농학석사 학위논문

# Investigating Antiviral Activities of Iodine Conjugated Organic Agents against Major Viruses Infecting Pepper

# 고추를 감염시키는 주요 바이러스에 대한 천연 유래 아이오딘 탑재형 유기태화 제제의 항바이러스 효능 검정

**2022 년 2 월**

서울대학교 대학원

농생명공학부 식물미생물학전공

장 수 연

**A THESIS FOR THE DEGREE OF MASTER OF SCIENCE**

# Investigating Antiviral Activities of Iodine Conjugated Organic Agents against Major Viruses Infecting Pepper

**BY**

**SUYEON JANG**

**Department of Agricultural Biotechnology**

**The Graduate School of Seoul National University**

**February 2022**

농학석사 학위논문

# 고추를 감염시키는 주요 바이러스에 대한 천연 유래 아이오딘 탑재형 유기태화 제제의 항바이러스 효능 검정

지도교수 김 국 형

이 논문을 농학석사 학위논문으로 제출함
2022 년 2 월

서울대학교 대학원
농생명공학부 식물미생물전공
장 수 연

장수연의 농학석사 학위논문을 인준함
2022 년 2 월

위 원 장 김 광 형 (인)

부위원장 김 국 형 (인)

위 원 손 호 경 (인)

**A THESIS FOR THE DEGREE OF MASTER OF SCIENCE**

# Investigating antiviral activities of iodine conjugated organic agents against major viruses infecting pepper

UNDER THE DIRECTION OF

DR. KOOK-HYUNG KIM

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

BY SUYEON JANG

MAJOR IN PLANT MICROBIOLOGY
DEPARTMENT OF AGRICULTURAL BIOTECHNOLOGY

FEBRUARY 2022

APPROVED AS A QUALIFIED THESIS OF RIRA JEONG
FOR THE DEGREE OF MASTER OF SCIENCE
BY THE COMMITTEE MEMBERS

**CHAIRMAN** Kwang-Hyung Kim

**VICE CHAIRMAN** Kook-Hyung Kim

**MEMBER** HoKyoung Son

# ABSTRACT

## Investigating Antiviral Activities of Iodine Conjugated Organic Agents against Major Viruses Infecting Pepper

SUYEON JANG
Major in Plant Microbiology
Department of Agricultural Biotechnology
The Graduate School of Seoul National University

Plant viruses cause significant financial losses due to the reduction of the quantity and quality of the major crops all over the world. These plant virus diseases are a growing concern due to the expansion of global trade and climate change. Climate change can affect the incidence and pathogenicity of plant viruses, consequently increasing the frequency and scale of disease outbreaks. Moreover, newly-recognized pathogens and new strains of established pathogens are annually being discovered. These are the reason why it is becoming increasingly important to develop managing strategies for out-breaking diseases. The control strategy for viral diseases relies primarily on non-chemical and cultural practices including cultivating tolerant or resistant varieties and controlling insect vectors because there are no commercially available viricides. Although several substances have been identified to be effective against several viruses, use of those

chemicals in fields was limited because i) the concentration of substances to control the virus is too high; ii) they caused interference with the plant growth and thus showed toxicity to the treated plants; and iii) amount of the compounds reached the target organisms were very low and the remaining bulk contaminates the surrounding environment. To solve these problems, the development of new substances including organic antiviral substances for managing virus diseases is increasing. In this study, I tested several iodine conjugated organic agents for their activity in managing virus disease on *Nicotiana benthamiana* and pepper plants against *Broad bean wilt virus 2* (BBWV2; *Fabavirus*), *Cucumber green mottle mosaic virus* (CGMMV; *Tobamovirus*), *Cucumber mosaic virus* (CMV; *Cucumovirus*), *Pepper mottle virus* (PepMoV; *Potyvirus*), *Pepper mild mottle virus* (PMMoV; *Tobamovirus*), *Tomato spotted wilt virus* (TSWV; *Tospovirus*), and *Tomato yellow leaf curl virus* (TYLCV; *Begomovirus*) which cause enormous damages in Korea. The candidate compounds were drenched on the test plants twice or thrice prior to or after virus challenge inoculations. I observed viral replication as well as symptom developments on systemic leaves. A quantitative analysis was also conducted to determine the relative levels of virus RNA replications upon treatment. After inoculation, samples were harvested on particular days post-inoculation, extracted total RNAs, and used for RT-PCR as

well as RT-qPCR analyses. In the case of CGMMV and PepMoV, compared to the untreated plant (control), the viral replication to the upper leave of the plant treated with the candidate agent was not suppressed, and the same results could be observed in RT-PCR results. This suggests that the replication and movement of the virus have not been significantly affected by treatments of candidate agents. Replication, as well as the development of symptoms, were reduced and/or retarded, respectively, against BBWV2, TYLCV, and TSWV in some agent-treated plants, especially on the upper systemic leaves. The same results could be observed in RT-PCR results, indicating that the replication and movement of the viruses were partially suppressed. In particular for TSWV and BBWV2, reduced replication of viral RNAs were confirmed through RT-qPCR analysis. In CMV, I observed that almost all plants showed weak symptoms compared to the control. This suggests that treatment of the target agent might reduce CMV RNA accumulation, and the expected results were observed by conducting RT-qPCR analysis. Altogether, my study confirmed that treatments with iodine, nitrogen, and sialic acid conjugated organic substances, which are natural-derived materials, might directly or indirectly affect resistance response of the host plants against several virus infections indicating that these iodine and sialic acid-based natural candidate materials can be used for managing virus diseases in fields.

**Keywords:** Iodine, Antiviral agents, Inhibition of virus replication, organic agent, Metal-organo complex

**Student number**: 2020-28266

# CONTENTS

**page**

# LIST OF TABLES

**page**

# LIST OF FIGURES

**page**

# INTRODUCTION

Plant viruses usually form spherical- or rod-shaped virions containing only one type of nucleic acid, either DNA or RNA. By successfully replicating their genomes and translating their gene products in the infected host plants, the virus particles and/or RNAs spread into adjacent cells through cell-to-cell movement and onto upper systemic leaves by long-distance movement and cause systemic infection of plants (Hull, 2009; Meshi et al., 1987). Although viruses are too small to be seen with a light microscope, they can cause threats to many economically important crops (Gergerich & Dolja, 2006).

Plant viruses affect plant growth and cause a wide range of distortions in leaves, flowers, and fruits, due to physiological stresses in the various infected plants (Takahashi, Fukuhara, Kitazawa, & Kormelink, 2019). Virus infections frequently induce significant economic losses caused by reduced crop yields and qualities. Recently, the incidence and pathogenicity of many plant viruses have been gradually increasing probably due to climate changes and the expansion of global trades. Moreover, newly-recognized pathogens and new strains of established pathogens are discovered annually.

Therefore, the need for viral disease control agents is also increasing. Plant virus disease control is primarily based on several strategies: i) Selection of virus-free plants and seeds; ii) Eliminating the infected sources like weeds, wild and susceptible host plants; iii) Reducing the vector population by using viricidesinsecticides, and iv) Developing the virus resistant or tolerant plants. These management strategies, however, offer no perpetual solution to a virus

disease problem. For example, efficiency of cultivating resistant varieties was limited over time due to the development of new viral strain(s) overcoming resistance by mutations and recombination events (Matthews & Hull, 2002; Sastry & Zitter, 2014). As for using insecticides, chemical substances have gradually lost their effectiveness because pests have developed resistance over time.

In addition, curing virus-infected plants is difficult since there are no commercially available viricide while bacterial or fungal diseases can be managed with antibacterial or antifungal agents (Rubio, Galipienso, & Ferriol, 2020); (Sastry & Zitter, 2014). Results of previous studies have identified several chemical compounds that were effective in inhibiting virus replication and movement, or inducing host defense mechanisms, such as ribavirin, 2-thiouracil (Bawden & Kassanis, 1954; Lerch, 1987). However, the use of those chemical compounds has several limitations: i) The concentration of substances to control the virus is too high; ii) They caused interference with the plant growth and thus showed toxicity to treated plants; and iii) Amount of the compounds reached the target organisms were very low and the remaining bulk contaminates the surrounding environment. Therefore, the identification and development of new eco-friendly substances are needed to manage virus diseases in fields.

Plants need nutrients for growth and development. Nutrients are sorted into primary nutrients, secondary nutrients, and micro-nutrients. They have a microscopic or extensive effect on biological molecules' chemical composition and essential metabolic function. Primary nutrients, also known

as macro-nutrients, are those usually needed in the most enormous amounts, i.e. carbon, hydrogen, nitrogen, oxygen, phosphorus, and potassium. Secondary nutrients are usually required in suitable amounts and they contain calcium, magnesium, and sulfur. Micro-nutrients are required in tiny amounts compared to primary or secondary nutrients such as boron, chlorine, copper, iron, manganese, molybdenum, and zinc (Mengel, Kirkby, Kosegarten, & Appel, 2001). Iodine is not included anywhere in these classifications of plant nutrients, and its importance as a nutrient affecting plants is not regarded as significant as compared to the other nutrients. For this reason, little was known about the role of iodine in plants, but as a result of studying the effect of iodine in various concentrations and forms on the growth of various crops, opinions have begun to emerge that iodine is also a micro-nutrient of plants (Pauwels, 1961).

As these views and results began to emerge, the possibility of iodine as a nutrient that can affect plants has been suggested. As it has been revealed to date, we therefore know that iodine is absorbed from roots or above ground structures (stomata and cuticular waxes), and when supplied at a well-defined concentration range, it influences plant growth and development and modulates the plant transcriptome (Kiferle et al., 2021; Medrano-Macías, Leija-Martínez, González-Morales, Juárez-Maldonado, & Benavides-Mendoza, 2016). Like that, iodine contributes to nutritional and bioactive aspects in plants in various ways. However, there are very little, if any, published studies on the direct impact of iodine on plant pathogens. Some studies have shown that iodine increases the number of antioxidants and

increases resistance to certain types of abiotic stresses, such as salinity and heavy metals (Leyva et al., 2011; Mehra, Pasricha, & Gupta, 2015). We also know that most of the factors that cause stress increase the concentration of free radicals at the cell level (Sun & Leopold, 1995). Since the induction of antioxidants is considered an important aspect of adaptive reactions that induce stress resistance in plants, this view suggests induction of resistance by iodine, which functions as an antioxidant (Medrano-Macías et al., 2016). In addition, research results suggest that modifications to iodine-induced cuticular waxes can change the pattern of interaction between pathogens and plants (Silva-Moreno et al., 2016). These findings involved in the plant response to biotic and/or abiotic stresses suggest that iodine may modulate defense responses against virus infections. Therefore, iodine might have sufficient value to be studied as a new antiviral substance. So, this study aims to find new resources of antiviral organic substances containing iodine by testing their potential antiviral activity against major pepper infecting viruses.

# MATERIALS AND METHODS

## 1. Plant material and virus inoculation

*Nicotiana benthamiana* and *Capsicum annuum* L. used in this study were grown at 25 ℃ in a growth chamber with 16 hours of light and 8 hours dark cycles. The plant viruses including broad bean wilt virus 2 (BBWV2), cucumber green mottle mosaic virus (CGMMV), pepper mottle virus (PepMoV), cucumber mosaic virus (CMV), tomato spotted wilt virus (TSWV), and pepper mild mottle virus (PMMoV) were mechanically inoculated by using virus-infected saps. Frozen virus-infected leaf tissue was ground using 0.05 M potassium phosphate (pH 7.4). Tomato spotted wilt virus (TSWV)-infected leaf tissue was ground using 0.05 M potassium phosphate (pH 7.0) and 5% sodium sulfate. Carborundum was powdered over the leaf surface before inoculation, followed by a quick rub of the sap all over the leaf surface (Pflieger, Blanchet, Meziadi, Richard, & Geffroy, 2015). Tomato yellow leaf curl virus (TYLCV) was infected by a leaf agroinfiltration method with *Agrobacterium tumefaciens* infectious clone.

## 2. Agent treatment

The candidate compounds diluted to each concentration were drenched on the test plants twice or thrice prior to or after virus challenge inoculations. Each virus was challenge inoculated by sap or agroinfiltration inoculations. Agents used in this study were based on iodine, but there are differences such as sulfur, nitrogen, and sialic acid being contained (Table 1).

### 3. Setting the standards for judgment of inhibitory activity

Compared with the control of each treatment, effect(s) of each treatment was determined by observing symptom development and by conducting semi-quantitative polymerase chain reaction (PCR). For visual observation, plants were kept for up to 4 weeks post-challenge inoculation. To differentiate replication levels for each virus, amplification cycles were selected for semi-quantitative PCR where expected band from 80% or more from positive control (untreated and virus-challenge inoculated) were detected.

### 4. Reverse transcription-PCR (RT-PCR) for virus detection

Total RNA was extracted from systemic leaves using RNAiso Plus (TaKaRa, Japan). About 2 μg of total RNA was used to synthesize complementary DNA (cDNA) with random hexamer and GoScriptTM Reverse Transcriptase (Promega, USA) in a reaction volume of 10 μl according to the manufacturer's protocols. RT-PCR was conducted using virus-specific primer sets. For RT-PCR, 20 ng of cDNAs were added to the reaction mixture consisting of 1 μl of 10 X PCR buffer, 0.8 μl of dNTP, 0.1 μl of Ex-Taq (TaKaRa, Japan), 0.5 μl each of forward and reverse primers (Table 2), 2 μl of cDNA (20 ng/ μl) and 5.1 μl of third-distilled H2O. PCR was performed with the following standard protocol: 95°C for 2 min, then 30 cycles of 95 °C for 20 sec, 50°C for 30 sec, and 72°C for 1 min, followed by 72°C for 10 min.

## 5. Quantitative RT-PCR (RT-qPCR) analysis for determining virus replication level

After agent treatment with virus-challenge inoculation, the leaves of treated plants along with controls were collected at several different time points. Isolated RNA was reverse transcribed into cDNA. RT-qPCR was performed on a CFX384 real-time PCR system (Bio-Rad, USA). The 10 µl of reaction mixture included 1 µl of cDNA (20 ng/ µl), 5 µl of 2X iQSYBR Green supermix (Bio-Rad, USA), 1 µl of each gene-specific primer set (Table 3), and 2 µl of DEPC-treated water. The RT-qPCR condition used in this experiment is as follows: 95 ℃ for 3 min (pre-denaturation), followed by 40 cycles of programmed amplification (denaturation 95 ℃ for 10 s; annealing & extension 55 ℃ for 30 s and melt curve 55 to 95 ℃, for 5s. Relative expression levels of genes were calculated by Bio-Rad CFX Manager software, version 1.6.541.1028 (Bio-Rad, USA), using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001), and were all normalized to the expression levels of *Eukaryotic initiation factor 5A2* (EIF5A2) and *β-tubulin* (***β***-TUB) (Wan et al., 2011).

## 6. Statistical analysis

Statistical analyses were performed through one-way analysis of variance (ANOVA) followed by Scheffe's test. Significant difference from each treatment group compared with the control was considered when *P*-values of less than 0.05 (typically *P-value* $\leq 0.05$).

**Table 1. List of agent`s main ingredients used for treatment**

| Agent | | Main ingredients |
|---|---|---|
| **PI** | | Iodine conjugated metal-organo complex |
| **LI** | | Liquid type of iodine conjugated metal-organo complex |
| **SI** | | Sulfur and iodine conjugated metal-organo complex |
| **M** | | Sialic acid and whey, blood meal |
| **A** | **A4** | Sialic acid and Nitrogen and iodine conjugated metal-organo complex |
| | **A5** | |
| **B** | **B1** | Sialic acid |
| | **B2** | Sialic acid and iodine conjugated metal-organo complex |
| | **B3** | Sialic acid and Sulfur and iodine conjugated metal-organo complex |
| | **B4** | Sialic acid and Nitrogen and iodine conjugated metal-organo complex |
| | **B5** | |

**Table 2. List of primers used RT-PCR**

| Viruses | | Primer sequence (5' –> 3') | Annealing temperature (°C) | Amplification size (bp) |
|---|---|---|---|---|
| BBWV2 | Fw | AAACAAACAGCTTTCGTTCCG | 50 | 380bp |
| BBWV2 | Rv | GCCATCTCATTGGCATGGA | | |
| CGMMV | Fw | GATGGCTTACAATCCGATCAC | 55 | 496bp |
| CGMMV | Rv | CCCTCGAAACTAAGCTTTCG | | |
| CMV | Fw | ATGGACAAATCTGAATCAACCAGTG | 55 | 754bp |
| CMV | Rv | GACTGGGAGCACTCCAGATG | | |
| CMV | Fw | AGACGTTAGCAGCTGGTCGT | 58 | 240bp |
| CMV | Rv | TCACCCACACGGTAGAATCA | | |
| PepMoV | Fw | TGTTCACTAGGCTCAGGAGT | 58 | 461bp |
| PepMoV | Rv | GACGACCCAAACACACTATT | | |
| PMMoV | Fw | ATGGCTTACACATTTCCAGT | 55 | 480bp |
| PMMoV | Rv | AGGAGTTGTAGCCCAGG | | |
| TSWV | Fw | ATGTCTAAGGTTAAGCTCACTA | 52 | 777bp |
| TSWV | Rv | TTAAGCAAGTTCTGTGAGTTTT | | |
| TSWV | Fw | TCCATAGCAATACTTCCTTTAGC | 55 | 848bp |
| TSWV | Rv | AGAGCAATTGTGTCAATTTTATTC | | |
| TYLCV | Fw | CGGAATTCACTATGTCGAAGCGACCAGG | 55 | 787bp |
| TYLCV | Rv | CGGGATCCTTAATTTGATATTGAATC | | |

**Table 3. List of primers used for qRT-PCR**

| Name | Forward primer (5'–3') | Reverse primer (5'–3') |
|---|---|---|
| β-tubulin | GAGGGTGAGTGAGCAGTTC | CTTCATCGTCATCTGCTGTC |
| EIF5A2 | CCTGTTATCGTGCTACTTTG | GTTTCATTGCCNTGCCAGAT |
| CMV | TGATTCTACCGTGTGGGTGA | CAGTTTGTTGTTGGCTTGGA |
| TSWV | ACTTGCCATAATGCTGGGAG | TGCTTTGCTTTTCAGCACAG |
| BBWV2 | TCACAGGTTATGCCGCTTGT | TCACTCGTCCCAAGCTGTTC |

# RESULTS

## 1. Treatment of the agent does not show side effect(s) on *N. benthamiana*

To investigate any possible side effect(s) caused by the drenching of the agent treatment on the plants, the agent was treated onto the *N. benthamiana* by drenching. After the drenching, any significant differences between the treatment and untreated control plants were observed for at least 10 days. No notable change on plants between control and treated plants was observed (Fig. 1) indicating that the treatment of the agents did not cause any significant side effect to the treated plants at the recommended concentration.

## 2. Preparatory experiment for confirming the antiviral activity for iodine conjugated organic agent alternative treatment in *N. benthamiana*

To verify the effectiveness of the agents and protocols prior to massive experiment, preliminary experiments were conducted only with CMV and BBWV2. Both CMV in the genus *Cucumovirus* (Moyle, Pretorius, Shuey, Nowak, & Schenk, 2018) and BBWV2 in the genus *Fabavirus* (Ferrer, Ferriol, Moreno, Guerri, & Rubio, 2011) cause the most significant damage to peppers in fields in Korea (Kwon & Chung, 2018) and thus were selected as target viruses. For CMV and BBWV2 inoculation, virus-infected leaf tissues were used for inoculation into *N. benthamiana*. The PI agent was drenched on the test plants twice prior to virus challenge inoculation for alternative treatments and the LI agent was drenched on the test plants twice after challenge inoculation. In the case of CMV and BBWV2, symptoms were observed on

the upper systemic leaves of both treated and untreated plants (Fig. 2A and C). However, the agents treated plants infected by the target virus did not show severe symptoms indicating both agents might inhibit the virus replication and movement. Therefore, to verify the replication of viral RNA, RT-PCR was conducted using virus-specific primers.

As predicted, I could not detect expected amplified DNA from CMV-infected plants' upper systemic leaves that were drenched with PI and LI agents (Fig. 2B). A similar result was also observed against BBWV2. Virus-specific amplified DNA fragment was not detected by alternative treatments of the two agents (Fig. 2D). These results suggest that alternative treatments of PI and LI agents have inhibitory activity against BBWV2 and CMV.

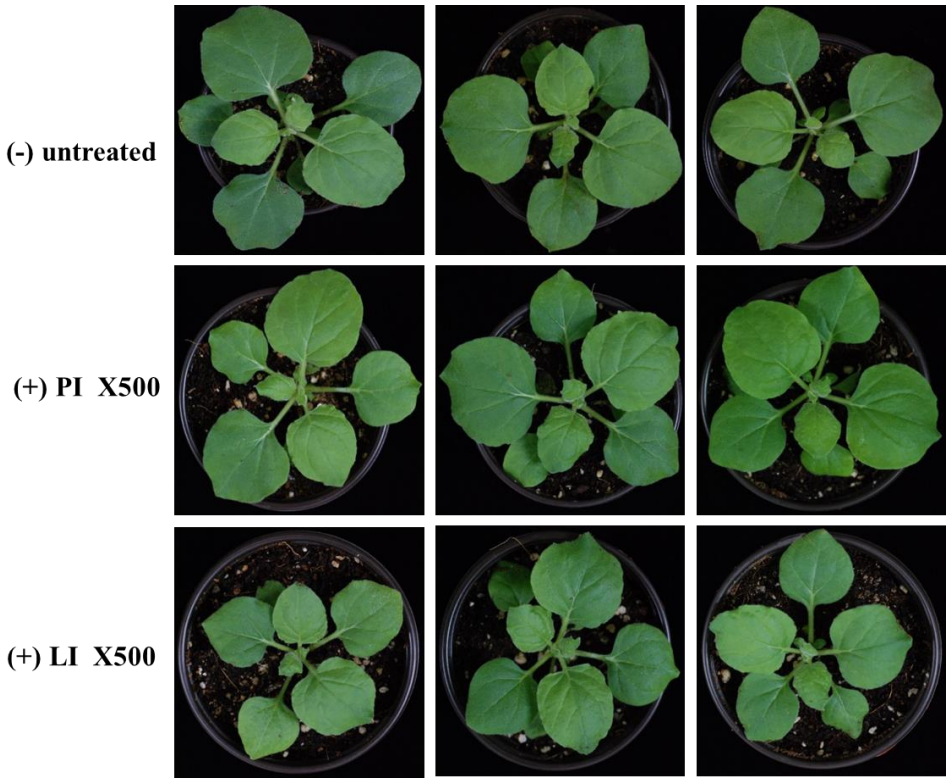



**Figure 1. Testing possible side effect(s) of the agents on *N. benthamiana*.** Each agent was diluted in X500, respectively, and was treated onto *N. benthamiana* plants by drenching method. Plants were observed at 10 days post treatment.

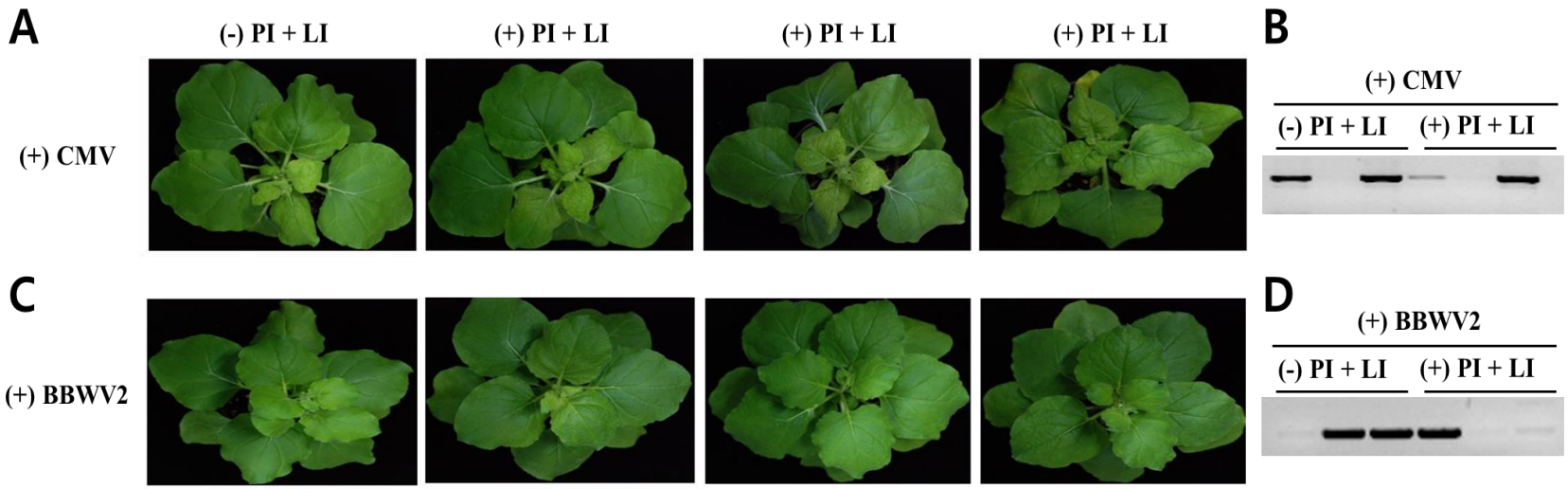



**Figure 2. Effect of PI and LI agents on CMV and BBWV2 infections in *N. benthamiana*.** For CMV and BBWV2 inoculation, virus-infected leaf tissue was used for inoculation into *N. benthamiana*. The plants inoculated by infected leaf tissue were observed under normal light (panels A and C). The viral RNA of CMV and BBWV2 in systemic leaves was detected by conducting RT-PCR (panels B and D).

### 3. Effect of cross treatments using powder and liquid types of iodine conjugated organic agents in *N. benthamiana* against virus challenge inoculations

As described above, PI and LI agent treatments showed inhibitory activity against CMV and BBWV2 infections. To further investigate the efficacy of the agent against several other plant viruses, BBWV2, CGMMV, and TYLCV that belong to different genera were used. While the agroinfiltration method with *Agrobacterium tumefaciens* infectious clones for TYLCV was used, virus-infected leaf tissue was used for BBWV2 and CGMMV inoculations into *N. benthamiana*.

Although cross treatments of PI and LI agents did not seem to affect virus replication in the additional experiments for BBWV2 by visual observation (Fig. 3A), virus-specific amplified DNA was not detected in some plants by RT-PCR analysis (Fig. 3B) indicating movement and replication of the virus in the systemic leaves was inhibited in some treated plants.

In TYLCV, infected plants cross treated with PI and LI agents did not completely inhibit the movement of the virus to the upper leaves (Fig. 3C). Mild symptoms, however, were observed on some agent-treated plants. To further confirm these results, RT-PCR was conducted in tested plants with TYLCV specific primer pairs. Virus specific band were not detected in some plants suggesting that cross treatments affect virus movement and replication (Fig. 3D).

In contrast, CGMMV infected plants did not show any inhibition of virus movement onto the upper systemic leaves of treated plant and difference in

amplified band by conducting RT-PCR using virus-specific primer set was not observed on plants between control and treated plants (Fig. 3E and F).

Inhibition efficiency against these four viruses by cross treatments of PI and LI agents in *N. benthamiana* was ranged from 33 to 66 % for BBWV2, CMV, and TYLCV while showing a 0 % inhibition against CGMMV (Fig. 3G). These results indicate that cross treatments of PI and LI agents effectively prevented BBWV2, CMV, and TYLCV infections in *N. benthamiana* plants.

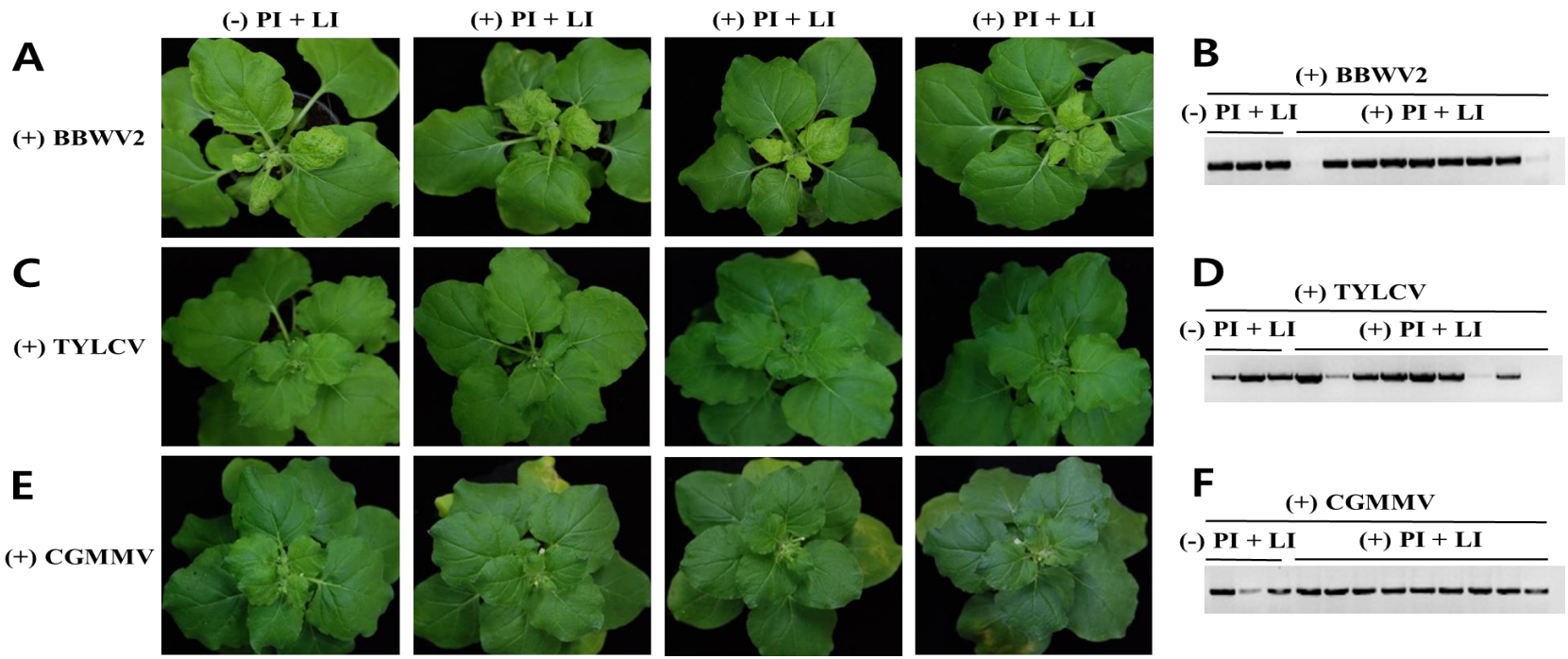



**Figure 3. Effect of cross treatments of PI and LI agents against BBWV2, TYLCV, and CGMMV infections in *N. benthamiana*.** For the inoculation, virus-infected leaf tissues were used for inoculation into *N. benthamiana*. Inoculated plants were observed under normal light (panels A, C, and E). Replication and movement of each virus in systemic leaves were detected by conducting RT-PCR using virus-specific primer sets (panel B, D, and F).

G

| Virus | No. of samples | Symptoms | | Inhibition efficiency(%) |
|---|---|---|---|---|
| | | *Non | **Weak | |
| **CMV** | 3 | - | 2 | 66.7% |
| **BBWV2** | 12 | - | 4 | 33.3% |
| **TYLCV** | 9 | 1 | 2 | 33.3% |
| **CGMMV** | 9 | - | - | 0% |

*Non: non-infected sample
**Weak: weak symptom

**Figure 3. Effect of cross treatments of PI and LI agents against BBWV2, TYLCV, and CGMMV infections in *N. benthamiana*.** Inhibition efficiency was calculated by counting plants that were either non-infected or infected with mild symptom development following cross treatments with PI and LI agents followed by challenge inoculation (G).

**4. Effect of iodine•sulfur conjugated organic agent and sialic acid•whey•blood meal conjugated organic agent blended treatment in *N. benthamiana* against virus challenge inoculations**

To further confirm the effect of the agent against virus infections, PepMoV of the genus *Potyvirus* (Luo et al., 2016) and PMMoV of the genus *Tobamovirus* (Secrist & Ali, 2018) were also added along with BBWV2, CMV, and TYLCV used in previous experiments. For treatments, SI and M agents were blended and were used for drenching the test plants thrice prior to virus challenge inoculation.

As for the testing effect of agent treatment against BBWV2 and TYLCV infections, mild symptoms were observed on agent-treated plants (Fig. 4A and C). To identify the replication of viral RNA, RT-PCR was conducted by using each virus-specific primer set. As expected, I could not detect amplified DNA band from the upper leaves of infected plants drenched with blended agents in some plants (Fig. 4B and D).

In the case of CMV, plants treated with blended agents did not completely inhibit the movement of the virus to the upper systemic leaves (Fig. 4G). The qualitative analysis confirmed that virus replication and movement were delayed in only one plant, suggesting that the blended agent was ineffective against CMV infection (Fig. 4H). A similar result was also observed against PMMoV infection (Fig. 4E and F).

As for PepMoV, virus-infected plants did not show any significant difference either in symptom development or in replication of virus on the

upper systemic leaves between plants treated with blended agents and control plants (Fig. 4H and 4I).

Protection and/or inhibition efficiency against these five viruses by blended SI and M agent treatment in *N. benthamiana* were ranged from 11 to 33 % for PMMoV, BBWV2, CMV, and TYLCV while showing a 0 % inhibition against PepMoV (Fig. 4J). My results also showed that blended agent treatment was most effective against BBWV2 infections in *N. benthamiana* plants. The overall efficacy of this blended agent treatment was less effective than cross treatments using powder and liquid types of iodine conjugated organic agents. Regarding the component composition of treated agents, it was found that the efficiency in formulations containing whey or blood meal was less effective and that formulations containing iodine works better against tested viruses in general.

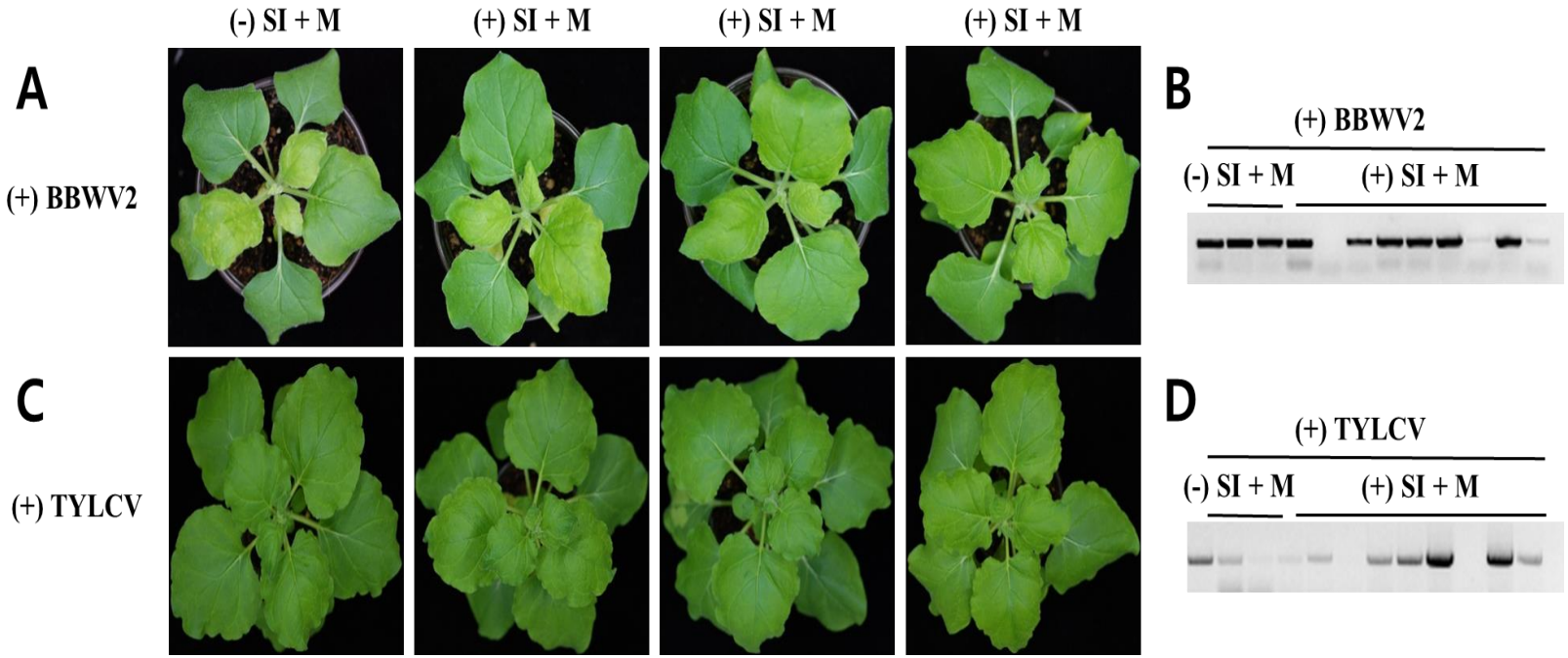



**Figure 4. Effect of the blended SI and M agent against five different virus infections in *N. benthamiana*.** BBWV2 (A) and TYLCV (C) were inoculated on *N. benthamiana*. The plants inoculated by infected leaf tissue were observed under normal light (panels A and C). The viral RNA of BBWV2 and TYLCV in systemic leaves was detected by conducting RT-PCR analysis using virus-specific primer sets (panels B and D).

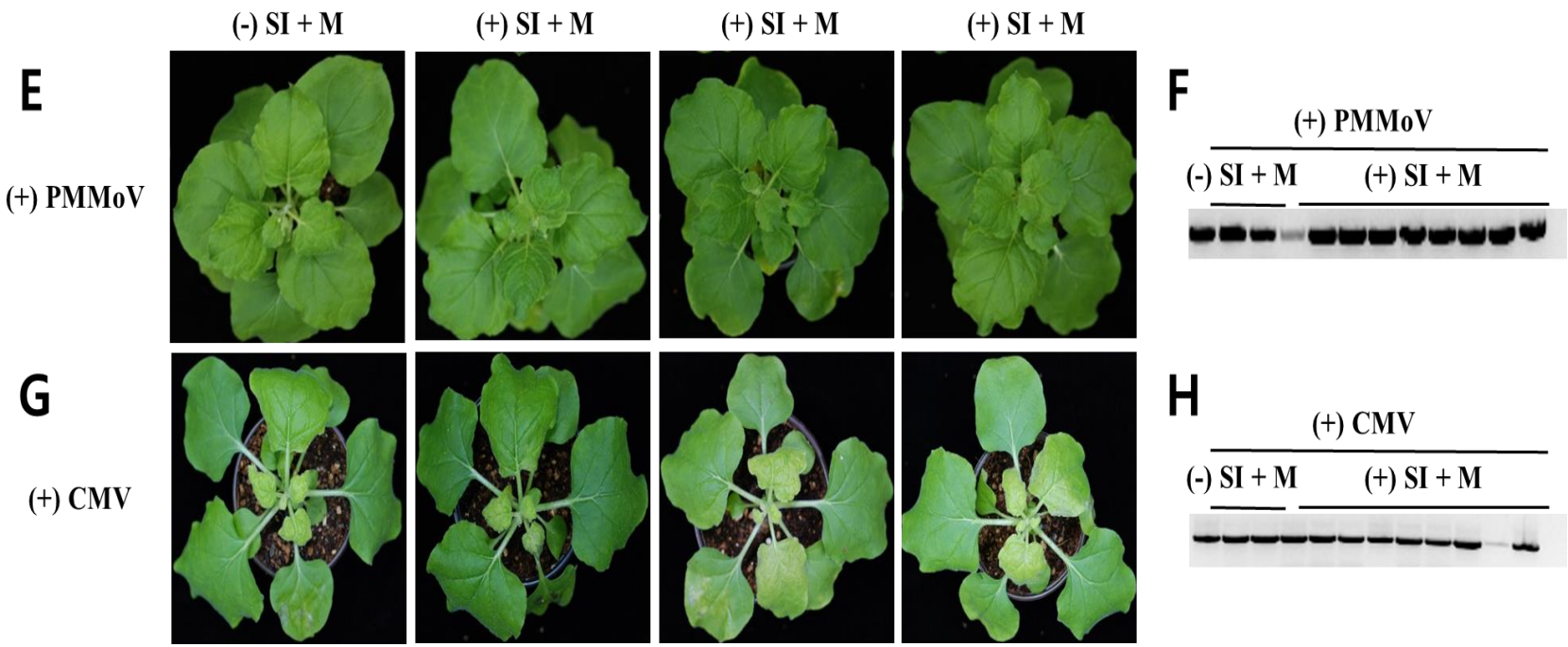
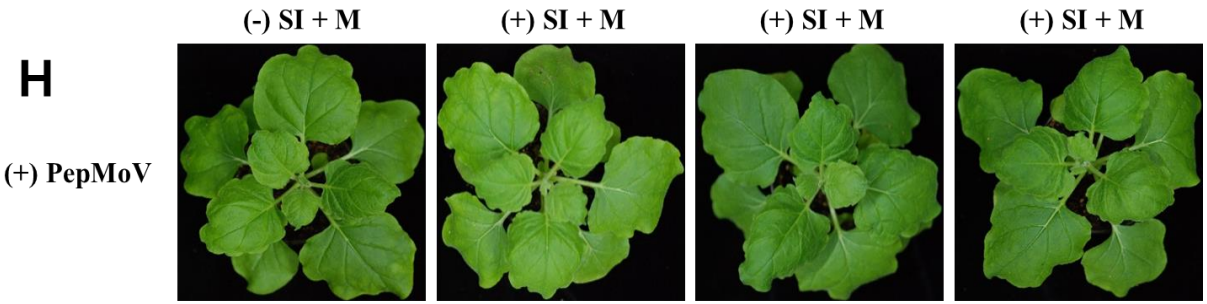
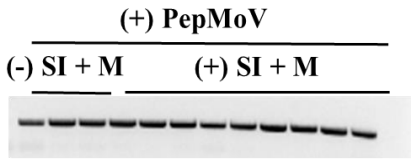



**Figure 4. Effect of the blended SI and M agent against five different virus infections in *N. benthamiana*.** PMMoV (E) and CMV (G) were inoculated on *N. benthamiana*. The plants inoculated by infected leaf tissue were observed under normal light (panels E and G). The viral RNA of PMMoV and CMV in systemic leaves was detected by conducting RT-PCR analysis using virus-specific primer sets (panels F and H).

H




I




J

| Virus | No. of samples | Symptoms | | Inhibition efficiency(%) |
|---|---|---|---|---|
| | | *Non | **Weak | |
| **BBWV2** | 9 | 1 | 2 | 33.3% |
| **TYLCV** | 9 | 2 | - | 22.2% |
| **CMV** | 9 | - | 1 | 11.1% |
| **PMMoV** | 9 | - | 1 | 11.1% |
| **PepMoV** | 9 | - | - | 0% |

*Non: non-infected sample
**Weak: weak symptom

**Figure 4. Effect of the blended SI and M agent against five different virus infections in *N. benthamiana*.** PepMoV (H) was inoculated onto *N. benthamiana*. The plants inoculated by infected leaf tissue were observed under normal light (H). The viral RNA of PepMoV in systemic leaves was detected by conducting RT-PCR analysis using virus-specific primer set (I). Inhibition efficiency was calculated by counting plants that were either non-infected or infected with mild symptom development following the blended SI and M agent treatment (J).

## 5. Effect of sialic acid based-agent treatments in *C. annuum* L. against virus challenge inoculations

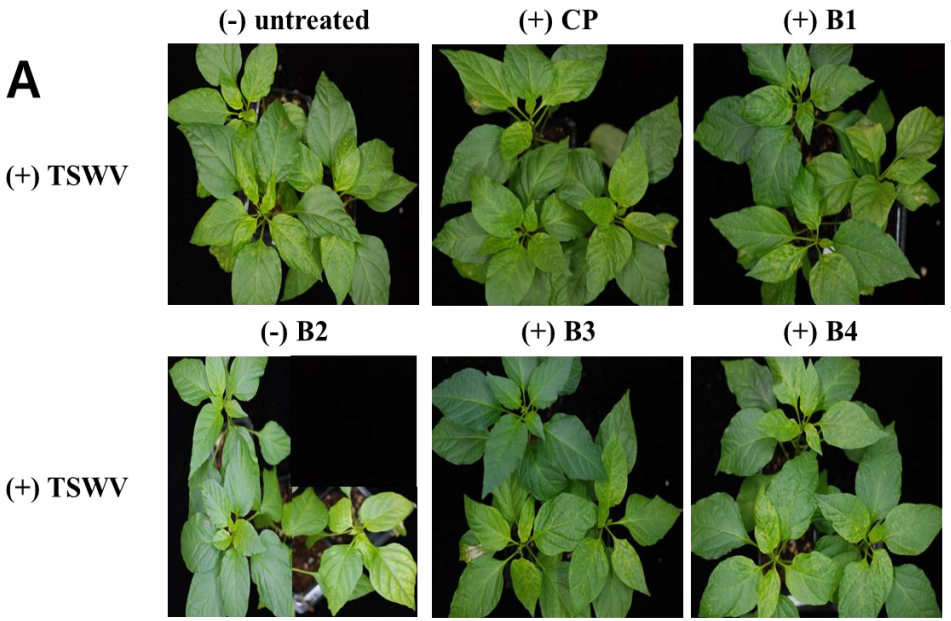
Based on the previous experiment results, B line agents were combined, and the experiment was conducted to confirm the effectiveness of agents against virus infections in pepper. A preliminary pilot study prior to full-scale research was conducted against TSWV of the genus *Tospovirus* (Moyle, Pretorius, Dalton-Morgan, Persley, & Schenk, 2016). Virus-infected *N. benthamiana* leaf tissue was used to inoculate *Capsicum annuum L*. for infection. For objective appraisal, I included one commercial product (CP) that has been announced to be effective against CMV and TSWV infections in my experiment and used it as an additional control to compare effect of treatments in preventing or inhibiting virus infections.

The B line agents were treated on the test plants once prior to virus challenge inoculation and twice after challenge inoculation by drenching method. As shown in Fig. 5C, inhibition efficiency against TSWV infection by B line agent treatment in *C. annuum* L. was ranged from 33 to 100 %, whereas inhibition efficiency of CP was around 33 %. In our view, the aggregated overall inhibitory activity at B line agents is equal or high to the efficiency value obtained with CP treatment. In particular, B3 and B4 treatment significantly prevented TSWV infections in *C. annuum* L. plants (Fig. 5, panels B and C).

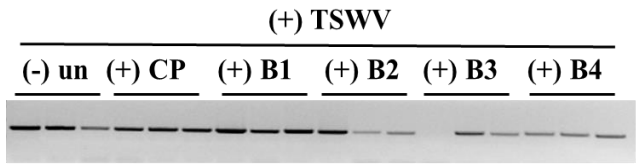
These results suggest that treatment of B line agents negatively affected the replication and/or movement of TSWV and thus inhibited virus replication or delayed symptom development. B line agents composed with sialic acid as

the base and addition of iodine, nitrogen, and sulfur depending on agents. Agent containing iodine and nitrogen (B4) was the most effective against TSWV infection. Accordingly, I conducted additional experiments according to differences in iodine and nitrogen component composition and interval.

A




B




C

| Agent | No. of samples | Symptoms | | Inhibition efficiency(%) |
|---|---|---|---|---|
| | | *Non | **Weak | |
| B1 | 9 | 2 | 1 | 33.3% |
| B2 | 9 | 2 | 3 | 55.6% |
| B3 | 9 | 2 | 6 | 88.9% |
| B4 | 9 | 1 | 8 | 100% |
| CP | 9 | 2 | 1 | 33.3% |

*Non: non-infected sample
**Weak: weak symptom

**Figure 5. Effect of B line agent treatments against TSWV infections in *Capsicum annuum* L.** (A) TSWV was inoculated on *C. annuum* L. For the inoculation, virus-infected *N. benthamiana* leaf tissue was used for inoculation into *C. annuum* L. The plants inoculated by infected leaf tissue were observed under normal light. (B) The viral RNA of TSWV in systemic leaves was detected by conducting RT-PCR analysis using TSWV-specific primer set. (C) Inhibition efficiency was calculated by counting plants that were either non-infected or infected with mild symptom development following the B line agent treatments.

## 6. Effect of sialic acid, iodine, and nitrogen based agents in *C. annuum* L. against virus challenge inoculations

With reference to the previous studies and preliminary tests, A and B lines containing sialic acid, nitrogen and iodine were selected to investigate effect against virus infections. For this, I tested BBWV2, CMV, and TSWV which were previously judged to be effective. Virus-infected *N. benthamiana* leaf tissue was used for inoculations into *Capsicum annuum* L. For the efficiency test, the first treatment was drenched on the 5th day after inoculation except for control plants, and then, the agents were treated with intervals of 5 days or 10 days. Likewise, RT-PCR was conducted by using each virus-specific primer set to identify the replication of viral RNA. For a more detailed qualitative analysis, semi-quantitative PCR was performed in this experiment. The number of cycles were selected based on cycles where I can detect the expected amplified band from 80% or more of plants from control plants. To determine whether the agent treatment caused milder symptom development either by affecting virus movement or RNA replication level, RT-qPCR was performed on the treatment judged to be effective from the visual observation. For this analysis, the upper systemic leaves of treated plants were collected.

As a result, the most significant inhibitory effects by the treatments against TSWV infection were as follows: A4 treatment every 10 days and A5 treatment every 5 days (Fig. 6, panels B and D). For RT-qPCR, the upper systemic leaves of treated plants were collected at 22 days post-inoculation. The accumulation level of TSWV RNAs was significantly reduced in the

plants treated with A4 every 10 days and A5 every 5 days agents compared to untreated control plants (healthy; Fig. 10A).

Overall, I observed inhibitory activity against CMV infection by most treatments as confirmed by RT-PCR analyses (Fig 7, panels B, D, F, and H). In particular, I observed remarkable inhibitory effect with B4 and B5 treatments (Fig. 7, panels F and H). To identify whether the agent treatment caused this inhibitory effect either by delayed movement or by inhibited replication, RT-qPCR was performed (Fig. 10B). The accumulation level of RNAs was significantly decreased in treated plants compared to control. These results indicate that treatment of A and B line agents affected CMV replication and thus delayed movement.

Unlike effects against TSWV and CMV infections, the efficiency of treatment was not significant against BBWV2 infection. Only mild effect was observed (Fig. 8). B4 agent treated every 5 days and B5 agent treated every 5 days showed some inhibitory effect among tested agent treatments (Fig. 8, panels F and H; Fig. 10C).

Altogether, it could be concluded that sialic acid which is main components of the agent along with iodine and nitrogen significantly affected TSWV and CMV replication and movement. These results also suggest that differences in effectiveness in managing virus infection depend on the composition and treatment interval.

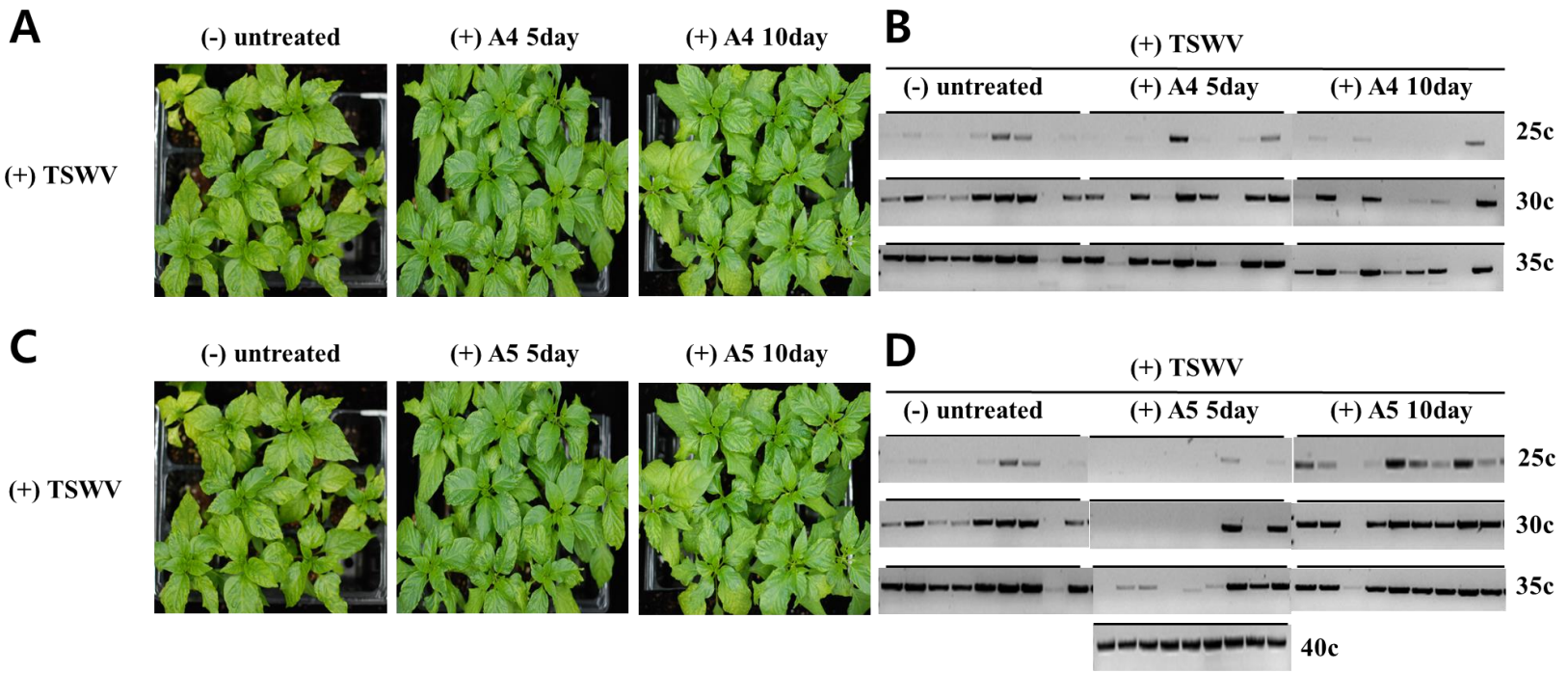



**Figure 6. Effect of A and B line agent treatments against TSWV infections in *C. annuum* L.** (A and C) TSWV was inoculated on *C. annuum* L. The plants inoculated by infected leaf tissue were observed under normal light. (B and D) The viral RNA of TSWV in systemic leaves was detected by semi-quantitative RT-PCR using TSWV-specific primer set.

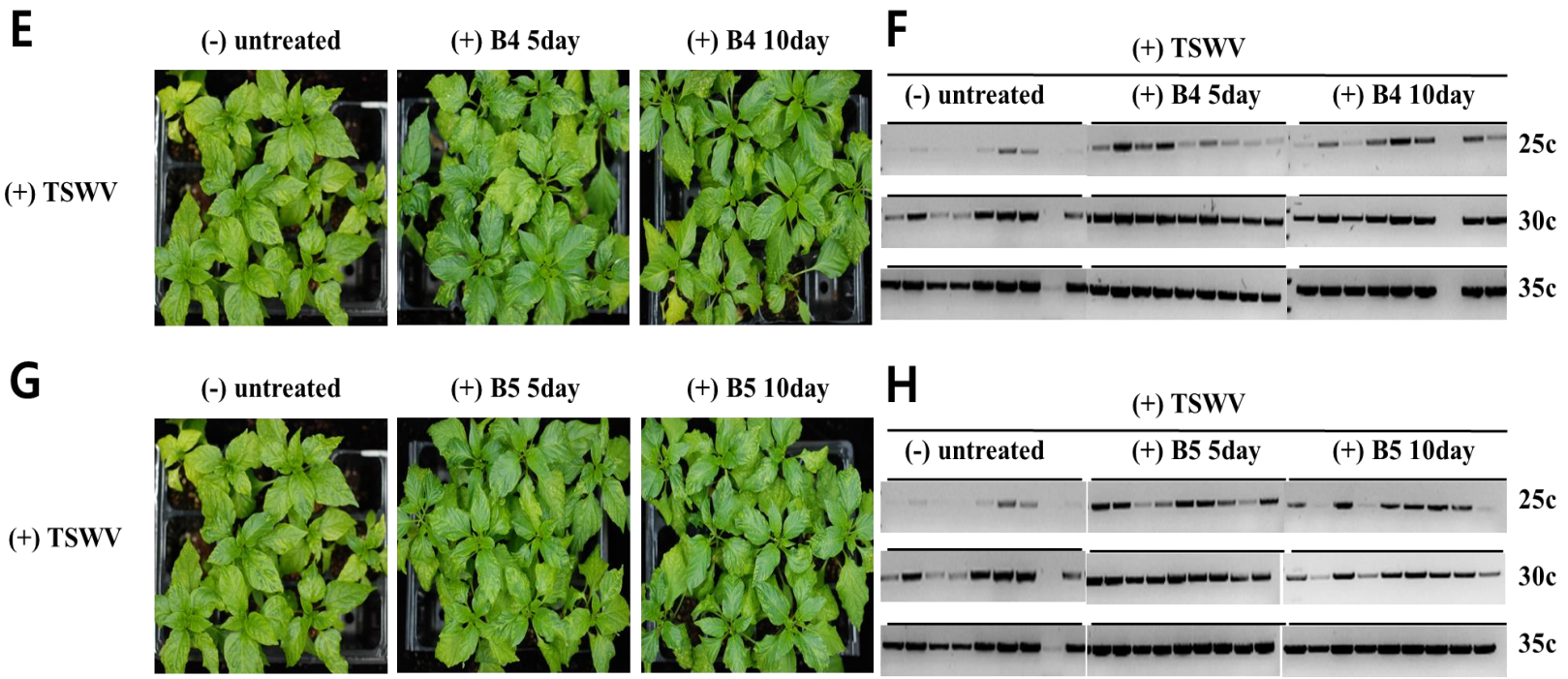



**Figure 6. Effect of A and B line agent treatments against TSWV infections in *C. annuum* L.** (E and G) TSWV was inoculated on *C. annuum* L. The plants inoculated by infected leaf tissue were observed under normal light. (F and H) The viral RNA of TSWV in systemic leaves was detected by semi-quantitative RT-PCR using TSWV-specific primer set.

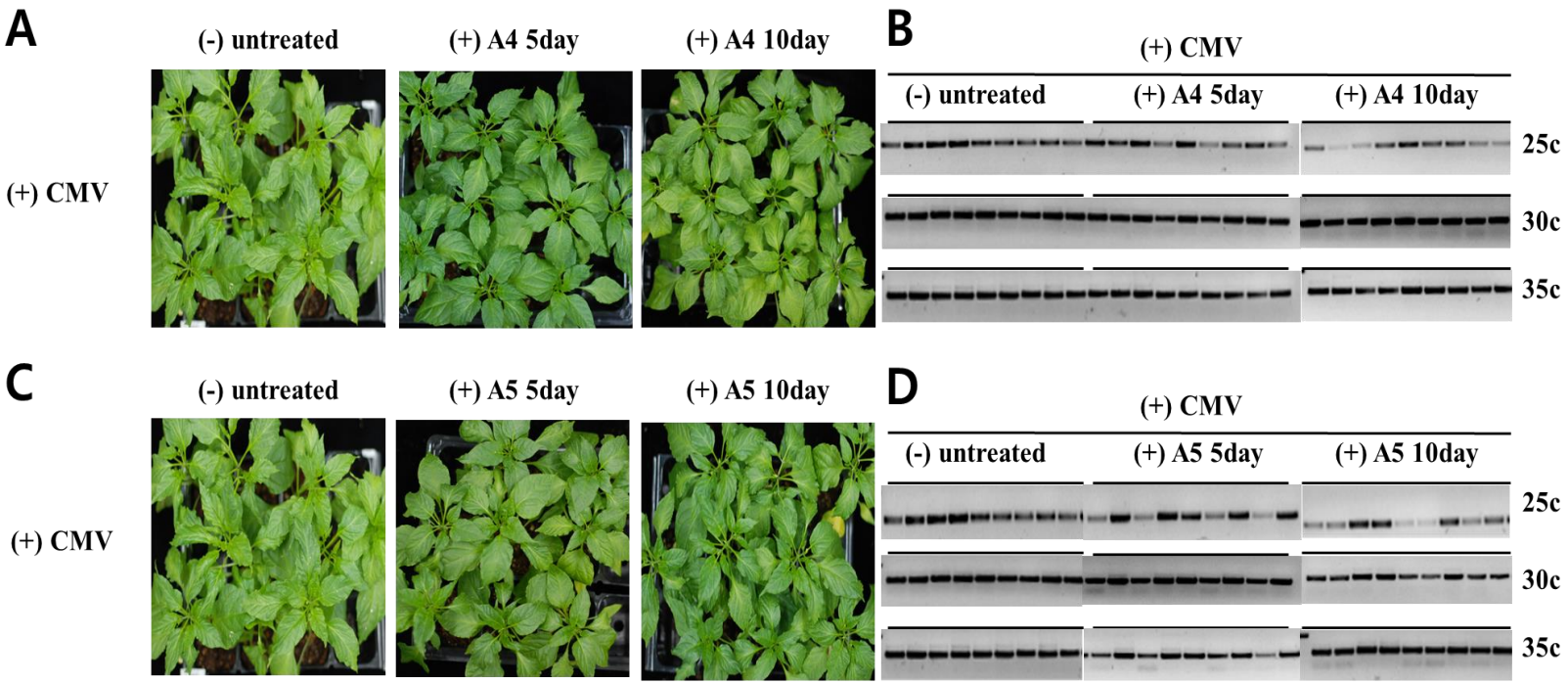



**Figure 7. Effect of A and B line agent treatments against CMV infections in *C. annuum* L.** (A and C) CMV was inoculated on *C. annuum* L. The plants inoculated by infected leaf tissue were observed under normal light. (B and D) The viral RNA of CMV in systemic leaves was detected by semi-quantitative RT-PCR using CMV-specific primer set.

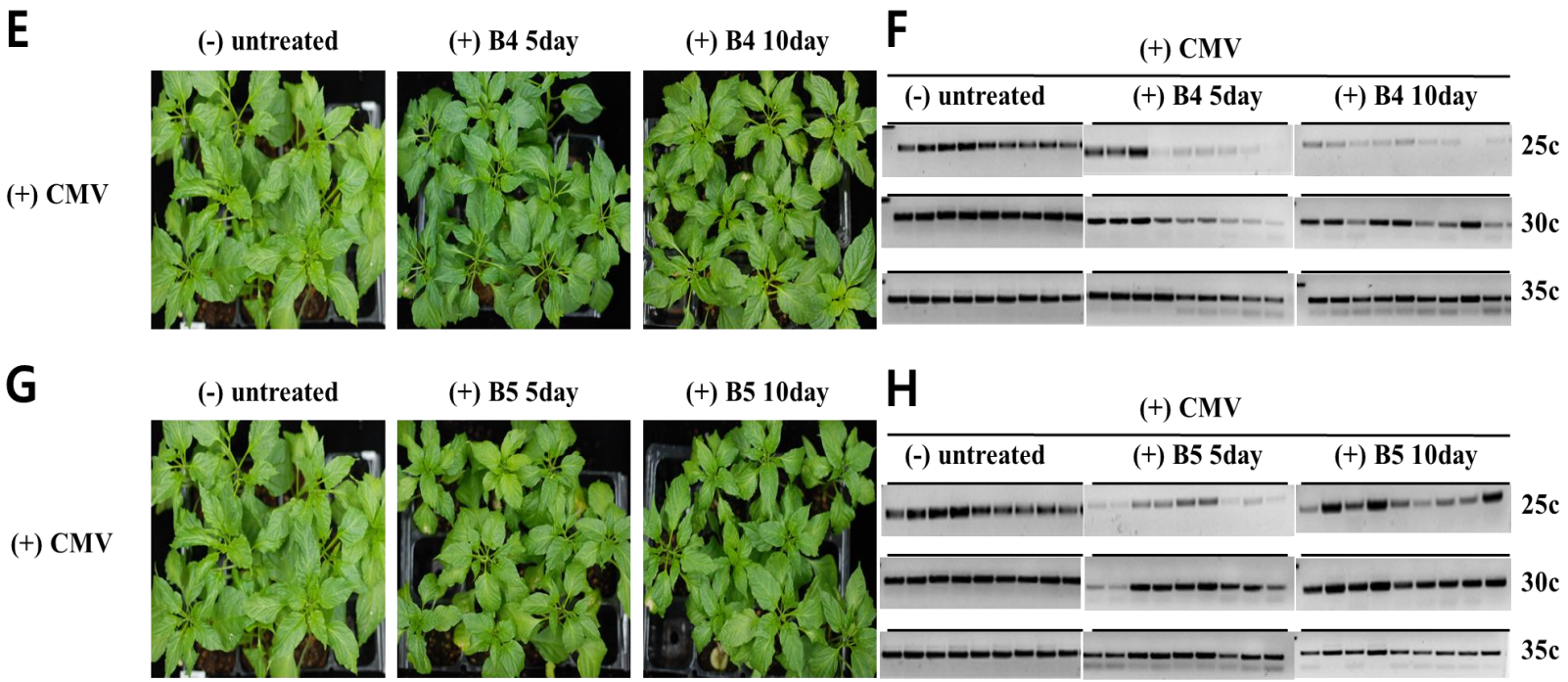



**Figure 7. Effect of A and B line agent treatments against CMV infections in *C. annuum* L.** (E and G) CMV was inoculated on *C. annuum* L. The plants inoculated by infected leaf tissue were observed under normal light. (F and H) The viral RNA of CMV in systemic leaves was detected by semi-quantitative RT-PCR using CMV-specific primer set.

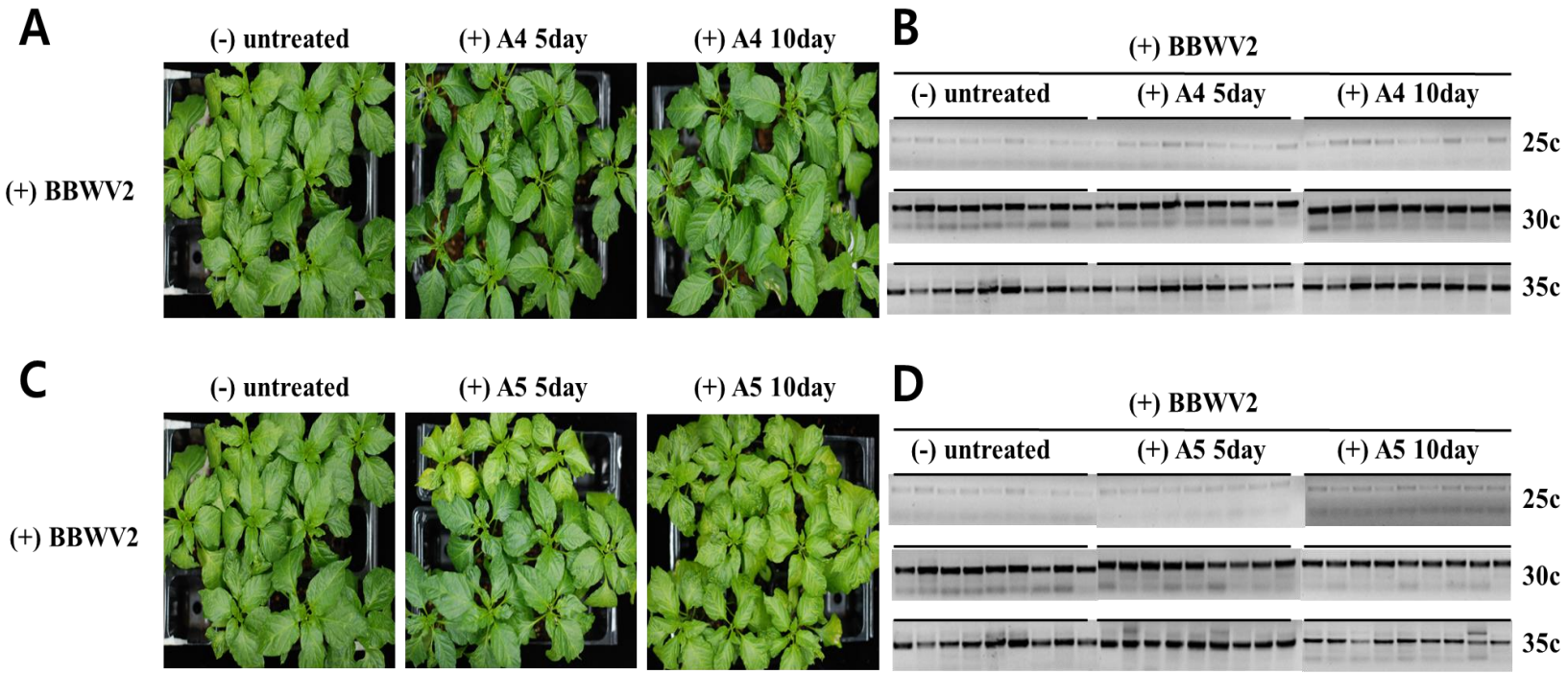



**Figure 8. Effect of A and B line agent treatments against BBWV2 infections in *C. annuum* L.** (A and C) BBWV2 was inoculated on *C. annuum* L. The plants inoculated by infected leaf tissue were observed under normal light. (B and D)The viral RNA of BBWV2 in systemic leaves was detected by semi-quantitative RT-PCR using BBWV2-specific primer set.

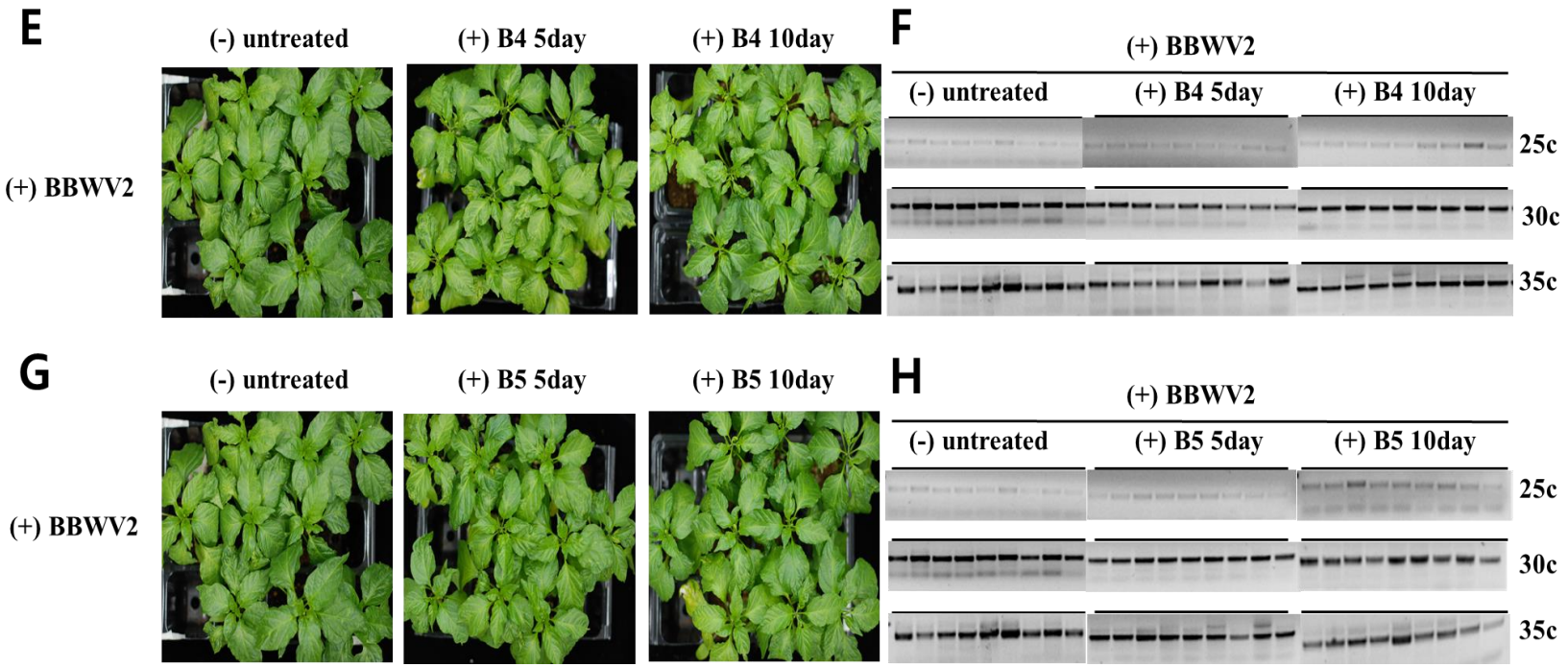



**Figure 8. Effect of A and B line agent treatments against BBWV2 infections in *C. annuum* L.** (E and G) BBWV2 was inoculated on *C. annuum* L. The plants inoculated by infected leaf tissue were observed under normal light. (F and H) The viral RNA of BBWV2 in systemic leaves was detected by semi-quantitative RT-PCR using BBWV2-specific primer set.

| Agent | | No. of samples | ‡35c BBWV2 | | Inhibition efficiency(%) | ‡25c CMV | | Inhibition efficiency(%) | ‡30c TSWV | | Inhibition efficiency(%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Non | **Weak | | *Non | **Weak | | *Non | **Weak | |
| A4 | A4•5day | 9 | - | - | 0% | - | 3 | 33.3% | 2 | 1 | 33.3% |
| | A4•10day | 9 | - | - | 0% | - | 6 | 66.7% | 3 | 3 | 66.7% |
| A5 | A5•5day | 9 | - | - | 0% | - | 4 | 44.4% | 6 | 1 | 77.8% |
| | A5•10day | 9 | - | 1 | 11.1% | - | 6 | 66.7% | 1 | - | 11.1% |
| B4 | B4•5day | 9 | - | 5 | 55.6% | 1 | 5 | 66.7% | - | - | 0% |
| | B4•10day | 9 | - | - | 0% | 1 | 8 | 100% | 1 | - | 11.1% |
| B5 | B5•5day | 9 | - | 1 | 11.1% | - | 9 | 100% | - | - | 0% |
| | B5•10day | 9 | - | 7 | 77.8% | - | 4 | 44.4% | - | 3 | 33.3% |

*Non: non-infected sample
**Weak: weak symptom
‡Nc: number of cycles

**Figure 9. Effect of sialic acid, iodine, and nitrogen based-agents on BBWV2, TSWV, and CMV infections in *C. annuum* L.** Inhibition efficiency was calculated by counting plants that were either non-infected or infected with mild symptom development following the sialic acid, iodine, and nitrogen based-agent treatments.

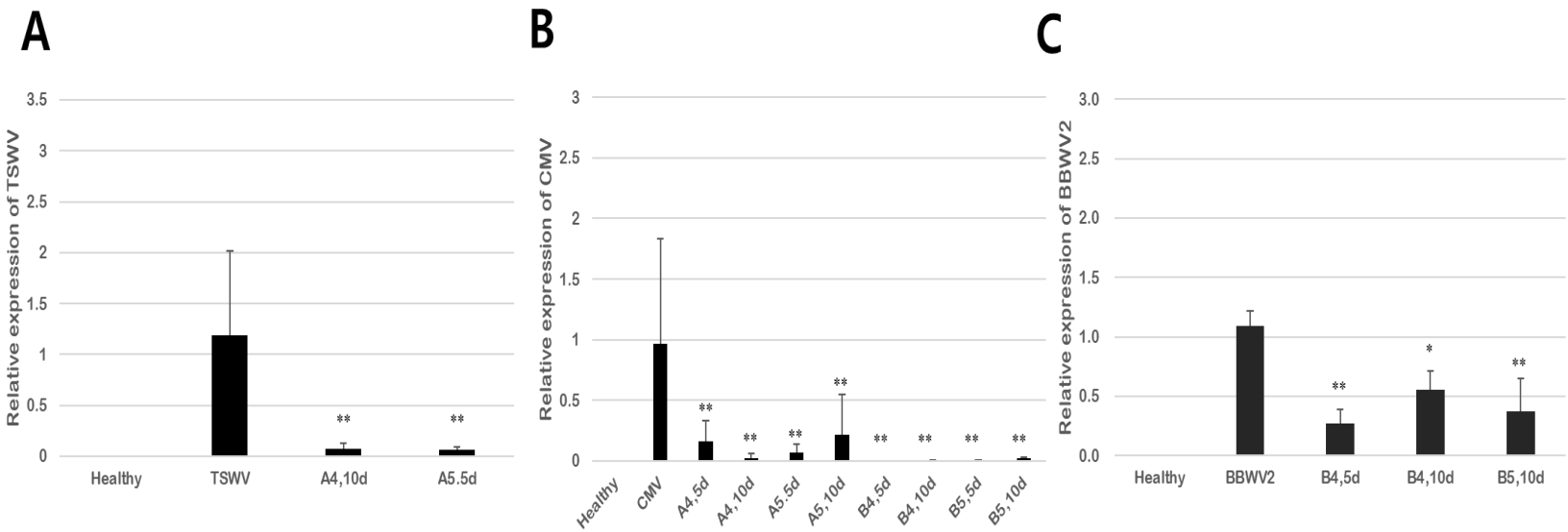



**Figure 10. The relative viral RNA accumulations of TSWV, CMV, and BBWV2 in *C. annuum* L.** One of the experimental groups was treated with only A line agents and the other was treated with B line agents after virus inoculation. (A) Relative viral accumulation in the agent-treated plants relative to the positive control (TSWV only infected plants). (B) Relative viral accumulation in the agent-treated plants relative to the positive control (CMV only infected plants). (C) Relative viral accumulation in the agent-treated plants relative to the positive control (BBWV2 only infected plants). All data were generated by conducting RT-qPCR using *β-tubulin* and *EIF5A2* as reference genes.

# DISCUSSION

New materials for virus control are needed in line with the global trend of rapidly increasing damage from plant virus infections. Therefore, while overcoming the limitations of previously discovered chemicals, interest in organic substances with antiviral effects is high. Among the various organic substances, we tried to find substances that can contribute nutritionally to plants and at the same time, induce resistance to viruses. Among them, I came to pay attention to iodine as the main substance. Originally, iodine was an element that did not receive much attention in terms of plant nutrition. However, as studies have recently begun to show that iodine can contribute to plant nutrition and resistance, it is necessary to think about new possibilities. Accordingly, an agent based on iodine was prepared, and an experiment was conducted on plant virus resistance.

Iodine was used as the main component, and sialic acid, nitrogen, sulfur, whey, and blood meal were added or subtracted. And also, there were differences such as blended treatment, alternative treatment, and cross treatment as necessary. The treated substances did not show side effects such as interference with the plant growth or toxicity, which are the most problematic parts when used antiviral chemical substances in *N. bentamiana* and *C. annuum* L. (Fig. 1). There are seven types of viruses used to evaluate resistance, including BBWV2, CGMMV, CMV, PepMoV, PMMoV, TSWV, and TYLCV and they are known to cause a lot of damage to peppers in Korea. As a result of the agent treatment, it was confirmed that some viruses were

suppressed in BBWV2, CMV, PMMoV, and TYLCV, and among them, it was confirmed that BBWV2, CMV, and TSWV were significantly inhibited in this experiment. Based on these experimental results, an additional experiment was conducted to confirm the efficiency of agents according to the difference between the iodine, sialic acid, and nitrogen composition and the treatment interval. As a result, the accumulation level of TSWV RNAs was significantly reduced in the plants treated with A4 every 10 days and A5 every 5 days agents compared to untreated control plants (Fig. 6). As for CMV, the remarkable inhibitory effect was observed with B4 and B5 agent treatments (Fig. 7). However, unlike TSWV and CMV, only limited efficacy was observed against BBWV2 infection (Fig. 8). These results suggest that differences in antiviral activity depend on the composition and treatment interval (Figs. 6, 7, 8, 9, and 10). Taken together, these results also indicate that these conjugated organic substances might directly or indirectly affect resistance response against virus infections in plants and that agents which made iodine, sialic acid, and nitrogen can be used as antiviral agents for several plant virus species. Research is needed on how iodine, which accounts for the most among agent components, affects the normal physiology of treated plants.

According to the results from previous studies in spinach, white clovers, tomatoes, perennial ryegrass, turnips, barley, flax, wheat, and mustard, plant growth was positively affected by iodine. However, there was no significant difference in growth in buckwheat while showing inhibitory effect on growth with all tested concentrations in oats and turnips (Pauwels, 1961). These

results described above clearly show that iodine can have a favorable effect on plant development for several plant species. It can be inferred that it may have changed complex resistance response by contributing to biologic activity of host plant species when iodine was treated at a specific concentration. In addition, some studies using tomatoes have suggested the possibility of iodine involvement in salicylic acid (SA) metabolism by aromatic iodine compounds (Medrano-Macías et al., 2016). Since peppers and tomatoes belong to the same family biologically, it could be speculated that peppers may have developed resistance by being associated with SA metabolism due to the same or similar mechanism. And also, in terms of the most well-characterized genes specifically regulated by iodine treatments in the shoot (Kiferle et al., 2021), the majority of the up- or down-regulated genes were commonly modulated by the presence of fungal infection, SA or synthetic analogues of SA, such as benzothiadiazole (Kouzai et al., 2018). This suggests that iodine treatments can contribute to the expression of several genes, mainly involved in the plant defense or resistance response and thus protect host plants against various stresses.

Based on these studies and inference, it was presumed that metal organo-iodine contributes to plant resistance mechanisms and it is judged to be of sufficient value to study plant resistance reactions caused by iodine. I think this study showed a new possibility of iodine as agent for managing virus diseases along with its contribution to plant resistance in the field. In addition, in terms of nutritional view, I think it made possible to think that it may be an element that contributes to a no small part of growth. However, since iodine is

not used alone and with other elements either added or subtracted, further experiments is required to see how these elements interacted or affected with iodine in managing virus diseases. Most importantly, in-depth researches are needed in terms of the mode of action on how iodine, which accounts for most of the agents, induces plant resistance.

# LITERATURE CITED


Bawden, F. & Kassanis, B. (1954). Some effects of thiouracil on virus-infected plants. Microbiology 10, 160-173.

Ferrer, R. M., Ferriol, I., Moreno, P., Guerri, J. & Rubio, L. (2011). Genetic variation and evolutionary analysis of broad bean wilt virus 2. Archives of Virology 156, 1445-1450.

Gergerich, R. C. & Dolja, V. V. (2006). Introduction to plant viruses, the invisible foe. The Plant Health Instructor, 478 pp.

Hull, R. (2009). Comparative plant virology: Academic press.

Kiferle, C., Martinelli, M., Salzano, A. M., Gonzali, S., Beltrami, S., Salvadori, P. A., . . . Perata, P. (2021). Evidences for a nutritional role of iodine in plants. Frontiers in Plant Science 12, 7.

Kouzai, Y., Kimura, M., Watanabe, M., Kusunoki, K., Osaka, D., Suzuki, T., . . . Toyoda, K. (2018). Salicylic acid-dependent immunity contributes to resistance against *Rhizoctonia solani*, a necrotrophic fungal agent of sheath blight, in rice and *Brachypodium distachyon*. New Phytologist 217, 771-783.

Kwon, S.-J. & Chung, B.-N. (2018). Incidence and occurrence pattern of viruses on peppers growing in fields in Korea. Research in Plant Disease 24, 67.

Lerch, B. (1987). On the inhibition of plant virus multiplication by ribavirin. Antiviral Research 7, 257-270.

Leyva, R., Sánchez-Rodríguez, E., Ríos, J. J., Rubio-Wilhelmi, M. M., Romero, L., Ruiz, J. M. & Blasco, B. (2011). Beneficial effects of exogenous iodine in lettuce plants subjected to salinity stress. Plant Science 181, 195-202.

Livak, K. J. & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25, 402-408.

Luo, X., Zhang, D., Zheng, L., Peng, J., Li, F., Zhang, S., & Liu, Y. (2016). Development of a reverse transcription loop-mediated isothermal amplification assay for the rapid detection of pepper mottle virus. Canadian Journal of Plant Pathology 38, 506-510.

Matthews, R. E. F. & Hull, R. (2002). Matthews' plant virology: Gulf professional publishing.

Medrano-Macías, J., Leija-Martínez, P., González-Morales, S., Juárez-Maldonado, A. & Benavides-Mendoza, A. (2016). Use of iodine to biofortify and promote growth and stress tolerance in crops. Frontiers in Plant Science, 7, 1146.

Mehra, M., Pasricha, V., & Gupta, R. K. (2015). Estimation of nutritional, phytochemical and antioxidant activity of seeds of musk melon (*Cucumis melo*) and water melon (*Citrullus lanatus*) and nutritional analysis of their respective oils. Journal of Pharmacognosy and Phytochemistry 3, 98-102.

Mengel, K., Kirkby, E. A., Kosegarten, H. & Appel, T. (2001). Potassium. In Principles of Plant Nutrition (pp. 481-511): Springer.

Meshi, T., Watanabe, Y., Saito, T., Sugimoto, A., Maeda, T. & Okada, Y. (1987). Function of the 30 kd protein of tobacco mosaic virus: involvement in cell-to-cell movement and dispensability for replication. The EMBO Journal 6, 2557-2563.

Moyle, R., Pretorius, L.-S., Dalton-Morgan, J., Persley, D. & Schenk, P. (2016). Analysis of the first complete genome sequence of an Australian tomato spotted wilt virus isolate. Australasian Plant Pathology 45, 509-512.

Moyle, R., Pretorius, L.-S., Shuey, L. S., Nowak, E. & Schenk, P. M. (2018). Analysis of the complete genome sequence of cucumber mosaic virus strain K. Genome Announcements 6, e00053-00018.

Pauwels, G. B. (1961). Iodine as a micronutrient for plants. Plant and Soil 14, 377-392.

Pflieger, S., Blanchet, S., Meziadi, C., Richard, M. M. & Geffroy, V. (2015). Bean pod mottle virus (BPMV) viral inoculation procedure in common bean (*Phaseolus vulgaris* L.). Bio-Protocol, 5, e1524.

Rubio, L., Galipienso, L. & Ferriol, I. (2020). Detection of plant viruses and disease management: relevance of genetic diversity and evolution. Frontiers in Plant Science 11, 1092.

Sastry, K. S. & Zitter, T. A. (2014). Management of virus and viroid diseases of crops in the tropics. In Plant virus and viroid diseases in the tropics (pp. 149-480): Springer.

Secrist, K. & Ali, A. (2018). First complete genome sequence of pepper mild mottle virus from chili pepper in the United States. Genome Announcements 6, e00331-00318.

Silva-Moreno, E., Brito-Echeverría, J., López, M., Ríos, J., Balic, I., Campos-Vargas, R. & Polanco, R. (2016). Effect of cuticular waxes compounds from table grapes on growth, germination and gene expression in *Botrytis cinerea*. World Journal of Microbiology and Biotechnology 32, 74.

Sun, W. Q. & Leopold, A. C. (1995). The Maillard reaction and oxidative stress during aging of soybean seeds. Physiologia Plantarum 94, 94-104.

Takahashi, H., Fukuhara, T., Kitazawa, H. & Kormelink, R. (2019). Virus latency and the impact on plants. Frontiers in Microbiology 10, 2764.

Wan, H., Yuan, W., Ruan, M., Ye, Q., Wang, R., Li, Z., . . . Liu, S. (2011). Identification of reference genes for reverse transcription quantitative real-time PCR normalization in pepper (*Capsicum annuum* L.). Biochemical and Biophysical Research Communications 416, 24-30.

# 고추를 감염시키는 주요 바이러스에 대한 천연 유래 아이오딘 탑재형 유기태화 제제의 항바이러스 효능 검정

장수연

## 초록

식물 바이러스는 전 세계적으로 주요 작물의 품질과 생산량에 영향을 끼쳐 상당한 경제적 손실을 초래한다. 이들 식물바이러스병은 국제 교류의 확대와 기후변화 등으로 인해 우려가 커지고 있는 상황이다. 이러한 기후 변화는 식물 바이러스의 발생과 병원성에 영향을 미칠 수 있으며, 결과적으로 질병 발생 빈도와 규모를 증가시킬 수 있다. 게다가, 신종 병원균과 변종 병원균이 매년 발견되고 있기 때문에 발발하는 질병에 관한 관리방법 수립의 중요성이 증가하고 있다. 바이러스성 질병에 대한 방제 전략은 주로 비화학적, 경종적 방법에 의존하는데 이는 상업적으로 이용 가능한 제제가 없으며, 주로 저항성 품종이나 매개충을 방제함으로써 관리가 되기 때문이다. 선행 연구에서, 항바이러스 효과가 입증된 여러 화학물질이 밝혀졌지만, 이들은 몇 가지 문제점을 내제하고 있다; i) 바이러스 증식 억제를 위해 필요한 화학물질의 농도가 너무 높기 때문에 사용에 있어 규제가 엄격하다. ii) 식물 생육에 있어 장애를 일으키고 약해를 보였으며, iii) 목표 유기체에

도달하는 화학물질의 양은 극도로 작고, 남은 화학물질은 주변 환경을 오염시켰다. 이러한 화학물질의 문제점들을 해결하기 위해 식물바이러스병 방제 및 관리를 위한 천연물 유래 신규 물질 개발이 증가하고 있다.따라서 본 연구에서는 천연 광물성 유래 아이오딘 유기태화 제제의 항바이러스성 효과를 검정하고 잠재적인 항바이러스 제제로의 개발 가능성을 확인하고자 하였다. 이를 위해, *Nicotiana benthamiana* 와 *Capsicum annuum* L. 에 한국에서 고추에 큰 피해를 입히고 있는 바이러스 7 종인 *Broad bean wilt virus 2 (BBWV2; Fabavirus), Cucumber green mottle mosaic virus (CGMMV; Tobamovirus), Cucumber mosaic virus (CMV; Cucumovirus), Pepper mottle virus (PepMoV; Potyvirus), Pepper mild mottle virus (PMMoV; Tobamovirus), Tomato spotted wilt virus (TSWV; Tospovirus), and Tomato yellow leaf curl virus (TYLCV; Begomovirus)*을 대상으로 효능 검정을 실시하였다. 후보 제제는 바이러스 접종 전, 후 관주처리 해준 뒤 바이러스의 증식 및 이동을 관찰하였다. 이에 CGMMV, PepMoV 의 경우, 대조군과 비교하여 후보제제를 처리한 식물 상엽으로의 바이러스 이동이 억제되지 않았으며, RT-PCR 결과에서 동일한 결과를 관찰할 수 있었다. 이는 바이러스의 증식과 이동이 억제되지 않았음을 뜻한다. 반면, BBWV2, TYLCV 와 TSWV 에서 일부 개체가 대조군에 비하여 증상 발생 정도가 약화된 것을 관찰할 수 있었다. RT-PCR 결과에서 동일한 결과를 관찰할 수 있었으며,

이는 바이러스의 증식과 이동이 일부 억제되었음을 나타낸다. 특히나, TSWV 와 BBWV2 에서는 일부 제제가 바이러스를 유의미하게 억제시킨다는 것을 RT-qPCR 을 통해 확인할 수 있었으며, CMV 에서는 대부분의 개체가 대조군에 비하여 증상 발생 정도가 약화된 것을 관찰할 수 있었다. 이는 대상 제제가 CMV 의 증식과 이동에 상당한 효과가 있음을 시사하며, 이와 동일한 결과를 RT-qPCR 결과에서 CMV RNA 축적 감소를 통해 확인할 수 있었다. 본 연구를 통해 유기태화된 아이오딘, 질소, 시알산 등이 식물 바이러스 감염에 대한 내성에 직, 간접적으로 영향을 미칠 수 있음을 확인했다. 이러한 결과는 새로운 분야에서의 천연물을 소재로한 바이러스 방제제의 후보 물질 개발 가능성을 제시할 뿐만 아니라 아이오딘, 시알산 등이 잠재적인 조절자로 식물의 내성 및 저항성에 영향을 미칠 수 있음을 시사한다.

**주요어:** 아이오딘, 항바이러스제, 바이러스 증식 억제, 유기제제, 유기태화

학 번: 2020-28266