliminated through external decontamination methods, underscoring the importance of understanding their specific localization to develop effective management strategies (Nallathambi et al., 2020).

Among the most studied viruses in tomatoes are tobamoviruses, which are widely recognized for their ability to spread via seeds, though the rate of transmission is low (Dombrovsky and Smith, 2017). Studies on tobamoviruses, such as tomato mosaic virus (ToMV) and TMV, have shown external contamination of the seed coat and occasional internal localization in tissues like the endosperm, with transmission to seedlings experimentally confirmed in grow-out studies (Broadbent, 1965; Taylor et al., 1961). The concentration

of ToMV in tomato seeds has been shown to depend significantly on the timing of plant infection, with earlier infections, such as those occurring 35 days after germination, leading to higher viral levels in the seeds (Chitra et al., 1999). Research has demonstrated that ToMV can contaminate tomato seeds at high rates, with seed infection exceeding 60 % in some cases (Kassim et al., 2019). However, seed-to-seedling transmission is significantly lower, typically not exceeding 10 % (Kassim et al., 2019). This highlights the restricted ability of the virus to transfer from seed coats to emerging seedlings.

Similarly to TMV and ToMV, ToBRFV exemplifies the transmission dynamics typical of tobamoviruses, with specific localization and low seed-to-seedling transmission rates. ToBRFV is predominantly found in the seed coat, both externally and internally, which serves as its primary reservoir. Studies using molecular and immunolocalization assays have consistently reported the absence of ToBRFV in the embryo, ruling out systemic vertical transmission to seedlings. Occasionally, ToBRFV has been detected in the endosperm, but this appears to be isolate- or condition-dependent (Avni et al., 2022; Davino et al., 2020; Fidan et al., 2024; Salem et al., 2022). Grow-out experiments and mechanical transmission studies have further explored ToBRFV's transmission mechanisms, consistently reporting seed-to-seedling transmission rates between 0.8 % and 2.8 % (Davino et al., 2020; Fidan et al., 2024). This supports the conclusion that infection primarily occurs via mechanical contamination of seedlings by infected seed coats during germination rather than systemic infection originating in the embryo. Efforts to mitigate ToBRFV transmission have focused on disinfection methods, including chemical sterilization and heat treatments, which have been effective in reducing surface viral loads while preserving seed germination viability (Fidan et al., 2024). However, ToBRFV particles internally localized within the seed coat or endosperm remain resistant to these treatments, emphasizing the importance of sourcing clean seeds and implementing preventive measures during seed production to limit the spread of this pathogen (Davino et al., 2020; Fidan et al., 2024). In addition to these well-studied tobamoviruses, tomato mottle mosaic virus (ToMMV) has also been confirmed in tomato seeds from various regions (Fowkes et al., 2022; Schoen et al., 2023; Tiberini et al., 2022). Although specific studies on its seed-to-seedling transmission are lacking, ToMMV is presumed to spread via seeds based on the transmission dynamics demonstrated for other tobamoviruses (EPPO, 2022a).

Another example of a mechanically transmissible virus, for which there is evidence of its association with seeds as a potential source of infection, is

PepMV, a *Potexvirus*. While primarily spread through mechanical means, research indicates that PepMV can also persist on contaminated seeds. Detailed dissections and real-time reverse transcription - polymerase chain reaction (real-time RT-PCR) analyses of tomato seeds have confirmed the presence of PepMV on the seed coat, while no traces were detected in the embryo (Ling, 2008). These findings align with the low rates of transmission through tomato seeds, observed at ≤ 0.057 % in large-scale grow-out experiments (Hanssen, Mumford et al., 2010).

Southern tomato virus (STV; family Amalgaviridae, genus *Amalgavirus*), which has been reported to cause symptoms under certain conditions (Gaafar et al., 2019; Harju et al., 2021), demonstrates systemic infection across all tomato seed tissues, including the seed coat, endosperm, and embryo (Hao et al., 2023), with high vertical transmission rates of 70–90 % (Sabanadzovic et al., 2009), making it significantly different from tobamoviruses and PepMV. Tombusviruses, such as tomato bushy stunt virus (TBSV), have also been occasionally reported to transmit through seeds (Nawaz et al., 2014). Although no studies have specifically examined the localization of tombusviruses within tomato seed compartments, research on TBSV biology highlights its ability to replicate and move systemically in host tissues. Its replication relies on specialized structures formed on peroxisomes, where the virus uses host cell membranes to create replication sites, potentially facilitating its movement into reproductive tissues (Gibson, 2009; Nagy, 2016). Despite these findings, the role of these mechanisms in seed transmission or localization within tomato seeds remains unexplored.

Nepoviruses, genus within the family *Secoviridae*, represent the other group of plant pathogens that can be transmitted through nematodes, and, notably, seeds (Harrison and Murant, 1996). The seed transmission of nepoviruses, such as tomato black ring virus (TBRV), underscores a vital pathway for their persistence and dissemination in agricultural systems. Studies indicate that TBRV exhibits seed transmission rates ranging from 1.69 % to 14.57 %, influenced by viral strain and tomato cultivar (Pospieszny, Borodynko-Filas et al., 2020). Seed surface disinfection experiments have minimized external contamination, suggesting that systemic transmission is linked to internal viral presence, but the specific localization of TBRV within seed compartments - such as the embryo, endosperm, or seed coat - has not yet been definitively determined (Pospieszny, Borodynko-Filas et al., 2020). Furthermore, defective interfering RNAs associated with TBRV have been shown to amplify seed transmission rates by up to 44 %, suggesting additional mechanisms that

could influence transmission dynamics (Pospieszny, Hasiów-Jaroszewska et al., 2020). However, data on whether defective interfering RNAs affect compartment-specific viral localization within seeds remains lacking.

The possibility of tomato seed transmission has also been documented for torradoviruses, which, like nepoviruses, belong to the *Secoviridae* family. Among these, tomato torrado virus (ToTV) has raised specific concerns about seed transmission. In Australia, strict plant import regulations led to the suspicion that ToTV might have been introduced via infected tomato seeds (Gambley et al., 2010). Experimental studies confirmed the seed transmission of ToTV, with a study reporting a transmission rate of approximately 0.4 % in over 17,000 seedlings grown from seeds collected from mechanically inoculated plants (Pospieszny et al., 2019). In their study, seeds were disinfected prior to planting to eliminate external contamination on the seed coat, indicating that the detected ToTV was likely located within the embryo or endosperm rather than on the seed surface. However, it is important to note that such a low transmission rate (0.4 %) may not conclusively indicate internal localization of the virus within the embryo. In contrast, research on a related torradovirus, tomato necrotic dwarf virus (ToNDV), found no evidence of seed transmission. A grow-out experiment using 169 seeds collected from six ToNDV-infected tomato plants failed to detect any infected seedlings, indicating that seed transmission, if it occurs, is extremely rare (van der Vlugt et al., 2015). Moreover, the severe symptoms observed in ToNDV-infected plants, such as reduced fruit production, further reduce the likelihood of seed-mediated transmission (van der Vlugt et al., 2015).

Viruses in the family *Bromoviridae* exhibit diverse mechanisms of seed transmission in tomatoes. Among these, pelargonium zonate spot virus (PZSV), classified in the genus *Anulavirus*, has demonstrated seed transmission with rates ranging from 11 % to 29 % (Lapidot et al., 2010). This high transmission rate suggests that PZSV likely localizes within internal seed structures, such as the endosperm or embryo, as surface disinfection treatments do not reduce transmission (Lapidot et al., 2010). Similarly, CMV, a member of the genus *Cucumovirus*, has been confirmed to be transmissible through seeds in tomatoes (Longe et al., 2022). A study by Longe et al. demonstrated CMV infection rates ranging from 0 % to 100 %, depending on the cultivar and seed source, emphasizing the importance of proper seed testing and selection to limit its spread (Longe et al., 2022). Another representative of *Bromoviridae*, tobacco streak virus (TSV), belonging to the genus *Ilarvirus*, has been shown to localize predominantly

in the endosperm (40 %−90 %) and less frequently in the embryo (10 %−50 %) in tomato seeds, suggesting systemic transmission via internal seed structures (Sdoodee and Teakle, 1988). In contrast, other ilarviruses, such as tomato necrotic streak virus (TomNSV), have demonstrated low seed transmission rates (0.33 %) without specific studies on their localization within seed compartments (Badillo-Vargas et al., 2016). The evidence underscores the epidemiological significance of *Bromoviridae* members, as seed-borne inoculum serves as a reservoir for virus dissemination, contributing to outbreaks in subsequent plant generations.

There are also many uncertainties regarding seed transmission in begomoviruses. While their primary dissemination mechanism involves insect vectors, such as whiteflies, evidence of begomoviruses associated with seeds has raised questions about their potential role in transmission through seeds. For instance, tomato yellow leaf curl Taiwan virus (ToLCTV) and tomato yellow leaf curl Thailand virus (TYLCTHV) have been shown to be seed-transmissible in tomatoes, with infection rates in progeny ranging from 36.87 % to 74.80 % depending on the cultivar (Chang et al., 2022). The viruses were detected in seed coats, cotyledons, and leaves of germinated seedlings, demonstrating their ability to invade reproductive tissues and persist in progeny (Chang et al., 2022). Similarly, Kil et al. (2016) demonstrated that TYLCV can also be transmitted from seeds to seedlings. Their study confirmed the presence of TYLCV in vegetative, floral, and seed tissues of infected tomato plants and its successful detection in seedlings germinated from infected seeds, with seed-to-seedling transmission rates reaching up to 84.62 % in some cases (Kil et al., 2016). However, the extent of seed transmission remains controversial, as highlighted by the findings of P é rez-Padilla et al., which challenge the idea of TYLCV seed transmission. Their study demonstrated that while TYLCV DNA was detectable in seeds from infected tomato plants, the virus was predominantly located externally on the seed coat (Pérez-Padilla et al., 2020). Importantly, surface disinfection drastically reduced the presence of viral DNA. Furthermore, extensive transmission assays involving over 3000 tomato plants across seven genotypes provided no evidence of actual seed transmission, suggesting that the detected viral DNA did not contribute to infection in progeny (Pérez-Padilla et al., 2020). Similar results were obtained for the tomato yellow leaf curl Sardinia virus (TYLCSV), which showed no seed transmission, as the virus detected in tomato seeds appeared to be due to surface contamination rather than true transmission to progeny (Tabein et al., 2021). Tomato mottle virus (ToMoV) and tomato golden mosaic virus (TGMV) were also evaluated for

seed transmission in tomatoes. Studies in the 'Florida Lanai' cultivar demonstrated no evidence of seed transmission, as seedlings raised from infected seeds neither displayed symptoms nor tested positive for the virus (Rajabu et al., 2018). Similarly, no seed transmission was observed for the tomato leaf curl New Delhi virus (ToLCNDV) in tomato, despite its seed-borne nature being confirmed in other hosts such as cucurbits (Sandra and Mandal, 2024). These findings underscore the variability of seed-associated behaviors in begomoviruses and highlight the importance of further research to resolve these inconsistencies and understand their epidemiological significance in tomato crops.

A similar pattern of uncertainty exists for other plant viruses. For example, in tomatoes, research has confirmed that TSWV is not seed-transmissible. Using serological (enzyme-linked immunosorbent assay; ELISA) and molecular (RT-PCR) techniques, studies have shown no evidence of TSWV in the embryo, seed coat, or in vitro germinated seedlings of infected tomato plants, even when the source plants exhibited clear symptoms of TSWV infection (Domínguez et al., 2019). This conclusively rules out seed-mediated transmission of TSWV in tomatoes. In contrast, findings in peppers have raised the possibility of seed transmission of TSWV. Studies have detected the virus in the endosperm of seeds using RT-PCR, real-time RT-PCR and transmission electron microscopy, though not in the embryo (Wang et al., 2022). While these results suggest a potential route for seed transmission of TSWV in peppers, their reliability remains uncertain and warrants further investigation. This contrast between tomatoes and peppers highlights the variability of seed transmission mechanisms across hosts and underscores the importance of rigorous, host-specific research to draw accurate epidemiological conclusions.

2.2 Pollen-mediated transmission

Pollen-mediated transmission represents an additional potential pathway for virus dissemination, as it facilitates both horizontal spread (between plants) and vertical transmission (to progeny through seeds) (Card et al., 2007; Fetters and Ashman, 2023). The study of Chang et al. has suggested that begomoviruses, such as ToLCTV and TYLCTHV, might utilize pollen as a vehicle for transmission. The study reported that ToLCTV and TYLCTHV could be detected in cross-pollinated tomato progeny, with infection rates reaching up to 77 % and 100 %, respectively, in certain cultivars, highlighting the potential for pollen-mediated transmission in these viruses (Chang et al., 2022). Additionally, the ilarvirus TSV is known

to transmit both horizontally and vertically via pollen, though studies specifically addressing its role in pollen-mediated transmission in tomatoes remain limited (Card et al., 2007). For tobacco rattle virus (TRV, genus *Tobravirus*), virus particles have been observed in premeiotic pollen mother cells and mature pollen grains of infected tomato plants, demonstrating its capacity for vertical transmission by infecting the developing embryo through pollen-derived pathways (Card et al., 2007). Similarly, for another Tobravirus, pepper ringspot virus (PepRSV), virus particles have been detected in pollen grains of infected tomato plants, suggesting a comparable potential for vertical transmission via pollen (Camargo et al., 1969). Another study has indicated that pollen may also play a role in the transmission of PZSV, as this virus has been detected in pollen grains and shown to enable vertical transmission through seeds. In this case, nearly 30 % of seedlings derived from hand-pollinated tomato plants exhibited infection symptoms (Lapidot et al., 2010).

In addition to viruses, viroids, particularly PSTVd and tomato planta macho viroid (TPMVd), can also be transmitted via pollen (Matsushita et al., 2018). Viroid RNA, detected in both generative and vegetative nuclei of pollen grains, can reach the ovary during pollen tube elongation, leading to vertical transmission through seeds, while TPMVd, unlike PSTVd, also spreads through pollen tubes into the style and ovary, enabling horizontal transmission in the maternal plant without requiring fertilization (Matsushita et al., 2018).

In the case of ToBRFV, although the virus is frequently detected in pollen and other reproductive tissues, it does not establish systemic infections in progeny through pollination. Instead, it acts as a surface contaminant, reducing pollen germination capacity by approximately 33 %, and is primarily spread mechanically by pollinators such as bumblebees (Avni et al., 2022). Similarly, PepMV is also transmitted by bumblebees, and unlike ToBRFV, it can actively infect plants by initiating infections in flowers, with the virus subsequently spreading systemically to other plant tissues, as demonstrated by Shipp et al. (2008). These examples underscore the critical role of bumblebees as vectors in greenhouse environments, where they facilitate the horizontal dissemination of viruses like ToBRFV, PepMV, and TMV during buzz pollination, carrying infectious viral particles within their hives and across plants (Levitzky et al., 2019; Okada et al., 2000; Shipp et al., 2008). CMV provides another example of how viruses can exploit plant-pollinator interactions to enhance horizontal transmission. By altering the volatile emission profiles of infected tomato plants, CMV increases their

attractiveness to bumblebees, leading to higher pollination rates (Groen et al., 2016). This strategy compensates for the potential fitness costs of infection, enhancing reproductive success and indirectly facilitating the spread of the virus (Groen et al., 2016). Such mechanisms emphasize the diverse ways viruses can manipulate ecological interactions for dissemination.

In addition to serving as a vector for within-species virus transmission, pollen can also facilitate cross-species virus spread, enabling the movement of viruses between closely related or ecologically associated plant species (Fetters and Ashman, 2023). For example, yellow tailflower mild mottle virus (YTMMV; Tobamovirus) has been shown to spread from indigenous *Anthocercis* species to invasive hosts such as *Solanum nigrum* and *Physalis peruviana*, particularly in environments where these plants grow in close proximity (Xu et al., 2022). Similarly, parietaria mottle virus (PMoV) can be transmitted via pollen from infected *Parietaria officinalis* plants to tomato and pepper crops, with evidence suggesting that nearby infected weeds act as reservoirs for agricultural viruses (Aramburu et al., 2010). This example highlights the ecological role of shared pollinators in facilitating pollen-mediated virus transmission, which is particularly relevant in tomato cultivation systems located near wild plant populations. The potential for spillover and spillback of viruses between wild and domesticated plants underscores the complexity of managing viral diseases in mixed ecosystems and emphasizes the importance of including pollen-mediated transmission in virus control strategies (Fetters and Ashman, 2023).

In this section, we focused on viruses for which seed or pollen transmission in tomatoes has been documented. However, it is worth noting that many other tomato-infecting viruses may have the potential to use these pathways, even though this has not yet been studied or confirmed. Some of these viruses are already known to spread through seeds or pollen in other plant species (Card et al., 2007; Dombrovsky and Smith, 2017; Fetters and Ashman, 2023; Pagán, 2022; Sandra and Mandal, 2024), emphasizing the need for further research to clarify their transmission dynamics in tomatoes. Understanding these mechanisms could reveal additional risks and inform strategies for better management of these viruses.

The persistence of tomato viruses associated with seeds and pollen poses significant challenges, particularly in the context of global seed trade. Even when transmission rates of viruses from seeds to plants are low, they can still facilitate the introduction of pathogens into new regions and amplify their impact under favorable conditions. This underscores the importance of stringent phytosanitary measures and advanced diagnostic methods to

mitigate these risks. A thorough understanding of how viruses persist and spread via seeds and pollen remains crucial for addressing the global dissemination of tomato viruses. The following section examines how tomato seed trade contributes to the worldwide distribution of tomato viruses, focusing on the role of infected seed lots in initiating outbreaks and spreading pathogens across international borders.

3. The influence of seed trade on the world-wide distribution of tomato viruses

It is expected that the tomato seed market will grow in terms of monetary value from one billion United States Dollar (USD) today up to 1.34 billion USD in 2030 (Mordor Intelligence, 2024). Therefore, the global seed market is an economically important segment. Tomatoes are one of the most popular fruit as they can be eaten fresh, dried or processed. Many processed tomato products are available such as preserves (whole peeled tomatoes, tomato pulp and juice, puree, paste and pickled tomatoes) dried tomato products and can be the base of many food products such as different types of sauces and ketchup as well as soups (Heuvelink, 2018). Depending on the end use of tomatoes, different varieties are needed. For example, tomatoes for the fresh market can vary in size from cherry or cocktail tomatoes, over plum tomatoes to classical round and beefsteak tomatoes (Heuvelink, 2018). Vine tomatoes are fruits that are sold when they are still attached to the fruiting stem and therefore need equal ripening of the fruit clusters. Tomatoes for fresh consumption are mainly produced under protected conditions, either in greenhouses or in open fields under cover, and hand-picked upon harvest (Heuvelink, 2018). Processed tomatoes are usually grown in open fields and harvested mechanically by machinery. Tomatoes are grown as annual crops in temperate climates. With an annual production of around 70 M tons, China was by far the largest tomato producing countries followed by India (20 M tons), Turkey (13 M tons) and the United States of America (USA) (12 M tons) (FAOSTAT, 2025). Not only the final use of tomato determines breeding aims for new varieties (such as fruit size and numbers, color, time of ripening, fruit firmness, fleshiness, sweetness and acidity, shelf-life), but also the resistance to biotic and abiotic stresses are important. This includes the resistance to viruses (tobamoviruses, potexviruses, begomoviruses, tospoviruses, cucumoviruses, etc.), bacteria (*Pseudomonas solanacearum/Ralstonia solanacearum, Clavibacter michiganensis, Pseudomonas syringae*, etc.), fungi (*Cladosporium fulvum*, *Verticillium* ssp, *Alternaria solani, Phytophthora infestans*, etc.), cold tolerance, heat and drought

resistance, and resistance to salt and physiological disorders such as blossom-end rot (Heuvelink, 2018). Traditional usage or taste preferences of tomatoes as well as local climatic conditions may also influence the choice of varieties by the growers for which breeders need to provide varieties that cater for those specific demands.

Tomato seed trade is very complex as breeding, selection of suitable variety candidates, propagation, processing and packaging of tomato seeds for the final user is distributed almost all over the world (Fig. 1). After packaging, the seeds are subsequently distributed to the final users – growers across the globe – through a network of regional subsidiaries and intermediate purchasers.

The most important tomato breeding companies originate from Europe but act globally to support the complex global markets. In general, target-specific breeding is carried out under greenhouse conditions in Central

Fig. 1 Global seed production and trade routes. This figure illustrates the complex commodity flows of tomato seeds. For example, target-specific breeding can be carried out under greenhouse conditions in Central Europe (1). The production of parental lines can be outsourced to Mediterranean countries with more favorable climatic conditions for bulking up seed stocks (2). Selected parental lines are shipped back to the central facilities (1, 3) for seed health testing and preparation for upscaled production of hybrid seeds in Asia, India or South America where climatic conditions allow fast production of vast amounts of seeds (4). Seeds are shipped back for commercial packaging in specialized facilities (5). Alternatively, regional subsidiaries e.g., (6) may test and package the seeds for regional markets (7). This figure is for illustrative purposes and does not endorse the trade route of specific companies nor countries. It does not depict the final sale and distribution of seed for local production within countries or regions. *Adapted from Dunkle (2014). Created in BioRender Ziebell (2025a).*

Europe (Fig. 1, ①). The production of parental breeding lines can be outsourced to countries with more favorable environmental conditions that allow quick building up of seed stocks such as conditions available in Southern European or Mediterranean countries (Fig. 1, ②). The selected parental lines are commonly shipped back to the Central European facilities, tested for seed health and prepared for upscaled production of hybrid seeds (Fig. 1, ③). This production often takes place in South America, India or Asia where the environmental conditions allow fast production of vast amounts of seeds (Fig. 1, ④). The produced seeds are shipped back to the central seed processing facilities for seed health testing (Fig. 1, ③) and are then shipped for commercial packaging for the final market to specialist facilities (Fig. 1, ⑤). Alternatively, the seeds may also be shipped, checked and packaged in regional subsidiaries, e. g. USA (Fig. 1, ⑥ and ⑦) before distributed to the growers via intermediate purchasers.

Each of these routes bears the risk that seeds contaminated with pests or pathogens are introduced to other regions. Many different legislative regulations are in place to prevent the spread of harmful organisms carried by seeds. These can be international standards (IPPC, 2017), regional standards (EPPO, 2021a) or international (European Commission, 2019b) and national regulation (Australian Government, 2025).

International Standard for Phytosanitary Measures (ISPM) 38 developed by the International Plant Protection Convention (IPPC) provides guidelines to national plant protection organizations (NPPOs) for identifying, assessing and managing risks associated with the global movement of seeds (IPPC, 2017). This involves a pest risk analysis to determine whether the traded seeds themselves would pose a threat to the environment of the importing country or whether seed-borne or seed-transmitted pests and diseases may occur on these specific seeds and could be establish in the importing country (further guidelines on pest risk analyses are given in ISPM2, ISPM 21 and ISPM 11 (IPPC, 2004, 2013, 2019)). Depending on the end use of the seeds (laboratory testing, planting under restricted conditions, field planting), the risk may be assessed differently. Recommendations are given to mitigate the risk of seed-borne and seed-transmitted pests and diseases during production which would include best-practice hygiene measures, such as the use of resistant plant varieties where available and appropriate, seed treatment, inspections and testing of plants (IPPC, 2017). For exports, phytosanitary certificates confirm that consignments comply with phytosanitary import requirements of the receiving country. Guidelines on phytosanitary

certificates are given in the international standard ISPM 12 (IPPC, 2022). Regional plant protection organizations such as the European and Mediterranean Plant Protection Organization (EPPO) or the North American Plant Protection Organization (NAPPO) give further guidelines on practical implementation of inspection, testing and reporting of findings (e.g., EPPO, 2019, 2021a, 2021b; NAPPO, 2025). Furthermore, international legislation, such as those laid out by the European Union (EU), regulates specifically the requirements for introducing seeds or planting material into specific territories (e.g., European Commission, 2019b).

There are also a variety of industry-based bodies which represent and lobby on behalf of the seed industry at an international level. The International Seed Federation (ISF) and the American Seed Trade Association (ASTA) are the most notable of these. Such bodies not only set their own guidelines for their members but actively develop seed testing protocols under the International Seed Health Initiative (ISHI) (ISF, 2020). These organizations also engage with policymakers to advocate industry interests and propose alternatives to phytosanitary certification (ISF, 2018, 2022, 2024a, 2024b).

Despite many standards and much legislation in place to prevent outbreaks with pests and pathogens, viruses such as ToBRFV still manage to move via trade. ToBRFV was first reported from Jordan in 2016 although it seemed to be present in Israel since 2014 (Luria et al., 2017; Salem et al., 2016). These reports were not of much concern until the first ToBRFV findings were reported from Germany in 2019 (Wilstermann and Ziebell, 2019). Soon after, ToBRFV was reported from many countries all over the world. Legislators introduced emergency measures to prevent further outbreaks and spread into pest-free areas (e.g., European Commission, 2019a). Mandatory seed and plants for planting testing was introduced for imported seeds as well as for movement within the EU; as for some countries ToBRFV contaminations were discovered on imported seed lots despite valid phytosanitary certificates, therefore the rates of mandatory testing were subsequently increased (European Commission, 2020).

Due to the complex trade pathways for tomato seeds, young plant production and final destination of young plants for fruit production, it has been difficult to directly link contaminated seeds to ToBRFV outbreaks. Van de Vossenberg et al. used full-genome data of different ToBRFV isolates collected at different times and geographic locations to establish common ancestries and potential pathways of spreading using Nextstrain (van de Vossenberg et al., 2020). In the latest version, genomes from contaminated

seeds could be included in the analyses and confirmed as pathway for introduction of ToBRFV (de Koning et al., 2025). However, despite a huge increase in sequence numbers compared to the initial analysis in 2020, the numbers of ToBRFV sequences originating from intercepted seeds are still comparatively low which may influence the interpretation of results. This may be due to the diagnostic sensitivity of the applied tests for seed testing that may detect lower concentrations of the virus than would be suitable for HTS. Nevertheless, these studies demonstrate the importance of rigorous plant and seed health testing for the prevention of seed-transmitted virus outbreaks.

4. The synergy of vertical and horizontal transmission in tomato virus outbreaks

While the transmission of tomato viruses through seeds serves as a critical mechanism for viral persistence and long-distance dissemination, the significance of this pathway often becomes apparent only when combined with other transmission methods. Mechanisms such as mechanical contact, vector activity, waterborne spread, and pollinator-assisted dissemination act synergistically with seed transmission to amplify infections within a single tomato-growing season. This interplay is particularly evident in tomato-infecting viruses, where seed transmission introduces latent inoculum that is subsequently amplified through efficient horizontal routes.

The tobamoviruses affecting tomatoes provide a clear example of this dynamic. While these viruses exhibit low seed-to-seedling transmission rates, often restricted to external seed contamination, they are exceptionally stable in the environment due to their robust virion structure, which allows them to persist for prolonged periods under various conditions, such as dry soils or water reservoirs (Broadbent et al., 1965; Dombrovsky and Smith, 2017; Mehle et al., 2023; Salem et al., 2023). ToBRFV, for instance, can persist on tools, gloves and greenhouse structures for weeks to months (Skelton et al., 2023). The robust nature of tobamoviruses ensures that mechanical transmission, facilitated by contaminated tools or surfaces, plays the most significant role in their dissemination (Ishibashi et al., 2023; Zhang et al., 2022a). The role of mechanical transmission is further supported by spatial observations, which show that ToBRFV infections start as small foci and grow into larger clusters through routine cultural practices in greenhouses, such as leaf removal and harvesting (González-Concha et al., 2021).

Experimental studies further confirm this rapid progression, demonstrating that even a small initial presence of ToBRFV infected tomato plants can lead to 100 % infection rates within 4–8 months under greenhouse conditions (Panno et al., 2020). This highlights how frequent plant handling and intensive production practices significantly accelerate viral dispersal (Salem et al., 2023). Additionally, the ability of tobamoviruses to remain infective in irrigation water and recirculating hydroponic systems enhances the potential for water-mediated transmission (Mehle et al., 2023; Zhou et al., 2024). In controlled experiments, water contaminated with ToBRFV was capable of infecting tomato plants through root uptake, with symptoms appearing after prolonged exposure, depending on root damage and viral load (Mehle et al., 2023). Moreover, monitoring of drain water in commercial greenhouses has demonstrated its potential as an early indicator of ToBRFV outbreaks, often before visible symptoms occur (Mehle et al., 2023). Beyond water, tobamoviruses like ToMV have been shown to persist in contaminated soils, further reinforcing their role as reservoirs that enable root-mediated infections in suitable environments (Aziz et al., 2019). Thus, the interplay between minimal vertical transmission and robust horizontal transmission modes—mechanical, waterborne, and soil-mediated—ensures the rapid amplification of infection in the intensive environment of greenhouse tomato cultivation.

PepMV follows a similar pattern, where its extremely low seed transmission rates are overshadowed by its efficiency in mechanical transmission (Hanssen and Thomma, 2010; Ling, 2008). Viral particles released from infected roots into hydroponic nutrient solutions facilitate systemic spread to neighboring tomato plants, much like the waterborne transmission described for tobamoviruses (Mehle et al., 2014; Schwarz et al., 2010). Furthermore, PepMV remains stable in nutrient solutions and on greenhouse equipment, significantly increasing the risk of localized outbreaks (EPPO, 2024b; Mehle et al., 2014). Its transmission potential is further expanded by the fungal vector *Olpidium virulentus*, which can facilitate PepMV spread when both the virus and fungus coexist in the root system, albeit with a low transmission rate of 8 %, posing an additional risk in greenhouse environments with recirculating irrigation systems (Alfaro-Fernández et al., 2010). Bumblebee pollination further enhances PepMV dissemination, as viral particles adhere to pollinators' bodies during buzz pollination, linking mechanical and pollinator-mediated pathways within tomato greenhouse systems (Shipp et al., 2008). This interaction of transmission pathways for PepMV, including pollinator involvement, was discussed in detail in the section *Tomato viruses*

transmitted by seeds and pollen, where we also highlighted the similar role of pollinators in e.g., tobamovirus transmission (Avni et al., 2022; Levitzky et al., 2019; Okada et al., 2000).

Insect-transmitted viruses like TYLCV and ToTV also demonstrate a strong reliance on horizontal spread. While vertical transmission of these viruses is negligible or absent, the tobacco or silverleaf whitefly (*Bemisia tabaci*) is a highly efficient vector. An illustrative case is TYLCV, which is transmitted in a persistent, circulative manner, traversing the vector's midgut, hemolymph, and salivary glands before being introduced into new plants during feeding (Ghosh and Ghanim, 2021; Pan et al., 2012). Specific interactions between the virus and vector proteins, such as viral coat protein binding to midgut receptors, facilitate this movement (Ghosh and Ghanim, 2021; He et al., 2020). TYLCV can persist within whiteflies for extended periods by replicating in the salivary glands, which contributes to increased viral loads and sustained transmission efficiency (He et al., 2020). Viruliferous whiteflies also exhibit altered behaviors, such as prolonged feeding and more frequent salivation, that optimize virus acquisition and inoculation into healthy plants (Li et al., 2021; Moreno-Delafuente et al., 2013). Notably, transmission efficiency varies among whitefly biotypes. The Q biotype has been shown to acquire and transmit TYLCV more effectively than the B biotype, with Q biotype achieving higher viral loads and faster acquisition rates (Pan et al., 2012; Wang et al., 2010). This differential efficiency contributes to the rapid spread of TYLCV in regions dominated by Q biotype populations (Pan et al., 2012).

Similarly, CMV, despite its occasional seed transmission, spreads primarily through aphids in a non-persistent manner, where short feeding bouts suffice for efficient virus acquisition and transmission (Ng and Perry, 1999). TSV, on the other hand, relies on thrips, primarily *Thrips tabaci*, for horizontal transmission, facilitated by mechanical inoculation through feeding wounds. This transmission mechanism is closely linked to its vertical persistence in pollen, allowing TSV to leverage reproductive structures for survival while thrips vectors amplify its spread across tomato crops (Klose et al., 1996; Sdoodee and Teakle, 1987; Sdoodee and Teakle, 1993).

Soil-borne viruses, such as TBRV and TRV, also demonstrate how vertical and horizontal transmission work together. For TBRV, nematodes of the genus *Longidorus* play a critical role in spreading the virus horizontally by transmitting it from infected plants to healthy ones, as demonstrated in studies highlighting their efficiency as vectors (Brown et al., 1989; Taylor and Murant, 1969). The ability of nematodes to transmit TBRV

depends on the specific interactions between the virus and the nematode vector, which are influenced by the virus's coat proteins and RNA structure (Brown et al., 1989). For TRV, nematodes such as *Trichodorus pachydermus* transmit the virus by retaining particles on their feeding structures, allowing the virus to efficiently enter plant roots during feeding (Taylor and Robertson, 1970).

The combination of vertical and horizontal transmission allows viruses to persist and spread more effectively, as even low rates of vertical transmission can allow reservoirs of infection to establish in newly planted crops. These initial infections serve as focal points for horizontal dissemination, where mechanical activities, waterborne pathways, insect vectors, and nematode-mediated spread enable exponential amplification of the virus within a single growing season (Fig. 2). Managing this interplay requires

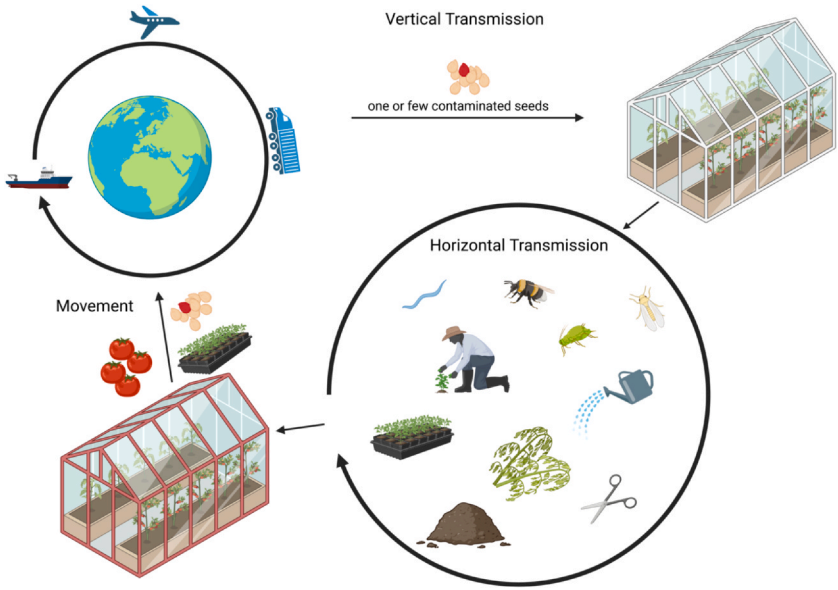

Fig. 2 The synergy of vertical and horizontal transmission in tomato virus outbreaks. Seed trade is a global business as tomatoes are grown virtually in all places of the world apart from Artica and Antarctica. A few seeds contaminated with viruses may lead to the emergence of an infected plant within a production site (greenhouse, protected outdoor cultivation, outdoor cultivation). Vectors such as insects, handling of plants and fruits by humans as well as plant debris and irrigation water transmit the virus diseases horizontally within the production site. Trade of young plants, fruits or seeds may spread again the viral diseases on a global scale. *Created in BioRender Ziebell (2025b).*

integrated strategies that address both pathways at the same time. While seed testing and disinfection are key to reducing vertical transmission, measures like cleaning tools, treating water, controlling vectors, and monitoring pollinators are crucial to stop the virus from spreading further. By tackling both transmission pathways together, the spread and impact of tomato viruses can be effectively reduced, ensuring healthier and more resilient production systems.

5. Effects of vertical transmission on tomato crop health and yield

The impacts of vertically transmitted viruses include direct effects on yield and vigor, leading to reduced fruit size or preventing fruiting entirely, as well as reduced marketable yield due to lower fruit quality or appearance. However, the impacts can also include indirect effects such as the additional costs of mitigation measures, including increased biosecurity protocols at the grower level and official phytosanitary controls, such as the emergency measures introduced to combat the spread of ToBRFV in the EU (European Commission, 2019a).

The importance of vertical transmission of different tomato viruses depends on the crop production system. In cooler temperate regions, tomatoes are primarily cultivated in protected environments, such as glasshouses and plastic tunnels, often using hydroponic systems. Within these environments, arthropod vectors of many viruses can be managed, but crops are routinely handled, which increases the risk of mechanically transmitted viruses such as potexviruses (e.g., PepMV), tobamoviruses (e.g., TMV and ToBRFV), and pospiviroids (e.g., PSTVd). Field-grown tomatoes, in contrast, are not handled as frequently as those in protected environments and are more commonly grown in warmer regions. In such conditions, viruses primarily transmitted by insect vectors, such as whitefly-transmitted begomoviruses (e.g., TYLCV, ToLCTV), tend to dominate. The challenge, however, is that commercial seed production often entails growing seed in multiple locations around the globe, including both tropical and temperate regions to feed a global market. Added to this, the additional labor requirement for activities such as emasculation and pollination of mother plants in hybrid seed production (Opeña et al., 2001) means that even in warmer climates, plants are manipulated by hand, creating a regular potential for contamination events to occur which could lead to infection with contact transmitted viruses.

Seed-borne infections can impact fruit production in multiple ways. Infected plants may produce fewer viable seeds, which can affect germination rates, while early infections may prevent fruiting, reduce vigor and yield, or result in unmarketable fruit. Broadbent, working on "tomato strain of TMV" (now recognized as ToMV), observed a relationship with severity of impact on seed related to the timing of infection (Broadbent, 1965). Plants infected later in the production cycle produced larger fruits, and these contained a lower proportion of necrotic seeds, compared to those infected earlier in the infection cycle. Broadbent observed that these necrotic seeds often failed to germinate. This observation suggests there is a dynamic relationship between the timing of infection in the mother plant and the direct impact on seed viability. For these contact transmitted viruses, it was also observed that infection did not occur until mechanical handling of seedlings occurred, in this case specifically pricking out of seedlings. The direct economic impact of infections arising from seed transmitted viruses have not been comprehensively studied, and as well as a lack of quantification impact studies at the virus level, impacts will also be dependent upon specific cultivar and growing system, which will complicate estimates of both yield and quality losses. James observed that crop loss and impact assessments were a neglected area of study, especially as such figures inform how much should be spent on research and control measures (James, 1983).

A study on the effects of PepMV in United Kingdom (UK) glasshouse grown tomatoes found no overall reduction in yield compared to uninoculated controls, but did report a reduction in fruit size, and a quality reduction with 6.5 % and 38 % of fruit in two trials failing to meet the highest quality standard (Class 1) (Spence et al., 2006). A second study found differences related to aggressiveness of isolates, whereby infection with one isolate gave no measurable yield loss or downgrading, whereas a more aggressive isolate resulted in 4 % yield loss, and 14 % downgrading of fruit (Peters et al., 2011). The additional complicating factor will be that most yield and quality impact trial studies compare artificially infected plants against uninoculated control plants, and therefore the differences arising from a season long dynamic outbreak scenario are difficult to quantify, i.e. where a limited number of plants begin the season as infected seedlings, and then infection progresses though the whole crop; for example, Panno et al. (2020) demonstrated how a whole crop would become infected by ToBRFV through natural spread of the virus over a season, however this study did not include a comparative impact assessment. For ToBRFV, it is recognized that

a crop can become severely diseased with up to 100 % plants showing symptoms of infection (Alkowni et al., 2019; Salem et al., 2016). A disease scoring scale was developed for the virus to improve current disease recording scales, and that study recorded a 25 % to 40 % reduction in fruit weight (González-Concha et al., 2023). However, diseased plants will still yield marketable fruit, and assessments of losses in "real" growing conditions at either the grower or regional crop level are lacking. Data gathered in Florida (USA) during 2019 estimated between 30 % to 70 % loss of yield, equating to an estimated $262 M loss of production (News Service of Florida, 2019).

Such yield and quality studies often overlook the overall impact on the production process of the additional management measures implemented to mitigate the impact of seed transmitted pathogens. These may be implemented by industry as "good practice measures" such as seed treatment or production process controls, including biosecurity measures and diagnostic checks during the seed production process. Contact transmitted viruses tend to have evolved some level of persistence outside of their living host, either on glasshouse surfaces (Mackie et al., 2015; Skelton et al., 2023) or in irrigation systems (Mehle et al., 2014; Mehle et al., 2023). Therefore, even when these viruses are seed-borne and transmitted on the seed coat, rather than seed transmitted in sensu stricto, they can be challenging to control within seed production systems once premises have become infected. In commercial production seed control measures include either chemical disinfection and/or thermal inactivation, and these have been shown to have some effect either on their own or in combination (Davino et al., 2020; Fidan et al., 2024; Samarah et al., 2021). Treating during the post-harvest seed processing stage is common practice within seed production systems. Soaking seeds in disinfectant solutions (e.g., HCl, trisodium phosphate, sodium hypochlorite) effectively reduces ToBRFV infectivity, while heat treatments show variable efficacy and UV light is less effective, with similar approaches mitigating viruses like PepMV (Fidan et al., 2024; Samarah et al., 2021; del Carmen Córdoba-Sellés et al., 2007) (see next section for further details). Additional measures which focus on a production system biosecurity accreditation approach are in operation for pathogens such as *Clavibacter michignanensis michiganensis*, in the guise of "Good Seed and Plant Practice" (Peusens and Lesprit, 2018). This audit-supported system of applying both engineered and behavioral biosecurity approaches should help minimize spread of other contact, however, as a voluntary self-funded scheme, most global tomato seed is not produced under this system (Constable et al., 2019).

In the cases where pathogens have been regulated and designated with quarantine status or as "regulated non-quarantine pests" (RNQPs), additional phytosanitary controls will apply to the production and movement of seeds. This may include inspection and testing at the place of production, statutory survey to demonstrate freedom from the pathogen at the place of production or in the region of production, specific treatments, border inspection and testing, or even prohibition (IPPC, 2017). Such approaches are only implemented after a rigorous pest risk analysis (IPPC, 2004, 2013, 2019). These regulatory controls have most recently been implemented for controlling the introduction of seed-borne ToBRFV in Europe, with the introduction of emergency measures in the EU (European Commission, 2019a). The most notable aspect of this was not only the requirement of a test result, but also a stipulated method for testing and sample size, aligned with the diagnostic standard produced by the EPPO (EPPO, 2022b). This regulation has resulted in every tomato seed lot entering the EU being tested at 3000 seeds per consignment. Due to the high cost of F1 hybrid tomato seeds, the value of the seed sample required for testing (e.g., 3000 seeds) often exceeds the actual cost of the diagnostic testing itself. Shipment destined to a high biosecurity region will require a higher number of seeds, and a greater associated diagnostic cost, for example Australia require 20,000 seeds for PepMV, ToBRFV, ToMMV and pospiviroids (Australian Government, 2025).

Whilst these relative sample sizes are based upon statistics from international standards such as ISPM 31 (IPPC, 2008), the underlying appetite for risk from an importing country will also influence the sample size as this is related to the confidence of detecting a low level of infection in a sampled seed lot. For pospiviroids and tobamoviruses there is evidence that at lower sampling rates, there may be contaminated seed lots missed during seed sampling. Dall et al. (2019) calculated that a 3000 sample size may miss 85 % of pospiviroids contaminated seed lots detected at the 20,000 sample size, and a similar study on tobamoviruses indicated up to 90 % of contaminated seed lots may be missed at the smaller sample size (Dall et al., 2023). However, the numbers of seed sampled has a cost implication for the seed company, and consequently a cost and resource implication for the plant health authorities testing the seed. The costs of this sampling and testing should be balanced against the costs of phytosanitary action from potential outbreaks. A further challenge is that there is not necessarily a direct relationship between detection of a viral target by molecular diagnostics, and its consequent likelihood of transmission

becoming an outbreak event. As diagnostic methods have become more sensitive and specific, the challenge is emerging to determine the risks associated with the detection of a virus. Many seed treatment methods may inactivate a virus, but these inactivated virus genomes may still be detected by PCR based tests (Davino et al., 2020). Often the methods applied, such as PCR or real-time PCR, do not detect an intact viral genome, only short fragments. Even most HTS bioinformatic pipelines may "reconstruct" fragmented genomes. This distinction is critical, as molecular detection methods identify the presence of viral nucleic acids, but do not confirm whether the virus is still infectious. A positive test result may therefore reflect the presence of non-viable viral particles that pose no risk of transmission. Therefore, novel diagnostic approaches to determining the viability of a detected pathogen may need to be allied to sampling volumes and strategies to maintain confidence in both the detection methods used and the seed being traded.

6. Mitigating the impact of vertically transmitted tomato viruses: Best practices and strategies

Drawing on prior discussions in this article, it is apparent that infections linked to seeds are essential for both the persistence and spread of certain tomato viruses. Even low levels of infected seeds can lead to outbreaks if not properly managed, as some virus genera, such as *Tobamovirus* and *Potexvirus*, can persist in seeds and initiate infections at early growth stages, spreading further through mechanical transmission. To mitigate these risks, a combination of preventive and control strategies is essential. Thus, the following measures are recommended (Sastry, 2013): (i) using 'virus-free' seeds by ensuring that only tested and certified seeds are utilized in cultivation—this approach incorporates strict certification programs and legislative regulations to control and monitor seed quality and movement; (ii) disinfecting seeds through chemical or physical means to eliminate surface or internal viral contamination, along with implementing cultural practices such as crop rotation, weed control, and sanitation to minimize viral reservoirs and limit further transmission; and (iii) developing and utilizing resistant plant varieties to reduce susceptibility to infection. Collectively, these measures not only reduce virus transmission but also mitigate the negative impact if an infection occurs, thereby contributing to sustainable tomato production.

6.1 Use of virus free seeds

The use of virus-free seeds is essential for preventing the introduction and spread of viral pathogens in agricultural systems (Jones, 2021). Given the global movement of seeds, regulatory frameworks such as ISPM 38, developed by IPPC, provide the scientific basis for risk assessments and set guidelines to minimize phytosanitary risks.

Comprehensive seed testing is a critical component in ensuring virus-free status. Reliable diagnostic tests enable the rapid detection of viral contaminants, supporting effective seed treatment measures. Equally important is the establishment of robust quarantine and certification programs. These programs enforce strict import and export restrictions by requiring that seeds be accompanied by phytosanitary certificates confirming that they have been tested and meet established health standards. Certification bodies such as the European Seed Certification Agencies Association (ESCAA), the Organization for Economic Cooperation and Development (OECD) Seed Schemes, and the International Seed Testing Association (ISTA), together with systems like the United States' National Seed Health System (NSHS), ensure that seeds are fully traceable and compliant with international standards. International initiatives led by bodies such as the ISF further harmonize these standards across borders. At the European level, harmonization efforts extend to member states of the EU, European Economic Area (EEA), and European Free Trade Association (EFTA). As illustrated in Fig. 1, Europe plays a coordinating role in the global tomato seed supply chain, with many breeding, testing, and processing activities linked to the region. This further underlines the importance of EU standards in shaping international seed production and trade.

By integrating rigorous diagnostic testing, effective seed treatment, and comprehensive certification measures, the use of 'virus-free' seeds minimizes the risk of seedborne disease outbreaks. This integrated approach supports sustainable agricultural practices by reducing the need for chemical interventions, preserving yield quality, and enhancing long-term crop resilience and food security.

6.2 Seed testing: Methods for virus detection

Detecting viruses transmitted through seeds is a critical step in managing their spread. Traditional detection methods, such as visual inspection and serological tests, have been widely used to identify infected seeds. Visual inspection involves examining seeds and seedlings for symptoms of viral infection, such as discoloration, stunting, or deformities (Jones, 2021).

However, it is often unreliable because many viruses transmitted through seeds do not produce visible symptoms, particularly during the early stages of infection. Serological tests, such as the ELISA, detect specific viruses in seeds by targeting viral proteins with antibodies and are relatively simple to perform (Adams and Clark, 1977). However, ELISA has limitations, including its inability to detect low levels of viruses or differentiate between closely related virus strains/species, often resulting in cross-reactions (Hull, 2013). Molecular diagnostic techniques have greatly improved the accuracy and sensitivity of virus detection. Among these, various PCR-based methods amplify viral genetic material, enabling the sensitive detection of viruses in seeds even when present at very low levels (Bartlett and Stirling, 2003). Despite advancements in detection technology, early detection of seedborne viruses remains challenging. The latent nature of some viruses and delays in symptom expression can allow them to spread widely before they are detected. Additionally, the high cost of advanced diagnostic tools may restrict their accessibility, especially for farmers in developing countries (Bartlett and Stirling, 2003). To address these challenges, it is essential to develop cost-effective and user-friendly diagnostic tools that allow for rapid and accurate virus detection. One promising approach is loop-mediated isothermal amplification (LAMP), a molecular technique that can be performed without sophisticated equipment and delivers results in a short time. LAMP assays have the advantage of being highly sensitive and specific while remaining accessible for routine use in various settings, including field diagnostics (Notomi, 2000).

Effective detection of seedborne viruses depends on several key factors, including the choice of diagnostic test, sampling methods, seed homogenization, and accurate result interpretation (Pushpangadan et al., 2012). The reliability of any method is influenced by these parameters, making standardization and validation essential. A literature review of diagnostic methods developed for viruses with confirmed or suspected seed and/or pollen transmission in tomatoes reveals a considerable number of available assays. These include both commercial and non-commercial tests, spanning ELISA kits, LAMP assays, and various PCR-based methods. While some of these tests are designed for specific viruses, others can detect multiple related viruses. Much of the available data focuses on virus detection in plant tissues, but certain methods have also been applied to seed and pollen matrices (Atik and Paylan, 2023; Berendsen et al., 2024; Faggioli et al., 2015; Fowkes et al., 2022; Gumus and Paylan, 2013; Kil et al., 2016; Lapidot et al., 2010; Ling et al., 2007; Ling, 2008; Matsushita and Tsuda,

2016; Pospieszny, Borodynko-Filas et al., 2020; Sdoodee and Teakle, 1988; Sui et al., 2017; Tiberini et al., 2022; Verhoeven et al., 2021). This diversity in application highlights the challenge of selecting the most suitable diagnostic approach and the need for careful validation to ensure accuracy across different sample types.

To ensure effective identification of harmful viruses in seed lots, a standardized approach is necessary given the vast and heterogeneous range of available diagnostic tests. Harmonization efforts are fundamental in determining the most reliable and widely applicable testing methods. Several national, transnational, and industry organizations, such as IPPC, EPPO, NSHS, ISTA, and ISF, provide guidelines and validated protocols based on internationally recognized criteria (EPPO, 2021d). These validation efforts ensure that diagnostic methods are robust, reproducible, and suitable for regulatory use. Among the available resources, EPPO's database on diagnostic expertise is one of the platforms where validated diagnostic tests, including those suitable for detecting viruses in tomato seeds, can be found. To further enhance reliability, test performance studies have been conducted in multiple laboratories to compare the effectiveness of different diagnostic methods and tests. These studies provide valuable data that can support the selection of assays for official phytosanitary controls conducted by plant protection organizations. As one of the available approaches, such studies help identify effective diagnostic methods/tests, ensuring that the techniques used are both sensitive and specific enough to detect viruses transmissible through seeds.

In addition to selecting appropriate diagnostic tests, proper sampling methodologies are critical. International standards, such as the IPPC's ISPM 31 ("Methodology for Sampling of Consignments"), provide guidelines for designing statistically sound sampling strategies to ensure compliance with phytosanitary regulations (IPPC, 2008). This standard helps NPPOs select appropriate sampling techniques to verify compliance with phytosanitary regulations. The methodology considers factors such as the percentage or proportion of infestation and the required confidence level, which can range from 10 % to 95 %. For example, for a batch of 1500 seeds, a sample size of 29 seeds is necessary to achieve a 95 % confidence level in detecting an infection rate of 0.1 % or higher, assuming the test is 100 % effective. Similarly, for a batch of 50,000 seeds, a sample of 3000 seeds is required for the same confidence level (see Appendix 5 of ISPM 31) (IPPC, 2008).

Validated seed health testing methods cover a wide range of viruses affecting different crops, including corn, leafy greens, cucurbits, legumes, and solanaceous crops. Specifically for tomato seeds, recommended

protocols are available through ISTA, NSHS, ISF, and EPPO for detecting *Pospiviroids* (EPPO, 2021c) as well as viruses, including PepMV, ToBRFV, and tobamoviruses in general. For viruses, the following provides more detail on the available detection protocols.

For PepMV, recommended protocols are provided by ISF and EPPO (EPPO, 2013; ISF, 2023), with the NSHS protocol being largely based on ISF guidelines (ISF, 2021). The recommended sample size in ISF protocol (ISF, 2021) (last updated in 2023) is 3000 seeds, with a maximum subsample size of 1000 seeds for real-time PCR and 250 seeds for ELISA and bioassay. An optional pre-screening step using ELISA or real-time PCR is available, and in the case of a positive result, confirmation through bioassay, on e.g., *Nicotiana benthamiana*, followed by ELISA is required. EPPO protocol PM7/113 (EPPO, 2013) outlines similar procedures, including a recommended sample size of 3000 seeds, with subsample sizes of 1000 seeds for real-time PCR and 250 seeds for ELISA, making it consistent with ISF guidelines. However, EPPO explicitly states that due to the highly variable sensitivity of bioassays, it is not recommended as a primary detection method for PepMV in seeds. While a positive bioassay result indicates viable PepMV, a negative result does not confirm the absence of the virus, further reinforcing its unreliability as a diagnostic tool (EPPO, 2013).

For tobamoviruses such as TMV and ToMV, recommended protocols are available from ISTA (ISTA, 2024) and ISF (ISF, 2019a), while NSHS (protocol So 5.1) follows the ISTA standard specifically for tomato. These protocols primarily rely on bioassays, with ELISA as an optional prescreening method. If ELISA yields a positive result, confirmation must be done through a bioassay. Among tobamoviruses, ToBRFV has specific detection protocols available through ISF (ISF, 2019b), EPPO (EPPO, 2022b), and NSHS (NSHS, 2024). Unlike general tobamovirus protocols, which rely primarily on bioassays with optional ELISA prescreening, ToBRFV detection emphasizes molecular methods. According to the ISF protocol, any ToBRFV positive real-time PCR results must be confirmed using a bioassay, similar to the approach for PepMV. In contrast, EPPO recommends only real-time PCR for testing seeds, as other methods are considered insufficiently sensitive for reliable detection of ToBRFV. EPPO provides three different real-time PCR protocols targeting different genomic regions of ToBRFV, with one recommended for initial screening. In the case of a positive result, confirmation must be performed using a second real-time PCR assay targeting a different genomic region.

Ultimately, the integration of harmonized diagnostic tools, robust sampling methodologies, and validated protocols contributes to more efficient seed health testing. However, differences between EPPO and ISTA protocols for PepMV and ToBRFV highlight the need for careful alignment of detection strategies. EPPO prioritizes real-time PCR as the primary detection method, considering bioassays as insufficiently sensitive for reliable diagnostics, while ISTA includes a bioassay as a key confirmation step. These differences reflect varying perspectives on the reliability and practicality of the bioassay in virus diagnostics. Continued international collaboration and data-sharing initiatives will be essential for future harmonizing of protocols and ensuring effective biosecurity measures in seed trade.

6.3 New detection approach: HTS and CRISPR

The high number of tomato-infecting viral species and frequent mixed infections with different species or variants of the same species (Choi et al., 2020; Rivarez et al., 2021; Xu et al., 2017) challenge standard diagnostic tests. Molecular and serological tests require prior knowledge of the target virus and are often unsuitable for detecting unexpected or unknown viruses or novel species (González-Pérez et al., 2024). HTS offers a powerful solution, identifying plant viruses regardless of their genome nature or structure (Roossinck, 2015). HTS can be used for the targeted detection of regulated pests and can also help identify pests causing novel diseases or diseases of unknown etiology that might pose a potential threat to plant health (Aritua et al., 2015; Barba et al., 2014; Malapi-Wight et al., 2016; Maliogka et al., 2018). In recent years, the discovery and first detections of viruses in tomatoes have increased, but these findings are often not paralleled by virus characterization studies—biological or ecological—of newly discovered tomato viruses (Rivarez et al., 2021). In-depth characterization studies are mostly performed for viruses with high phytosanitary and economic importance (Rivarez et al., 2021). This gap highlights the need for broader virome studies and improved surveillance efforts - a point further emphasized by recent reviews of HTS applications in plant biosecurity (Fox et al., 2025).

By using HTS, a great number of viruses have been detected in seeds (Fox et al., 2015; Galipienso et al., 2021; Harju et al., 2021; Hussain et al., 2022; James et al., 2023; Maachi et al., 2021; Rivarez et al., 2021; Vučurović et al., 2021) but in most cases, little information is provided as few studies have been conducted to determine whether seeds could serve as a transmission pathway to offspring. Nevertheless, HTS could represent a

powerful tool in epidemiological studies, for instance, allowing extensive HTS-based tomato virome studies (Xu et al., 2017; Ma et al., 2020), and demonstrating the potential to detect tomato-infecting tobamoviruses in the environment outside their hosts (Bačnik et al., 2020). Furthermore, HTS technologies open new possibilities and opportunities in routine diagnostics, including (a) understanding the status of a pest in a region through surveillance programs, (b) certifying nuclear stock and plant propagation material, (c) (post-entry) quarantine testing to prevent the introduction of pests into a country or area, and (d) monitoring imported commodities for new potential risks (Olmos et al., 2018). The ability to detect viruses independently of prior knowledge makes HTS a valuable tool for phytosanitary risk assessment and biosecurity measures. However, major challenges in using HTS for virus detection still arise from the lack of established methodological standards, especially regarding bioinformatic tools, input material, nucleic acid preparation, and the overall cost of the analysis (Kutnjak et al., 2021). Attempts to standardize the methodological workflow are in progress (e.g., the EPPO standard PM7/151 (EPPO, 2022c)). A recently published Addendum lists examples of validated workflows and bioinformatic pipelines which may help to include HTS in the ISO/IEC 17021-accredited process, which is sometimes a prerequisite for using HTS in official analyses (EPPO, 2024a).

Another emerging approach for the species-specific identification of viruses is based on CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats - CRISPR-associated protein) technology (Alon et al., 2021). The CRISPR-Cas is an innate immune system present in many bacteria and archaea (Jiang and Doudna, 2017), mostly utilized for genome editing (Hwang et al., 2013; Jinek et al., 2012; Shalem et al., 2014). More recently, it has been shown that some Cas proteins can be used for the detection of specific nucleic acid sequences (Chen et al., 2018; Gootenberg et al., 2017; Harrington et al., 2018). Recently, CRISPR-Cas12a was applied for the detection of RNA (Aman et al., 2020; Jiao et al., 2021) and DNA plant viruses (Mahas et al., 2021), suggesting its potential application in the detection of plant viral diseases. For instance, it has been used to detect tobamoviruses (Alon et al., 2021) and other vertically transmitted viruses in tomato (Bernabé-Orts et al., 2022; Prasad et al., 2022; Shymanovich et al., 2024), as well as pospiviroids (Zhai et al., 2024). In most cases, the output signal is based on fluorescence emission; however, a recent study demonstrated that a visual output, rather than a fluorescent one, can be used, further simplifying the detection procedure

(Alon et al., 2021). Such outputs may be colorimetric, or by using lateral flow strips, like common pregnancy tests (Broughton et al., 2020; Choi et al., 2021). Collectively, these approaches will allow onsite, rapid, and accurate detection without the need for specialized training or equipment. A future combination of this approach with isothermal amplification could provide a platform for efficient and user-friendly ways for the detection of closely related pathogen species or strains, and resistance-breaking variants.

6.4 Approaches for virus inactivation in seeds

In this section, we review various methods employed to inactivate viruses associated with seeds, with the aim of preventing virus transmission to emerging plants. During seed maturation, various processes in the seed coat and embryo tend to inactivate most viral particles, leaving a small fraction that remains infectious and capable of initiating infection in the emerging plant (Davino et al., 2020)—either via direct transmission from the embryo (as observed with alfalfa mosaic virus (AMV)) (Bailiss and Offei, 1990) or when the infected seed coat detaches during germination, exposing the emerging seedling to viral particles and facilitating infection (Pesic and Hiruki, 1986). Although literature suggests that virus transmission from the external seed coat is generally rare, even a low rate of transmission can significantly contribute to further virus dissemination through physical contact during handling or seedling transfer (Johansen et al., 1994; Sastry, 2013). The following subsections outline different disinfection strategies, including chemical, thermal, and innovative treatments.

6.4.1 Chemical seed disinfection

Chemical seed disinfection focuses on eliminating viral contaminants that are often present on the external surfaces of seeds, frequently associated with fruit pulp residues. Seed cleaning is typically carried out by fermenting the fruit pulp adhering to the seeds with pectolytic enzymes, detergents, specific chemicals, or by mechanical separation. These methods help reduce viral inoculum before applying more targeted chemical treatments.

For instance, in the case of tobamoviruses such as TMV, tomato seed infection can be significantly reduced by treating the pulp with chemicals such as HCl (soaking for 30 min, followed by washing and drying of the seeds), teepol (10 %), trisodium orthophosphate (10 %), or sodium carbonate solution (10 %) (Alexander, 1960; Broadbent, 1965; Crowley, 1958; Howles, 1978; McGuire et al., 1979; Nitzany, 1960). A combined treatment involving pectolytic enzymes and dilute HCl, followed by drying at

80 °C for 24 h, has proven effective in reducing TMV inoculum to negligible levels (Laterrot and Pecaut, 1965). Additionally, 1 % HCl treatment has been effective in disinfecting tomato seeds contaminated with ToMV, leading to healthy crops (Pradhanang, 2009). In some cases, a sequential combination of treatments was employed. For example, a 1 % aqueous solution of trisodium orthophosphate (15 min) followed by 0.52 % sodium hypochlorite (30 min) successfully eradicated TMV from tomato seeds while preserving seed viability (Gooding, 1975). Alternative chemical treatments, such as salicylic acid (0.05 M) and neem oil (5 %), have also been evaluated. Applied to either seeds or seedlings, these treatments effectively reduced tobamovirus concentrations (Madhusudha et al., 2004; Murphy and Carr, 2002; Singh et al., 2004).

Recent research, especially in response to the global outbreak of ToBRFV, has led to the reassessment of chemical disinfection methods (Fidan et al., 2024; Ling et al., 2022; Salem et al., 2022; Samarah et al., 2021; Zamora-Macorra et al., 2023). For example, soaking seeds in a 2.5 % sodium hypochlorite solution for 15 min resulted in ToBRFV being undetectable by real-time RT-PCR in a limited test sample (100 seeds) (Davino et al., 2020). Similarly, protocols for the chemical treatment of seeds infected with PepMV, TMV, ToMV or pepper mild mottle virus (PMMV) have been documented (Berke et al., 2005; Ling, 2010), with one study successfully eradicating PepMV from tomato seeds by immersing them in a 10 % trisodium phosphate solution for 3 h without adversely affecting seed germination (Singh et al., 1989).

6.4.2 Thermal seed disinfection

Most attempts to eliminate virus from seeds by heat treatment have been done with high temperatures for relatively short periods or at low temperatures for longer periods by means of hot water or dry heat treatments. Tobamoviruses such as TMV have been reported to be successfully eliminated by subjecting dried, infected seeds to temperatures of 70 °C for 3 days or 80 °C for 1 day (Broadbent, 1965; Rees, 1970). In the case of ToBRFV, one study showed that heating at 72 °C for 72 h or using hydropriming did not disinfect the seeds at all (Samarah et al., 2021), while another study found that treatments at 80 °C for 24 h, 75 °C for 48 h, and 70 °C for 96 h successfully disinfected the seeds—with treatment at 65 °C for 120 h leaving 20 % of the seeds infected (Davino et al., 2020). Although heat treatment often delays germination, the seeds remain viable. However, some studies have reported different results. For example, Kang et al. found

that heating infected seeds at 64 °C, 72 °C, or 80 °C for 48 or 72 h reduced seed germination—especially at higher temperatures and with longer durations (Kang et al., 2014).

6.4.3 Novel phhysical approaches for seed disinfection

In recent years, several studies have investigated innovative methods for seed treatment. In one study, an ozone-based treatment reduced the concentration of seed-borne viruses by approximately 32 % without negatively affecting seed germination (Paylan et al., 2014). Another study demonstrated that ToBRFV could be inactivated using cold plasma ozone treatment even in a greenhouse hydroponic system (Zhou et al., 2024). Experiments using a UV-C–based seed treatment were also conducted; however, this approach proved unsuccessful in reducing ToBRFV infectivity, as seeds exposed to UV-C radiation at 254 nm for 30 min were sown and the resulting plantlets tested positive for ToBRFV by ELISA (Fidan et al., 2024).

6.5 Cultural practices for reducing virus persistence and transmission

Cultural practices play a crucial role in minimizing the impact of vertically transmitted tomato viruses by preventing their persistence in agricultural environments and limiting their spread (Palti, 1981; Tomlinson, 1987). One of the most effective strategies is crop rotation, which helps break the virus life cycle by reducing the presence of infected plant residues in the soil (Palti, 1981). This is particularly important for viruses such as TMV, which can remain infectious for over two years in root debris. Over time, natural degradation by soil microorganisms, including fungi and bacteria, reduces virus viability (Smith and Dombrovsky, 2020). In some cases, composting tomato residues has successfully eliminated ToMV, likely due to a combination of biological decomposition and heat inactivation (Avgelis and Manios, 1989). However, standard composting may be insufficient for complete virus inactivation, necessitating additional preventive measures (Richter et al., 2019).

Proper field management is another key approach. Avoiding continuous or intensive cropping of tomatoes or other susceptible plants in the same area reduces the risk of infection buildup (Sastry, 2013). The proximity of infected or susceptible crops can lead to severe virus problems, especially when mechanical or vector-mediated transmission is involved. Timely rouging (removal) of infected plants, particularly in small plantings with low virus incidence, further helps contain the spread (Hanson et al., 2000; Sastry, 2013).

Weed and wild host control is also essential, as many weeds act as reservoirs for seedborne viruses and may harbor them for extended periods (Duffus, 1971; Hanson et al., 2000). Some weeds support seed transmission (Chatzivassiliou et al., 2007; Salem et al., 2022), allowing the virus to persist in the soil and infect new crops in subsequent growing seasons. Additionally, volunteer tomato plants and weeds compete with cultivated crops for nutrients and water while serving as sources of inoculum for viral diseases. Removing these alternative hosts eliminates virus reservoirs, reduces virus spread via seeds, and prevents vector reproduction, thereby limiting transmission pathways.

Since no chemical treatments can cure infected plants (EPPO, 2024c; Smith and Dombrovsky, 2020; Tomlinson, 1987), strict sanitation measures must be implemented to prevent mechanical transmission. In protected environments such as glasshouses, sanitation is particularly critical after the destruction of infected plants (Dombrovsky et al., 2022; Richter et al., 2019; Smith and Dombrovsky, 2020). Cleaning greenhouse surfaces with water and detergent to remove organic matter, followed by disinfection with virucidal agents, helps prevent reinfection (Richter et al., 2019; Wilstermann and Ziebell, 2019). Numerous disinfection protocols are available, particularly for tobamoviruses, which are highly stable and easily spread through contaminated surfaces, tools, and hands (García-Estrada et al., 2022; Paludan, 1992). Key hygiene measures include disinfecting hands, pots, tools, and equipment with virucidal disinfectants, placing disinfectant mats at greenhouse entrances, and treating non-metallic equipment with bleach. Additionally, soil disinfection through solarization or chlorine treatment, where feasible, can help reduce the risk of virus persistence. Avoiding the cultivation of host plants for at least one year in infected fields further aids in breaking the virus cycle.

Management options to reduce the probability of spreading pospiviroids are similar to those described for viruses. In fact, cultural practices, which show low efficiency when implemented as standalone measures, provide an effective barrier against outbreaks if they are implemented within a fully integrated management process (EFSA Panel on Plant Health, 2011).

By integrating these cultural practices with seed disinfection and the use of 'virus-free' seeds, growers can significantly reduce the impact of vertically transmitted tomato viruses/viroids. Preventing the accumulation of viral inoculum in the field, controlling alternative hosts, and maintaining strict sanitation collectively contribute to a more resilient and sustainable tomato production system.

6.6 Genetic resistance to seed-transmitted tomato viruses

Genetic resistance offers a sustainable approach to managing seed-transmitted tomato viruses, reducing reliance on costly and labor-intensive cultural and chemical control measures. Breeding programs aim to develop tomato varieties with intrinsic resistance, which limits virus transmission, reduces crop losses, and ensures stable yields (Picó et al., 2002). Resistance mechanisms can prevent virus replication, restrict movement within the plant, or suppress symptom expression (Sastry, 2013). Dominant resistance genes typically encode proteins that recognize viral components and trigger immune responses, while recessive resistance genes involve loss-of-function mutations in host factors necessary for viral replication (de Ronde et al., 2014; Garcia-Ruiz, 2018; Hashimoto et al., 2016).

Breeding for resistance typically involves crossing resistant and susceptible plant varieties, followed by selection of resistant offspring (Yu et al., 2019). Backcrossing is often used to retain desirable agronomic traits while incorporating resistance. Marker-assisted selection enables faster identification of resistant genotypes (Das et al., 2017). More advanced strategies include genetic engineering and genome editing (Hasan et al., 2021).

Tobamovirus resistance has been crucial in tomato breeding for decades, with resistant varieties successfully controlling ToMV and TMV since the 1970s (Carr, 2024). Two dominant resistance genes, Tm-1 from *Lycopersicum hirsutum* and Tm-2/Tm-2^2 from *L. peruvianum*, inhibit viral replication and trigger immune responses, respectively (Carr, 2024; Erickson et al., 1999). However, ToBRFV can overcome these resistance mechanisms, causing outbreaks worldwide (Salem et al., 2016). To combat ToBRFV, researchers are screening cultivated and wild tomato germplasm for resistance traits (Jewehan et al., 2022), identifying quantitative trait loci (QTLs) (Ashkenazi, et al., 2018, 2020; Gilan, et al., 2023; Hamelink, et al., 2019; Millenaar, et al., 2021) and discovering novel resistance genes, including an nucleotide-binding leucine-rich repeat (NB-LRR) protein from *S. habrochaites* and tobamovirus multiplication protein 2 A (TOM2A), a homolog of a tobamovirus susceptibility gene (Fujisaki et al., 2008; Hamelink, et al., 2019; Tsujimoto, 2003; Ykema, et al., 2021). Efforts to modify Tm-2^2 to recognize ToBRFV movement proteins have led to resistant variants (Lindbo, 2022; Rivera-Márquez et al., 2022; Spiegelman, et al., 2022), which may be introduced via transgenic or gene-editing approaches.

HTS has also become a key tool in resistance breeding, enabling the identification of viral variants that overcome existing resistance genes and

revealing the genetic basis of resistance in wild and cultivated tomato germplasm. By providing genome-wide data on both viruses and host plants, HTS accelerates the discovery of novel resistance genes and supports the development of targeted breeding strategies (Carr, 2024). Building on these insights, gene-editing technologies such as CRISPR-Cas9 and RNAi (RNA interference) are now being applied to engineer virus resistance more precisely. Studies on ToBRFV show that the TOM1 (tobamovirus multiplication 1) gene is essential for tobamovirus replication in *Arabidopsis*, and its disruption confers resistance in tomato (Ishikawa et al., 2022; Jogam et al., 2023). Similarly, for TYLCV, CRISPR-Cas9 editing of SlPelo (on the Ty-5 locus) suppresses virus proliferation and prevents disease symptoms (Pramanik et al., 2021; Tashkandi et al., 2018). By integrating HTS-driven gene discovery with gene-editing approaches, researchers can accelerate the development of resistant tomato varieties, reducing dependence on conventional breeding methods. However, using CRISPR-Cas9 comes with challenges, such as the risk of unintended genetic changes (off-target effects), strict regulations that vary between countries, and the high costs associated with developing and approving gene-edited crops (Tiwari et al., 2023). In the United States, crops edited with CRISPR-Cas9 that do not contain foreign DNA (transgenes) are generally not subject to the same strict regulations as genetically modified organisms (GMOs) (Yamamoto et al., 2018). Instead, they are regulated similarly to conventionally bred crops. In contrast, the EU currently applies stringent regulations to CRISPR-edited crops, treating them under the same legal framework as GMOs. However, regulatory discussions are ongoing, and future policies may differentiate between different types of gene-edited crops (Callaway, 2018). Public perception and regulatory decisions will shape future applications of gene-editing technologies in agriculture (Wang et al., 2019).

The durability of resistance remains a major challenge, as viral evolution can lead to resistance breakdown. Polygenic resistance, involving multiple resistance loci, is expected to be more robust than single-gene resistance and should be prioritized in breeding programs (Lindhout, 2002). Advances in bioinformatics and sequencing technologies are improving the identification of resistance genes, allowing for more targeted breeding efforts (Rivera-Márquez et al., 2022). Combining genetic resistance with 'virus-free' seed production, cultural controls, and sanitation will be critical for long-term virus management. Continued investment in breeding research, along with global cooperation in virus surveillance and genetic resource sharing, will further strengthen efforts to mitigate viral threats in tomato

production. However, tolerance to virus infection remains a challenge in breeding programs. Tolerant plants may not show visible symptoms but can still harbor and transmit viruses through seed, potentially contributing to high seed transmission rates and complicating phytosanitary control efforts.

6.7 Integrated pest management - IPM

Integrated Pest Management (IPM) represents a comprehensive approach to controlling vertically transmitted tomato viruses through the integration of multiple preventive and control strategies. Unlike single-method approaches, IPM seeks to minimize virus incidence while ensuring agricultural sustainability and economic viability (Picanço et al., 2007; Ramasamy and Ravishankar, 2018; Walgenbach, 2018). By emphasizing a combination of cultural, biological, mechanical, and chemical controls, IPM aims to reduce reliance on pesticides and mitigate environmental risks associated with virus management (Han et al., 2024; Stenberg, 2017).

A key principle of IPM is prevention, which involves limiting the introduction and establishment of seedborne viruses in tomato production systems. An integrated plan for a safe and effective IPM strategy is crucial, requiring strong collaboration between farmers, policymakers, and researchers. Effective regulation and oversight are necessary to ensure the correct implementation of IPM. In the United States, the Federal Integrated Pest Management Coordinating Committee (FIPMCC), coordinated by the United States Department of Agriculture (USDA), oversees national IPM initiatives, ensuring science-based management approaches are implemented across agricultural sectors. Similarly, in the EU, each member state develops a coordinated national action plan for sustainable pesticide use, as outlined by the European Commission (https://food.ec.europa.eu/plants/pesticides/sustainable-use-pesticides/national-action-plans_en; accessed 26.02.2025).

IPM is a complex strategy incorporating multiple control measures (Bueno et al., 2021). Cultural controls, such as crop rotation, removal and destruction of infected plants, and separation of seedlings from existing crops, help to minimize virus spread. Biological control methods, including the introduction of natural predators, parasitoids, and microbial insecticides such as *Bacillus thuringiensis* and *Beauveria bassiana*, aid in controlling insect vectors that transmit viruses (Basit et al., 2024; Gomis-Cebolla and Berry, 2023; Gupta et al., 2024; Sinno et al., 2021). Mechanical controls, such as physical barriers, sticky traps, and pheromone traps, further help to reduce vector populations and virus transmission risk.

Chemical control, including disinfectants and pesticides, is employed in IPM programs when no effective alternatives are available, or when alternative methods alone are insufficient to prevent pest populations from reaching damaging levels. To improve sustainability, continuous monitoring, surveillance, and evaluation of IPM strategies are necessary, along with the integration of new technologies (Smith, 2020). Biopesticides derived from natural sources present an environmentally friendly alternative to synthetic chemicals, contributing to vector control, virus infection reduction, and resistance induction in plants (Alexandersson et al., 2016; Besati et al., 2024; Hernández-Santiago et al., 2020; Smith, 2020; Wang and Fan, 2014).

Precision agriculture technologies have further revolutionized the management of tomato viruses within IPM frameworks (Kebe et al., 2023). Remote and proximal sensing technologies, including drones, satellite imagery, and various sensors, enable real-time crop health monitoring and early virus detection. Decision support systems that integrate data from multiple sources, such as weather forecasts, crop models, and pest monitoring, provide valuable recommendations for optimized management strategies (Meno et al., 2024; Rajabpour and Yarahmadi, 2024; Tonle et al., 2024).

The successful implementation of IPM has been demonstrated in various crops, leading to reduced virus pressure and improved productivity. For instance, the use of IPM strategies to manage wheat streak mosaic virus (WSMV) in wheat has resulted in significant reductions in virus incidence and yield losses (Seifers et al., 1997). In tomato crops, IPM could be effective in managing early outbreaks of ToBRFV and implementing containment measures in production sites (Chanda et al., 2021; Ling et al., 2022), though long-term control remains challenging (Caruso et al., 2022; Zhang et al., 2022b). Successful IPM applications have also been observed for TSWV (Batuman et al., 2020), TYLCV (Gilbertson et al., 2007; Gilbertson et al., 2011; Riley and Srinivasan, 2019) and ToMV (Shanmugam et al., 2024), particularly in cases where insect vectors play a key role in virus transmission (de Oliveira, 2023; Walgenbach, 2018).

By integrating preventive strategies, surveillance technologies, biological and mechanical controls, and targeted chemical applications, IPM offers a sustainable solution for managing seedborne tomato viruses. Through ongoing research, innovation, and policy support, IPM enhances crop resilience while safeguarding agricultural productivity and environmental health.

7. Future directions and research needs

7.1 Gaps in current knowledge

Despite significant advances in understanding the vertical transmission of tomato viruses, several knowledge gaps remain. A comprehensive understanding of the molecular and ecological mechanisms governing seed and pollen transmission is still lacking for many viruses. While some viruses, such as ToBRFV, have been extensively studied, others with suspected but unconfirmed seed or pollen transmission routes require further experimental validation. Harmonized methods for assessing seed transmissibility should also be developed.

Additionally, the interactions between vertical and horizontal transmission remain an underexplored area. The extent to which vertically transmitted viruses contribute to secondary spread within cropping systems, and how environmental and agronomic factors influence this process, needs to be quantified more precisely. Future studies should also investigate the role of mixed infections and their impact on vertical transmission efficiency and plant health.

7.2 Importance of interdisciplinary research

The study of vertical transmission in plant viruses requires an interdisciplinary approach, integrating virology, plant physiology, genetics, and epidemiology. Closer collaboration between plant pathologists, breeders, molecular biologists, and bioinformaticians is essential for developing improved diagnostic tools, resistant tomato varieties, and a deeper understanding of virus-host interactions.

While advancements in HTS have significantly improved virus detection, the biological and epidemiological significance of many newly detected viruses remains poorly understood. Furthermore, the integration of ecological and agronomic perspectives will help refine management strategies. Understanding how farming practices, climate change, and trade regulations influence the spread of seed- and pollen-transmitted viruses will be crucial for sustainable and resilient tomato production.

7.3 Emerging threats and preparedness

The increasing globalization of the seed trade raises concerns about the introduction and dissemination of novel viral threats. Climate change may also alter vector distribution patterns, thereby influencing the epidemiology of both vertically and horizontally transmitted tomato viruses. Predictive modeling should be prioritized to assess potential shifts in virus prevalence and transmission dynamics under changing climatic conditions.

Another major challenge is the emergence of resistance-breaking viruses and strains, such as ToBRFV overcoming Tm-2^2 resistance in tomato. Continuous monitoring and the development of durable resistance through advanced breeding techniques, including CRISPR-based genome editing, will be essential for managing these evolving threats.

7.4 Diagnostic challenges and solutions

While HTS has revolutionized virus detection, it is still not widely accessible, and its implementation remains challenging for routine diagnostics in official laboratories. The development of cost-effective, and user-friendly diagnostic methods is needed to improve seed health monitoring. Moreover, current seed-testing protocols often struggle with detecting low viral loads or differentiating between infectious and non-infectious viral particles.

Refining detection methods to distinguish between active and inactive viruses will enhance the reliability of phytosanitary measures. Additionally, harmonization of diagnostic standards across regulatory agencies will facilitate international trade while ensuring effective virus management.

7.5 Strategies for proactive management and global cooperation

Proactive management strategies should include the implementation of IPM approaches that combine strict seed health certification, resistant cultivars, and robust biosecurity measures. Investment in seed treatment technologies, such as thermal and chemical disinfection, will also help reduce the risk of seed-borne viral outbreaks.

Global collaboration is crucial for the early detection and containment of emerging viral threats. Strengthening international regulatory frameworks, such as those established by ISTA, EPPO, and IPPC, will support coordinated efforts in virus surveillance and response.

8. Conclusion

This review has highlighted the significance of vertical transmission in the epidemiology of tomato viruses, emphasizing the need for more effective detection methods, breeding of resistant cultivars, and integrated management strategies. While horizontal transmission mechanisms play a dominant role in virus dissemination, vertical transmission remains a key factor in the persistence and global spread of tomato viruses through seed trade.

Key research priorities should focus on advancing diagnostic technologies, exploring new-generation resistance breeding approaches, and strengthening international phytosanitary regulations. The complex interplay between vertical and horizontal transmission modes underscores the importance of holistic management strategies that address both pathways simultaneously.

To ensure the sustainability of global tomato production, interdisciplinary research, regulatory harmonization, and investment in innovative virus control measures will be essential. By combining advancements in molecular detection, breeding innovations, and proactive biosecurity policies, the agricultural sector can mitigate the impact of tomato viruses and support more resilient production systems worldwide.

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