Chapter

Aspects in *Tobamovirus* Management in Intensive Agriculture

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Abstract

In the recent years, disease spread of old and newly evolved tobamoviruses has occurred worldwide, affecting production of various vegetable and ornamental crops. The tobamoviruses are highly stable plant viruses that could cause severe disease symptoms. The well-known tobamovirus *Cucumber green mottle mosaic virus* (CGMMV) has recently caused severe damages in the cucumber, melon, and watermelon cucurbitaceous crops, worldwide. Similarly, a recent widespread of the newly identified tobamoviruses, *Tomato mottle mosaic virus* (ToMMV) and *Tomato brown rugose fruit virus* (ToBRFV), has reduced the solanaceous crop production. The primary route of tobamoviral infection is through mechanical means. These viruses adhere to agricultural facilities, contaminate the soil, infect seeds, and spread via beneficial pollinators and irrigation water. Mechanical plant injury suffices to initiate viral infection. Practicing hygiene by plant growers and in nurseries is currently the main strategy for mitigation of tobamoviral infection. Promoting the production of solanaceous vegetable crops genetically resistant to ToMMV and ToBRFV infection is a promising approach. However, CGMMV-resistant sources of cucurbitaceous vegetable crops are scarce. Conferring resistance to rootstocks and cross-protection strategies are newly implemented approaches that could alleviate tobamovirus disease spread in both solanaceous and cucurbitaceous crops.

Keywords: Solanaceae, Cucurbitaceae, primary infection, secondary spread, strobilurins, resistant rootstocks, cross-protection

1. Introduction

In the recent years, there has been a growing concern regarding disease damages and losses occurring in vegetable crop production. Plant viruses constitute the major causal factor for the diseases. Tomato plants, belonging to the Solanaceae family, and cucumber, melon, and watermelon plants, belonging to the Cucurbitaceae family, have shown the most severe disease symptoms. These symptoms are primarily attributed to infections by viruses belonging to the *Tobamovirus* genus, in the *Virgaviridae* family. The prevalent route of tobamovirus infection is via mechanical plant manipulations [1]. The tobamoviruses are highly stable and kept infectious in soil containing buried virus-contaminated plants [2], on various agricultural facility tool surfaces, in seeds [3], and upon adhering to beneficial pollinator body parts [4, 5]. Two tobamovirus species that had a conspicuous effect on vegetable crop production in various countries and caused severe disease symptoms in host plants are the *Tomato brown*

rugose fruit virus (ToBRFV) that infected solanaceous plants [6, 7] and *Cucumber green mottle mosaic virus* (CGMMV) that infected cucurbitaceous plants [8]. An important strategy to reduce viral infection of cultivated crops is to practice hygiene during planting and to divide the planting procedures between workers. The use of appropriate chemicals for disinfection of trellising ropes, planting trays in nurseries, and the various agricultural tools, before planting, is highly recommended [9]. Importantly, the applications of highly sensitive methods to disclose virus-infected seeds [6, 10] increase the probability to sow virus-free seeds. The various maneuvers currently available for tobamoviral disease management and future strategies to alleviate tobamoviral infections are discussed below.

2. Tobamovirus worldwide spread

Viruses belonging to the *Tobamovirus* genus are positive-sense single-stranded RNA viruses that infect a wide range of plant species. *Tobacco mosaic virus* (TMV), first described by Mayer in 1886 [11], is the prototype of this genus, in the *Virgaviridae* family. Tobamoviruses infect vegetable crops mostly solanaceous and cucurbitaceous plants, ornamental plants, weeds, and medicinal plants. In the recent years, the spread of tobamoviruses that infect two major cultivated vegetable crops, the solanaceous and cucurbitaceous plants, has increased. The *Tomato mottle mosaic virus* (ToMMV) that infected tomato plants (*Solanum lycopersicum*) had spread in America and Spain [12, 13]. In the Middle East, ToBRFV had broken the highly durable resistance-conferring allele *Tm*-2^2 [6] that was introgressed into *Lycopersicon esculentum* from *L. peruvianum* [14]. Phylogenetic tree analysis showed that ToBRFV and ToMMV were clustered in separate clades [6]. ToBRFV infection of tomato plants has recently occurred in Mexico [15], Germany [16], and the USA [17]. A worldwide infection of the cucurbitaceous plants has occurred due to the spread of the tobamovirus CGMMV, first reported by Ainsworth in 1935 [8, 18]. Excluding few reports on CGMMV-resistant plants, commercial cultivars resistant to CGMMV are scarce [19, 20].

3. Genome organization

The genome organization of the tobamoviruses ToBRFV and CGMMV resembles in general that of TMV [21, 22]. The virus single-stranded RNA genome encodes four known proteins: short (126 or 129 kDa) and long (183 or 186 kDa) replicase-associated proteins. The long component is the outcome of a translational read-through of a termination codon of the short component. In addition, a movement protein (MP) of ~30 kDa and a coat protein (CP) of ~17 kDa are translated from sub-genomic RNA. A putative fifth 54 kDa protein resides between the two replicase-associated proteins [23]. Recently, in Solanaceae-infecting tobamoviruses, a sixth protein of 4–5 kDa has been identified, which is encoded by a region in the genome overlapping the open reading frames (ORFs) of the MP and the CP [24–26].

4. Particle pathogenicity and systemic disease spread

ToBRFV infection of solanaceous plants induced pathogenic systemic symptoms of narrowing leaves and yellow and brown spotted fruits. CGMMV infection of cucurbitaceous plants resulted in systemic mottle mosaic leaves and fruits as well as yellowing fruit flesh combined with necrotic peduncles (**Figure 1**). Increased severity of the symptoms could occur due to a variety of mixed infections. For example,

the solanaceous tomato plants infected by both ToBRFV and the abundant tospovirus *Tomato spotted wilt virus* (TSWV) showed severe fruit necrosis (**Figure 1d**), and the cucurbitaceous cucumber plants infected by both CGMMV and the *Pythium* species *P. spinosum* showed plant wilting and collapse [27] (**Figure 1j**).

The virulence factors that caused the severe symptoms occurring upon tobamovirus infection have not been established yet excluding the virulence factor of TMV upon infection of *Nicotiana benthamiana* that was identified as the orf6-expressed protein, which occurs in Solanaceae-infecting tobamoviruses [24]. Similarly, the mechanism of *Tm*-2^2 resistance breaking by ToBRFV has not been discovered yet. The viral MP is the avirulence factor recognized by the plant resistance-conferring protein *Tm*-2^2 [28]. Mutational analysis of the MP revealed that a change of two amino acids could overcome the resistance conferred by the *Tm*-2^2 allele [29]. However, the MP modifications that have occurred during the evolvement of ToBRFV are still unknown, although in bioinformatics approach several potential mutations were identified in the MP of ToBRFV. In the Cucurbitaceae-infecting CGMMV, a single amino acid substitution at the replicase site resulted in symptom attenuation [30], conferring a role for the replicase in viral virulence mechanism.

Figure 1.
Tomato brown rugose fruit virus (ToBRFV) and Cucumber green mottle mosaic virus (CGMMV) infected vegetables. (a–d) ToBRFV-infected plants; (e–j) CGMMV-infected plants. (a) Narrowing tomato leaves with mosaic pattern. (b, c) Yellow and brown spotted tomato fruits. (d) Brown spotted and necrotic tomato fruits infected by both ToBRFV and Tomato spotted wilt virus. (e) Mottle mosaic pattern on watermelon leaves. (f) Yellowing and necrotic watermelons. (g) Necrotic peduncle. (h) Mosaic pattern on melon leaves. (i) Various manifestations of mottle mosaic melons. (j) Collapse of cucumber plants infected by both CGMMV and Pythium spinosum.

The tobamovirus CP molecules constitute the capsid of the virion, which is ~300 nm long and 18 nm wide. For viral RNA encapsidation, *ca*. 2000 CP subunits form a right-handed helix in which each subunit binds three nucleotides. There are electrostatic interactions between charged amino acid residues that contribute to CP subunit interactions and particle stability, which is strengthened by hydrophobic contacts in the capsid [31] and carboxylate interactions between subunits [32]. Tobamovirus particles can survive 90°C heating and years of storage [31, 33].

Tobamovirus encapsidation is necessary for long-distance movement of the virus in the plant but is dispensable for cell-to-cell movement [34, 35]. Since viruses are localized in the symplast, it is necessary to maneuver cell-to-cell movement. Viral MP binds RNA and increases the plasmodesmata size exclusion limit [36]. The virus could then be transmitted via the widened cytoplasmic continuity that was formed between cells [37]. In addition to the MP effect on viral cell-to-cell movement, a role for the short replicase-associated protein in cell-to-cell viral movement was also observed, although the mechanism of the replicase effect is still unclear [38].

Tobamovirus CP is required for long-distance viral dissemination [34, 39]. It is required for viral movement across the boundary between vascular parenchyma and companion cells [40]. It is not quite clear, however, whether the CP is necessary for interactions with host factors [39]. In the phloem, virus particles follow photoassimilate transportation [41]. However, mechanisms of entry and egress from the phloem differ [42, 43]. For example, egress from the phloem involves the activity of the host plant pectin methylesterase [44].

Viral genome replication that occurs in the epidermis and mesophyll cells induces plant resistance programs such as the RNA silencing process [45, 46]. Plant RNA silencing is triggered by viral double-stranded RNA precursors, which are processed by RNase III Dicer-like protein to small interfering RNA duplexes (siRNA), 21–24 nucleotide long [47, 48]. The siRNAs are stabilized by HUA ENHANCER1 (HEN1), which catalyzes methylation at the 3′ end, generating 2′-*O*-methylated siRNAs [49]. The methylation prevents uridylation and degradation of the siRNA duplexes [49, 50]. Single-stranded siRNAs direct ARGONAUTE (AGO) protein residing in RNA-induced silencing complex (RISC) to silence posttranscriptionally complementary RNA by endonucleolytic activity [51]. Importantly, small RNA duplexes that are formed by the plant silencing mechanism function also as silencing signals that spread via the plasmodesmata between cells and systemically through the phloem [52, 53]. Establishing silencing process systemically can lead to degradation of newly infecting viruses prior to viral replication [54]. Viruses counteract the plant silencing process by the expression of silencing suppressors [55, 56]. The tobamovirus short protein associated with the replicase, such as the 126 kDa protein of TMV [57] or ToMV [58] interferes with the methylation of the siRNA duplexes catalyzed by HEN1 and thereby induces degradation of the siRNAs [54]. Viral RNA silencing suppressors are therefore positive factors in viral long-distance movement [39]. However, the contribution of the replicase-associated protein to viral entry into the phloem is not clear yet but could not be attributed to suppression of RNA silencing [33].

5. Modes of infection

5.1 Primary infections

5.1.1 Seeds

Tobamoviruses are seed-borne viruses, although the average of reported seed to seedling transmission ratios was only 4.1% [3]. Low viral transmission ratios in

grow-out experiments are commonly indicative of uninfected embryos. Seed coat contamination could occur due to physical attachment between the seeds and the fruit flesh. However, there were also reports on ToMV infecting the endosperm of tomato plant seeds [1]. The tobamovirus passage through the maternal seed coat to the endosperm, which originates from both maternal and paternal sources, is enigmatic in the face of the uninfected embryos [3]. The consequence of endosperm infection is problematic, in particular, for considerations of the appropriate seed disinfection procedures. CGMMV-infected cucurbitaceous seeds are the most challenging for disinfection practices. Seeds from symptomatic CGMMV-infected cucurbit plants showed tobamoviral infection of both the seed coat and the perisperm-endosperm (PE) envelope underlying the seed coat [59, 60], which is characteristic to cucurbits [61, 62]. Importantly, the PE envelope is comprised of endospermic cells on top of which noncellular lipid and callose layers were formed [61, 62]. A similar question is raised therefore regarding CGMMV occurrence in the PE envelope in the face of the uninfected embryos [59, 60]. Tobamoviral dispersal emerging from infected seeds could occur via physical manipulations of the seeds upon sowing, seed to seedling transmission, and seed coat contamination of the soil.

5.1.2 Soil

Tobamovirus soil contamination primarily occurs due to buried plant debris originated from tobamovirus-infected crops [63]. Using a serological method for ToMV detection, a high primary infection ratio, of up to 80%, apparently occurred in the tomato plants grown in the contaminated soil [63]. Under field conditions as well, ToMV was detected in soil containing tomato debris of crops originated from a previous year planting [2]. ToMV was also recovered from forest soil in which the mineral fraction had more virus than the organic fraction [64]. Clay in the soil adsorbs a high fraction of tobamovirus particles [65], which could be visualized by scanning electron microscopy. Similarly, CGMMV inoculum buried in the ground for overwintering contaminated the soil [66]. Soil contamination was apparent by CGMMV detection in the soil supernatant, by inoculating uninfected cucurbit plants with the soil supernatant and by planting uninfected cucurbit plants in the contaminated soil [66]. Various soil types mediate CGMMV dispersal in various efficiencies, which could be attributed to root damage in the case of rock containing soil.

5.1.3 Beneficial pollinators

Bumblebees (*Bombus terrestris*) are essential beneficial pollinators of tomato crop cultivation. Bumblebee hives that were placed in ToBRFV-infected tomato-growing areas were ToBRFV contaminated [5]. The virus was detected in the hive components: the comb, the enveloping cotton, and the nectar. ToBRFV in the hives was infectious as studied by inoculating the laboratory test plant *Nicotiana tabacum* cv. Samsun with virus purified from the hive comb. ToBRFV adhered to the bumblebee body parts, primarily the abdomen, suggesting that ToBRFV could be transmitted by buzz pollination. Importantly, bumblebee hives from ToBRFV-infected tomato greenhouses placed in a new greenhouse of uninfected tomato plants constituted carriers of a primary infectious inoculum. ToBRFV infection ratios of the newly infected tomato plants were 12–60%. The ToBRFV infection ratios were positively correlated with bumblebee activity [5].

5.2 Secondary disease spread

Tobamovirus disease spread is abiotic. Mechanical manipulations during crop cultivation and the commonly associated plant injury constitute a major route for

tobamovirus disease spread. Low concentrations of tobamovirus contamination could establish the disease spread in a growing area due to mechanical manipulations [1]. For effective tobamovirus disease transmission, leaf or root injury seems imperative. Although the most common way of tobamovirus disease spread is via physical attachment, root-to-root viral transmission ratios in tobamovirus-contaminated soil are low [67]. Similarly, seeds sown in tobamovirus-contaminated soil showed low infection ratios when compared to seedling planting, which could involve plant injury [68]. However, high concentrations of the contaminating tobamoviruses and repeated exposure to the infectious source reduce the impact of injury as a necessary determinant in tobamovirus disease spread [63, 65].

5.2.1 Irrigation water

Humidity preserves tobamovirus particle viability in soil. Infectious tobamovirus particles of TMV and ToMV were found in environmental waters such as ponds and streams. The occurrence of the tobamoviruses was visualized by electron microscopy, and the infectivity of the tobamovirus particles was examined in a biological assay on laboratory test plants [69]. A quantitative and sensitive approach to detect the tobamoviruses in environmental waters was also applied using sensitive reverse-transcription real-time PCR analysis [70]. Apparently, there were environmental water samples that tested positive for ToMV without the usual sample concentration step. Dispersal of the Cucurbitaceae-infecting tobamovirus CGMMV via irrigation water was tested, for example, in laboratory facilities in the Volcani Center, Israel. In the middle of a planting tray of cucumber (*Cucumis sativus*) plants, one plant was sap-inoculated with the virus and was then separated from adjacent plants by an open plastic vessel to prevent any mechanical transmission of the virus via any other way than that of the irrigation dripping system that was applied. The results inspected a month later showed that CGMMV infection ratios ranged between 36 and 91%, while the control plants were CGMMV free (Dombrovsky and Darzi, unpublished data). CGMMV transmission efficiency via dripping and flooding irrigation systems was examined in a glasshouse experiment. The distances of CGMMV infection of watermelon (*Citrullus lanatus* Thunb.) plants by dripping and flooding irrigation systems were 1.9 and 2.3 m, respectively [71]. CGMMV was also detected in a river close to a farm of CGMMV-infected muskmelon (*Cucumis melo*) and watermelon plants [72].

5.2.2 Plant manipulations

Contaminated hands, pruning shears, knives, trellising ropes, and grafting procedures are the most common means for effective tobamovirus transmission. Attempts to quantitate the contribution of mechanical contact to tobamovirus disease spread revealed that a high number of repeated contacts between TMV-infected tobacco leaves and uninfected plants were positively correlated with increased tobamovirus transmission efficiency [63]. Interestingly, there was no correlation between the TMV quantity in the source leaves and the efficiency of viral transmission. Several characteristics of the source of infection could also affect transmission efficiency, such as leaf age and the viral source, whether it was the outcome of systemic spread or it was the primary inoculated material. However, the effects of these parameters on TMV disease spread were not conclusive [73]. Quantitating the disease transmission ratios of the Cucurbitaceae-infecting CGMMV in cucumber plants was conducted, for example, by touching the plants with CGMMV-contaminated hands. CGMMV contamination analyzed serologically 3 weeks post the infection procedure spread down the row, sequentially,

and the infection ratio was 86% [68]. The contribution of agro-technical work of pruning and trellising to CGMMV disease spread was monitored in an experiment conducted in commercial cucumber greenhouses [68]. In the greenhouses, 5–11 scattered CGMMV-infected plants, which constituted 0.4–0.5% of the plants, were identified, and a survey was conducted on the effects of the intensive agro-technical activities on CGMMV disease spread. The percent increase in infected plants due to agro-technical practice for 40 days, in the various greenhouses, was in the range of 11–32% [68].

5.2.3 Beneficial pollinators

Beneficial pollinators do not only constitute a primary source of tobamovirus disease spread, as was observed in ToBRFV spread analysis [5], but could also promote secondary viral spread between tobamovirus-infected and uninfected plants. Hives containing bumblebees (*Bombus terrestris* L.) placed in a greenhouse of TMV-infected tomato plants (*Lycopersicon esculentum* L. cv. Momotaro) spread the TMV infection to adjacent uninfected tomato plants planted in the greenhouse. TMV viral particles attached to the bumblebee body parts were visualized by electron microscopy and tested positive for TMV in a serological assay. TMV viral particles isolated from the hive components were infectious, as analyzed in a biological assay using *Nicotiana glutinosa* seedlings for inoculation [74]. Regarding the Cucurbitaceae-infecting tobamovirus CGMMV, the honeybee *Apis mellifera* promoted disease spread between infected melon seedlings, which constituted a primary viral source, and adjacent uninfected plants [4]. Efficient secondary viral transmission between the plants occurred when the uninfected plants were placed on the path of the honeybee foraging track, between the beehive and the CGMMV-infected plants.

5.2.4 Weeds

Volunteer plants such as weeds could have an important role as tobamovirus reservoirs that may constitute a source of infection. Apparently, weed species could constitute asymptomatic reservoirs of the Cucurbitaceae-infecting tobamovirus CGMMV [75, 76]. Among the weed species susceptible to CGMMV infection that did not show any conspicuous symptom development and their susceptibility to CGMMV infection which was confirmed by laboratory mechanical inoculations were *Molucella laevis*, *Amaranthus graecizans*, and the medicinal plant *Withania somnifera* [76]. Overwintering of tobamoviruses in the weed hosts could promote the tobamovirus spread between cultivated crops of consecutive growing seasons.

6. Management strategies

6.1 Sensitive tobamovirus detection methods

In order to prevent the occurrence and establishment of tobamovirus primary infectious source introduced by virus-infected seeds, the appropriate detection methods should be applied. Viral RNA extraction (using Viral RNA Extraction Kit; Bioneer) from ToBRFV-infected tomato (*S. lycopersicum*) seeds (Luria and Dombrovsky, unpublished data) and CGMMV-infected cucumber (*C. sativus* Derben) and melon (*C. melo* Raanan) seeds were successfully executed [60]. Next-generation sequencing (NGS) platform has been successfully applied for detection of the tobamoviruses ToBRFV [6] and CGMMV [77]. The NGS detection method

is highly sensitive when compared to the most commonly used serological assays (enzyme-linked immunosorbent assay, Western blot) and the genome sequence analysis performed after PCR amplification. ToBRFV-infected tomato seeds mixed with uninfected seeds in a ratio of 1:600 were successfully detected by the NGS method (Luria and Dombrovsky, unpublished data). Viral RNA extractions, which are easy to perform and need a small amount of starting material, were successfully applied in the NGS analysis [78]. The use of the new technology based on a single-molecule sequencing such as the Oxford Nanopore sequencing platform was successfully applied for detection of plant viruses and bacteria and the detection of the tobamovirus ToBRFV in infected tomato seeds. The sensitivity ratio for detection of ToBRFV-infected tomato seeds by applying the Oxford Nanopore sequencing platform was 1:200 virus-infected seeds mixed with uninfected seeds [10]. Application of sensitive methods for tobamovirus detection in seeds is most critical and needs to be developed for CGMMV-infected cucurbitaceous seeds, in which the viral particles accumulate in the PE envelope underlying the seed coat [59, 60].

6.2 Alleviating soil-associated tobamovirus infectivity

Soil fumigation with pesticides such as methyl bromide, which had an effect on a wide range of plant pathogens, was successfully used in various crop production facilities. However, since the elimination of its use due to its high toxicity and the detrimental effect on the ozone, several other chemicals such as chloropicrin (trichloronitromethane) had been used [79]. Unlike methyl bromide, many newly used chemicals had no effect on weeds or plant debris, and their efficiency in inactivation of tobamoviruses was questioned. A good alternative to those chemicals are products based on strobilurin fungicide, which originally occurred in the mushroom *Strobilurus tenacellus* [80]. Synthetic compounds such as pyraclostrobin (F 500) that protected the tobacco plant *N. tabacum* (cv. Xanthi nc) against TMV infection could be used in soil preplant treatment. Other chemicals that are based on natural products are alkaloids such as phenanthroquinolizidine that can be extracted from several plant families such as the Vitaceae family [81]. Several formulas of the alkaloid exhibited anti-TMV activity [81]. Similarly, quassinoids isolated from *Brucea javanica* showed anti-TMV activity [82]. Recently, the strong antiviral effect of synthesized bioactive tricyclic spirolactones, which are based on natural polycyclic compounds, has been demonstrated on TMV infection of tobacco plants [83]. Interestingly, the antibiotic Ningnanmycin showed anti-TMV activity both by induction of plant resistance against virus and by inhibition of the virus virulence [84].

Soil steaming treatment using low temperatures (50–60°C) for short time periods (several minutes) had been useful for inactivation of most plant pathogens while preserving soil microflora and minerals [85]. However, these steaming conditions could not be applied for disinfection of tobamovirus-contaminated soil [60]. Interestingly, a combination of mild steaming conditions with chemicals such as potassium hydroxide that are exothermal when reacted with water was effectively applied to disinfection of TMV-infected soil. TMV infectivity ratios were reduced to 3.0%. The increase in the persisted heat in the soil and the higher soil pH could have affected TMV stability [86]. Importantly, regarding CGMMV-contaminated soil, application of intermediate medium composed of CGMMV-free compost, which was prepared from cattle feces, into planting pits prior to planting melon (*C. melo*) seedlings, significantly reduced the initiation of primary infectious foci in the growing area. When combining removal of newly identified infected plants at early growth stage, before trellising, with the implementation of intermediate medium, CGMMV infection ratio at 60 days post planting was 0.3% [60]. Recently, growers have

implemented growth bags that separate between plants, to grow various vegetable crops in order to reduce tobamovirus disease spread via soil (**Figure 2**).

Grafting vegetable crops on *Tobamovirus*-resistant rootstocks could also separate the cultivated crops from the contaminated soil. In particular, *Solanum gilo* accessions were found resistant to the tobamoviruses TMV, ToMV, and the *Pepper mild mottle virus* (PMMoV). Unfortunately, CGMMV-resistant rootstocks scarcely occur and are difficult to find. Using CGMMV-tolerant rootstocks for grafting cucumber plants in field experiment in Northern Israel resulted in viral infectivity ratios of 0.4–0.8% (2 rows, 250 plants in each row). Concomitant growth of ungrafted cucumber plants had CGMMV infectivity ratios in the range of 16–44% (5 rows, 320 plants in each row) (Dombrovsky and Koren, unpublished data). Importantly, it is preferable to use rootstocks that do not cause any reduction in crop yield. Rotations in crop cultivation could reduce buildup of primary infectious tobamovirus inoculum from contaminated soil [87]. For example, it is possible to plant tomato plants after pepper plant plantings since PMMoV does not infect tomatoes. However, because ToMV infects pepper plants, planting tomato crops should not be followed by pepper plant plantings [87]. Importantly, alternating rice and watermelon cultivation reduced CGMMV infection ratios of the watermelons by tenfold (amounting to 7.3%) when compared to consecutive watermelon cultivation [88].

6.3 Hygienic and careful planting procedures

The use of sodium hypochlorite solution on planting facilities and even on seeds was recommended for disinfection of tobamoviruses. However, seeds treated with the hypochlorite solution could show low germination ratios. Trisodium phosphate treatment (TSP) of tobamovirus-infected seeds was also implemented. Importantly, these commonly used disinfection procedures could not be applied for disinfection of tobamovirus-infected cucurbitaceous seeds in which the virus penetrated the seed coat and accumulated in the PE envelope underlying the seed coat [59, 60]. TSP (10%) treatment, combined with heating to 72°C for 72 hours, did not disinfect CGMMV-infected melon (*C. melo* cv. Raanan) seeds. The CGMMV detected in the treated seeds using serological assays and PCR analysis was infectious in a biological assay [60]. However, sodium hypochlorite solution and the new stabilized chlorine product, which has the active ingredient $C_3Cl_3N_3NaO_3$, could be useful for general tobamovirus disinfection of planting facilities [8] such as shears, knives, trellising ropes, planting trays, and irrigation pipes. Careful planting procedures should be implemented, avoiding the infliction of any injury to the plants. Dividing tasks between workers during planting could also reduce secondary tobamovirus

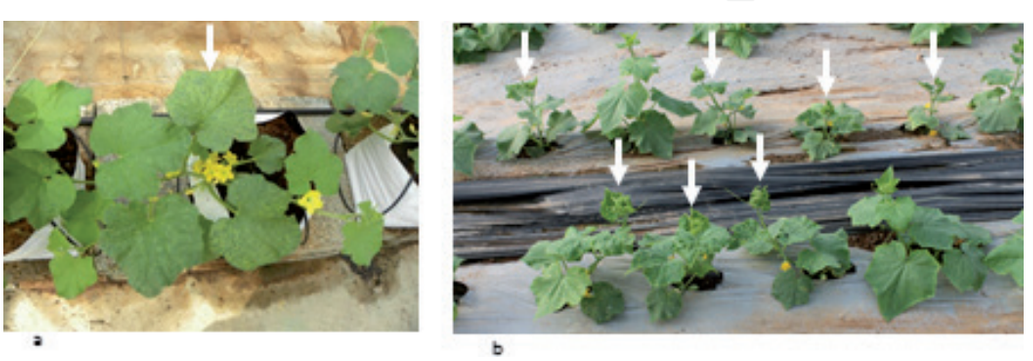

Figure 2.
Limited Cucumber green mottle mosaic virus (CGMMV) infection spread in melon plants grown in growth bags. (a) Melon plants grown in growth bags. (b) CGMMV spread through soil and irrigation water in regular planting of cucumber plants. Arrows mark CGMMV-infected plants.

infection spread. Weeding the weeds that might be reservoirs of tobamoviruses [76] and supplementing new bumblebee hives for tomato plant cultivation [5] could be important in preventing primary inoculum of tobamovirus infection. Identification and removal of tobamovirus-infected plants in crop cultivation facilities at early stages, before trellising, could reduce secondary tobamovirus disease spread.

6.4 Near-future management strategies

In the face of the genetic tobamovirus resistance occurring in tomato plants for many years, such as that of the durable *Tm*-2^2 resistance allele [14], a search for ToBRFV-resistant tomato plants could be successful. However, CGMMV-resistant genetic sources for introgression into commercial cucurbitaceous vegetable crop cultivation are scarce [19, 20]. Similarly, sources for CGMMV-resistant rootstocks are limited. Engineering transgenic watermelon rootstocks by transformation of the rootstock *Citrullus lanatus* (Twinser) cv. Gongdae with CGMMV CP gene conferred viral resistance to the plants [89], similar to the phenomenon observed in TMV CP-mediated resistance against TMV [90]. Another approach that could confer viral resistance to susceptible host plants while avoiding the production of transgenic crops is the use of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 genome editing technology [91]. This mutagenesis system is targeted by guide RNAs to a desired site in the plant cell genome, where Cas9 endonuclease causes double-stranded DNA breaks. The system exploits host cellular repair mechanisms to confer heritable high fidelity change in the genome. Host endogenous genes, such as the *Arabidopsis thaliana TOM1* and *TOM3* genes, and their homologues in tomato and melon plants, are necessary for tobamovirus replication [92, 93]. The host proteins translated from these genes comprise a complex with the tobamovirus replication protein [94]. These host proteins could be targeted by the CRISPR/Cas9 system. Importantly, RNA silencing of these host genes conferred tobamovirus resistance in *Nicotiana tabacum* [95] and in tomatoes [96]. Interestingly, RNA silencing, which is systemic [52, 53, 97], could be transmitted from rootstocks to scions. Hence, engineering rootstocks alone for tobamovirus resistance by RNA silencing of these host genes could confer resistance to the non-transgenic grafted tomato or melon plants. When using the biocontrol approach, plant defense mechanisms that specifically target the infecting tobamovirus, such as the RNA silencing, could be initiated by infecting the susceptible plants with a stable attenuated virus clone or a mutagenized variant of the virus. For example, several attenuated strains were successfully applied to protect tomato plants against ToMV infection, pepper plants against PMMoV infection [98], and muskmelon and cucumber [99] plants against CGMMV infection. However, mutagenized clones might not always be stable, and symptoms might develop in the susceptible plants. Therefore, in order to implement the cross-protection approach, for example, against ToBRFV that infects tomato plants harboring the *Tm*-2^2 resistance allele, it might be beneficial to infect the tomato plants with the stable ToMV that does not show symptoms in the resistant tomato plants. For that purpose, ToMV needs to infect systemically the *Tm*-2^2 resistant tomato plants.

7. Conclusions

In the recent years, tobamovirus disease spread has been one of the core causes for severe damages observed in various vegetable and ornamental crop productions. Concomitantly, there has been an increase in suggested strategies for tobamovirus disease management. The basic approach of implementing hygienic behavior

while planting has been improved by dividing the planting procedures between workers combined with soil disinfection or the use of intermediate tobamovirus-free medium. This new approach reduced tobamovirus infection substantially. Concurrently, new improved soil disinfectants based on naturally occurring products such as the strobilurin fungicide or plant alkaloids have been produced, eliminating possible harmful side effects of the disinfectants on animals and the environment. Improved methods for detection of tobamovirus-infected seeds have been developed as well. In addition, applicable in the near future are methods exploiting new molecular biology techniques, such as genome editing, to develop tobamovirus-resistant plants. Similarly, methods that engage the plant defense system to invoke resistance to tobamoviruses have been developed, such as the use of attenuated viral strains for plant infection or the use of engineered resistant plants as rootstocks.

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