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Evaluation of the Weeds around *Capsicum annuum* (CA) Cultivation Fields as Potential Habitats of CA-Infecting Viruses

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***Capsicum annuum* (CA) is grown outdoors across fields in Jeollabuk-do, South Korea. The weeds surrounding these fields were investigated regarding the infection of 11 viruses infecting CA during the year 2014-2018. In the reverse transcription polymerase chain reaction diagnosis, 546 out of 821 CA samples (66.5%) were infected by nine viruses, and 190 out of 918 weed samples (20.7%) were infected by eight viruses. Correlation analysis of the mutual influence of the viruses infecting CA and weeds during these 5 years showed that five viruses had significant positive correlations with the infection in both CA and weeds. Over the study period, the weeds infected by cucumber mosaic virus (CMV) in the previous year were positively correlated with the incidence of CMV infection in CA in the current year, although the correlation was lower for tomato spotted wilt virus (TSWV) compared to CMV. The CMV infection percent was 14.0% in summer annuals, 11.4% in perennials, and 7.8% in winter annuals. However, considering the overwintering period without CA, the infection percent was 5.2% higher in winter annuals and perennials than that in summer annuals, indicating that winter annual and perennial weeds served as the main habitats for insect vectors. The TSWV infection percent in weeds was 10.4% in summer annuals, 6.4% in winter annuals, and 6.2% in perennials. The weeds surrounding CA fields, acting as the intermediate hosts, were found to be the potent sources of infection, influencing the spread and diversity of CA-infecting viruses. The results of this study can contribute to prevent viral infection in agricultural fields.**

Keywords **:** *Capsicum annuum*, cucumber mosaic virus, tomato spotted wilt virus

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Capsicum annuum L. (CA) of the Solanaceae family is an important economic crop for farm household income. In South Korea, the CA cultivation area in 2021 was 37,761 ha (total crop production: 92,757 tons), with the largest area in Gyeongsangbuk-do at 8,902 ha (23.6%), followed by Jeollanam-do at 5,800 ha (15.4%), and Jeollabuk-do at 4,333 ha (11.5%) (Korean Statistical Information Service, 2022a).

In the region of Jeollabuk-do, the field cultivation of CA among the plains is concentrated mainly in Jeongeup-si, Gochang-gun, and Imsil-gun in order of most extensive area (Korean Statistical Information Service, 2022b), where stable production is a challenge due to repeated cultivation, climate change, and outbreaks of thrips, anthracnose, and viral disease. Among these, the virus causes diseases that deteriorate the quality and commercial value of CA. Moreover, the viruses are relatively difficult to control, so they pose one of the largest challenges to CA cultivation (Kwon et al., 2018). There are 68 reported viruses known to infect CA worldwide (Kenyon et al., 2014). Among them, the following 16 viruses are known to affect CA cultivation in South Korea: alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), broad bean wilt virus 2 (BBWV2), pepper mottle virus (PepMoV), potato virus Y (PVY), pepper severe mosaic virus (PepSMV), chilli veinal mottle

virus (ChiVMV), pepper vein chlorosis virus (PVCV), tomato spotted wilt virus (TSWV), impatiens necrotic spot virus (INSV), potato virus X (PVX), tobacco mosaic virus (TMV), tobacco mild green mosaic virus (TMGMV), tomato mosaic virus (ToMV), pepper mild mottle virus (PMMoV), and bell pepper mottle virus (BPMV) (Choi et al., 2002, 2005, 2010; Im et al., 1991; Kim et al., 1990, 2012; Kwak et al., 2013; Lee et al., 2004). The influence of five of these viruses on the local CA industry is negligible since PepSMV and BPMV have not been reported to cause infection in CA in South Korea, with only their base sequences identified. In contrast, PVCV (1990), ChiVMV (1991), and PVX (1991) have not caused any incidence after the first incidence in their respective years (Im et al., 1991; Kim et al., 1990; Kwon et al., 2018).

From the perspective of the pathophysiology of plant viral disease, the weeds around crop cultivation fields play a key role in the spread of plant viral disease. Weeds are known as the intermediate host to the virus, the site of overwintering, and the habitats of insect vectors that spread the virus (Kwon et al., 2016). Several studies (Arli-Sokmen et al., 2005; Kaliciak and Syller, 2009; Korbecka-Glinka et al., 2021) have been conducted, especially the study by Hobbs et al. (2000) on the weeds around Solanaceae plants as the infection source of CMV in Illinois, USA. However, the number of such studies is comparatively low, considering the crucial role of weeds in the incidence of viral disease.

Thus, this study investigated the viral infection in CA and weeds around CA showing suspicious symptoms across the CA cultivation fields in Jeollabuk-do, South Korea, from 2014-2018. The relevant correlations were also analyzed. The 11 viruses whose infection percent was determined in this study included the nine main CA-infecting viruses; TMV, TMGMV, CMV, TSWV, BBWV2, PMMoV, PepMoV, PVY, and AMV, which have continuously caused infection in South Korea, and two additional viruses; beet western yellows virus (BWYV; its first incidence was in paprika in 2010) (Park et al., 2011) and tomato chlorosis virus (ToCV; its first incidence was in tomato in 2015, in Nonsan-si, Iksan-si, Jeju-si, Hampyeong-gun, and Hwasun-gun) (Kil et al., 2015). Correlation analyses were also performed based on the annual infection frequency in CA and weeds around CA regarding the infected weeds as the intermediate host and the subsequent incidence of viral infection in CA in the following year concerning winter climate conditions. The infected weeds were analyzed by species and life cycles.

Materials and Methods

Study site and sample collection. The incidence and type of viral disease patterns in field-grown CA and weeds around CA were analyzed. The study sites were in the main cultivation fields of Jeollabuk-do, South Korea; Gochang-gun (9 sites), Jeongeup-si (9 sites), and Imsil-gun (9 sites). The study period was June-August in 5 years from 2014-2018. The investigation was divided into the early growth and post-harvest phases in compliance with the Application Guidelines for Crop Pest Monitoring and Control (Rural Development Administration, 2014). The samples were collected mainly from leaves (three leaves per plant) suspected of viral infection through visual examination of the symptoms of the disease in CA and weeds around CA. The number of plants during sample collection was not constant due to the differences in viral disease incidence across different fields. The total number of collected samples over the 5 years was 821 for CA and 918 for weeds around CA.

Viral RNA extraction and reverse transcription polymerase chain reaction analysis. The diagnosis of viral disease was based on reverse transcription polymerase chain reaction (RT-PCR) analysis. First, the collected sample was ground using liquid nitrogen, and the total RNA was extracted using the PowerPrep Viral DNA/RNA Extraction Kit (Kogenebiotech, Seoul, Korea). Each RNA was screened using the primers specific to the 11 viruses (Table 1). For nucleic acid amplification, the TOPscript One-step RT PCR DryMIX kit (Enzynomics, Daejeon, Korea) was used, and the reaction condition was as follows: 50°C, 30 min; 95°C, 10 min; <95°C, 30 s; 50-55°C, 30 s; 72°C, 1 min >35 cycles; 72°C, 5 min. Each PCR product was checked for infection through electrophoresis in 1.2% agarose. Electrophoresis included the 100 bp DNA Ladder (Invitrogen, Carlsbad, CA, USA) to identify the size of each PCR product. The WSE-5300 Printgraph CMOS I (Atto, Tokyo, Japan) was used for measurement.

Incidence of viral infection in CA and weeds. To analyze the incidence of viral infection in CA and weeds, the infection percent (%) was calculated using the formula in Odum (1971) (Eq. 1). The infection percent was estimated for 821 CA and 918 weed samples collected during 5 years (2014-2018) by checking the state of infection via RT-PCR analysis.

Table 1. List of CA-infecting virus detection primers used in this study

Virus	Primer	Primer sequences	Temperature (°C)	Amplified size (bp)
TMV	TMV-up TMV-down	CTACTGTCGCCGAATTCGATTCG TTTAGAATTCATCTTGACTACC	50	531
TMGMV	CPTMG-S CPTMG-R	TCGAGTACGTTTTAATCAAT ATTTTAGGAAATCTCACAAC	50	524
CMV	CMV DP u1 CMV DP d2	CGTCGTGGTTCCCGCTCCG AGCGCGCATCGCCGAAAGAT	55	473
TSWV	TSWV 6F TSWV 6R	GAGATTCTCAGAATTCCCAGT AGAGCAATCGTGTCAATTTTATTC	55	459
BWYV	BWYV-95F BWYV-784R	CGAATCTTGAACACAGCAGAG TGTGGGATCTTGGATAGG	55	690
BBWV	BBWV2 1-1u BBWV2 1R	AAACAAACAGCTTTCGTTCCG GCCATCTCATTGGCATGGA	55	380
PMMoV	PMMoV 6F PMMoV 6R	CAGTTTCCAGTGCCAATCAATTA GTTGTAGCCCAGGTGAGTCCACTC	55	456
PepMoV	PepMoV-U1 PepMoV-D1	AATGGCACGTCCCCAAA TCTCTCTCATGCCAACTACGA	55	705
PVY	PVY-N40 PVY-C10	GCATACGACATAGGAGAAACTG TATGATAAAAGTAGTACAGG	55	550
ToCV	ToCV-M-4F ToCV-M-4R	AGAAGATCCGCGCTAATGCTAA GGTCATCTTCCCAAACACGA	55	479
AMV	AMV-a AMV-b	CGCATGGGTAGGAGCTGTGAAGAC CTGGTGGGAAAGCTGGTAAAC	55	440

CA, *Capsicum annuum*; TMV, tobacco mosaic virus; TMGMV, tobacco mild green mosaic virus; CMV, cucumber mosaic virus; TSWV, tomato spotted wilt virus; BWYV, beet western yellows virus; BBWV, broad bean wilt virus; PMMoV, pepper mild mottle virus; PepMoV, pepper mottle virus; PVY, potato virus Y; ToCV, tomato chlorosis virus; AMV, alfalfa mosaic virus.

$$\text{Infection percent (\%)} = \frac{\text{No. of infection-confirmed samples}}{\text{No. of total samples}} \times 100 \quad (1)$$

Correlation analysis on viral infection in CA and weeds. The correlation of viral infection between CA and weeds around CA was analyzed. Based on the viral infection frequency of the study period (5 years), we analyzed six viruses out of eight that simultaneously infected CA and weeds, excluding TMGMV and PMMoV, which are known to be transmitted by seeds. Next, the correlation of annual viral infection was analyzed for CMV and TSWV, whose continuous infection in CA and weeds was confirmed for the study period based on the annual infection frequency of CMV and TSWV in CA and weeds. To analyze the correlation, Spearman's rank correlation coefficient ρ (rho) was used with the level of significance set at $\alpha = 0.05$. The IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA) was used for statistical analyses.

Causal analysis based on climate conditions and weed species and life cycles. Data on climate conditions were used to analyze the cause of the large annual variation in the incidence of viral disease in CA. As viral infection is closely associated with the overwintering of insect vectors, the mean, minimum, and maximum temperatures of the overwintering period (from December to February of the following year) during 2014-2018 were analyzed.

Each weed species was identified to analyze the influence of weeds as the primary infection source of the viral disease in CA. The collected weeds were listed and annotated based on the National Biological Species Information System (http://www.nature.go.kr) and the Illustrated Book of Weeds (National Institute of Agricultural Sciences, 2017). The identified weeds were divided based on life cycles into summer annuals, winter annuals, and perennials, and the respective infection percents were analyzed.

Results and Discussion

Incidence of viral infection in field-grown CA. To investigate the incidence of viral infection in CA across the fields in Jeollabuk-do, a total of 821 CA samples were collected from Imsil-gun, Jeongeup-si, and Gochang-gun during the 5 years from 2014 to 2018. In the RT-PCR analysis of 11 viruses, it was found 546 CA plants (66.5%) were infected by nine viruses (Table 2).

The highest infection percent among all study sites was observed for BBWV2 and PMMoV at 35.9% and 31.3%, respectively, while CMV also showed a high percent of 26.9%. The infection percents for TSWV and PepMoV were 20.0% and 19.9%, respectively. BWYV had an infection percent of 6.6%, and PVY had 1.9%. TMGMV had 0.6%, and ToCV had 0.4%. No infection by TMV or AMV was detected during the study period.

The results indicated infection by nine viruses, seven of which were previously reported by Kwon et al. (2018): CMV, BBWV2, TSWV, BWYV, PVY, PepMoV, and PMMoV. These viruses were detected during 2015-2016 in Jeollabuk-do (Iksan-si, Imsil-gun, Gochang-gun, and Wanju-si). Additionally, six of the viruses were reported by Choi et al. (2005): PMMoV, TMGMV, PepMoV, BBWV2, TSWV, and CMV. These viruses were detected during 2001-2004 in various regions of South Korea. Furthermore, one virus, ToCV, was reported by Kil et al. (2015), and its first incidence in tomatoes in South Korea was detected in 2013.

The incidence of BBWV2, PMMoV, CMV, and TSWV was detected annually. Among these, BBWV2, PMMoV, and CMV showed a high level of incidence, with 295, 257, and 221 cases, respectively. On the other hand, the incidence of TSWV was relatively low, with n = 164. However, the incidence of PepMoV, BWYV, PVY, ToCV, and TMGMV was not detected annually (Table 2). This finding aligns with the study conducted by Kim et al. (2012) over a period of 5 years from 2007 to 2011, where the incidence of PepMoV, PVY, TMGMV, and other viruses was not detected annually. Therefore, the annual infection frequency of these five viruses, namely PepMoV, BWYV, PVY, ToCV, and TMGMV, was low in CA cultivation fields.

Incidence of viral infection in weeds around CA fields. To study the incidence of viral infection in weeds around CA fields, 918 samples were collected from Imsil-gun, Jeongeup-si, and Gochang-gun during the 5 years from 2014-2018. In the RT-PCR analysis of 11 viruses, 190 weeds (20.7%) were infected by eight viruses (Table 3).

The highest viral infection percent across all study sites was shown by PMMoV and CMV at 10.9% and 7.2%, respectively, and the infection percent of BBWV2 was 6.2%. For TSWV, PepMoV, BWYV, ToCV, and TMGMV, the infection percent was 3.9%, 0.7%, 0.2%, 0.2%, and 0.1%, respectively, and no infection by TMV, PVY, or AMV was detected during the study period. The infection frequency was the highest at 100 cases for PMMoV, although no infection was detected in 2017. For CMV with a broad scope of hosts, the incidence during the 5 years was n = 66, with

Table 2. Frequency and infection percent of virus in CA

Virus	Survey year						Infection percent (%)
	2014	2015	2016	2017	2018	Total	
BBWV2	52	105	31	44	63	295	35.9
CMV	50	56	54	47	14	221	26.9
PepMoV	15	27	113	8	0	163	19.9
BWYV	9	36	0	4	5	54	6.6
PVY	12	0	4	0	0	16	1.9
TSWV	15	27	58	33	31	164	20.0
ToCV	0	0	3	0	0	3	0.4
PMMoV	41	113	39	1	63	257	31.3
TMGMV	0	0	5	0	0	5	0.6
Non-infection	51	55	23	92	54	275	-
Total	106	180	186	182	167	821	-

CA, *Capsicum annuum*; BBWV 2, broad bean wilt virus 2; CMV, cucumber mosaic virus; PepMoV, pepper mottle virus; BWYV, beet western yellows virus; PVY, potato virus Y; TSWV, tomato spotted wilt virus; ToCV, tomato chlorosis virus; PMMoV, pepper mild mottle virus; TMGMV, tobacco mild green mosaic virus.

Table 3. Frequency and infection percent of viruses in weeds around CA fields

Virus	Survey year						Infection percent (%)
	2014	2015	2016	2017	2018	Total	
BBWV2	42	4	0	0	11	57	6.2
CMV	31	15	10	1	9	66	7.2
PepMoV	6	0	0	0	0	6	0.7
BWYV	0	2	0	0	0	2	0.2
TSWV	0	5	29	1	1	36	3.9
ToCV	0	0	2	0	0	2	0.2
PMMoV	41	45	3	0	11	100	10.9
TMGMV	0	1	0	0	0	1	0.1
Non-infection	75	124	187	200	142	728	-
Total	153	172	225	202	166	918	-

CA, *Capsicum annuum*; BBWV 2, broad bean wilt virus 2; CMV, cucumber mosaic virus; PepMoV, pepper mottle virus; BWYV, beet western yellows virus; TSWV, tomato spotted wilt virus; ToCV, tomato chlorosis virus; PMMoV, pepper mild mottle virus; TMGMV, tobacco mild green mosaic virus.

the infection detected annually. For TSWV, the infection frequency was n = 36 for 4 years, excluding of 2014. For PepMoV, BWYV, ToCV, and TMGMV, the infection frequency was ≤6 cases (Table 3).

The incidence pattern in weeds showed that, while the infection percent was low compared to CA, the viruses PMMoV, CMV, BBWV2, and TSWV mainly infected the weeds around CA, as with CA itself. This implied that weeds and wild plants frequently functioned as viral habitats to affect the viral diseases in crops of nearby fields, which agrees with the study by Hasiów-Jaroszewska et al. (2021) reported that weeds and crops can spread viral diseases to one another.

Correlation of viral infection in CA and weeds. A correlation analysis was performed on the mutual influence of viruses infecting CA and weeds around CA within the agricultural ecosystem during the study period (2014-2018) (Table 4). Except for ToCV and BWYV, which displayed low infection frequency, significant positive correlations were shown by four viruses between CA and weeds. The correlation was the highest between weed-infecting CMV and CA-infecting CMV at $\rho = 0.32^{**}$, followed by weed-infecting BBWV2 and CA-infecting BBWV2 at $\rho = 0.26^{**}$; weed-infecting PepMoV and CA-infecting PepMoV at $\rho = 0.17^{**}$; weed-infecting TSWV and CA-infecting TSWV at $\rho = 0.16^{**}$. The results indicated that the main CA-infecting viruses could have been influenced by the infection of weeds around CA, thereby implicating the potential spread of viral infection. Thus, it is conjectured that the weeds around CA are the intermediate host and a potent infection source that influence the spread and diversity of the virus

Table 4. Correlation analysis of virus types infecting CA and weeds

Virus	Pe_ CMV[a]	Pe_ TSWV	Pe_ BWYV	Pe_ BBWV2	Pe_ PepMoV	Pe_ ToCV
We_CMV	**0.32****	0.13**	0.08*	0.21**	0.13**	–0.02
We_TSWV	0.13**	**0.16****	–0.06	–0.10**	0.33**	–0.01
We_BWYV	0.08*	–0.03	–0.01	0.07	–0.03	–0.01
We_BBWV2	0.33**	0.04	0.10**	**0.26****	0.04	–0.02
We_PepMoV	0.14**	0.17**	0.32**	0.12**	**0.17****	–0.01
We_ToCV	0.08*	0.10**	–0.01	–0.04	0.10**	–0.01

CA, *Capsicum annuum*; CMV, cucumber mosaic virus; TSWV, tomato spotted wilt virus; BWYV, beet western yellows virus; BBWV 2, broad bean wilt virus 2; PepMoV, pepper mottle virus; ToCV, tomato chlorosis virus.

$^{*}P < 0.05$, $^{**}P < 0.01$.

[a]Pe and We mean pepper and weed, respectively.

(Rist and Lorbeer, 1989; Toyoda et al., 2004).

Influence of weeds as primary infection source of CMV and TSWV in CA. CMV and TSWV were the viruses with high correlation coefficients and continuous CA and weed infections during the study period (2014-2018), and hence, the potential cause of their spreading was analyzed. First, we determined whether the weeds infected by CMV affected the incidence of CMV in CA in the following year after winter (from December to February of the following year). The CMV-infected weeds in the previous year were positively correlated with the CMV infection frequency in CA in the current year. The correlation was highly significant during the 5 years of the study period (Table 5). A similar trend was found for TSWV, although the level of correlation was lower (Table 6).

The cause of the sudden fall in correlation coefficients in 2018 for both CMV and TSWV was analyzed in connection with the winter climate conditions. In 2018, the maximum temperature was 4.5°C, which was lower by 1.7°C than the average maximum temperature during the 5 years of the study period at 6.2°C. The mean annual temperature was also the lowest at –0.55°C. The average minimum temperature during the 5 years was –3.12°C, and that in 2018 was –4.75°C, which was lower by –1.63°C to indicate a low winter temperature range (data not shown).

In a study analyzing the correlation between winter temperatures and overwintering of *Diuraphis noxia* in southern parts of Alberta, Canada, the aphid population steadily decreased at temperatures from 0 to –10°C on the soil surface. In another study, the mortality of female imago of *Tetranychus urticae* in apple orchards was 72-80% on average during overwintering. Considering these findings, the fall of viral infection percent in 2017-2018 was determined to be due to the increased mortality of insect vectors at low winter temperatures (Butts, 1992; Lee et al., 2015b). In contrast, the average temperature during the overwintering period in 2014-2017 was 1.2-2.3°C. This is a higher range than in 2018 at –0.55°C, with a highly significant correlation with the incidence of CA viral infection in the following year. Based on this result, and according to the study by Szostek and Schwartz (2015) which reports that *Thrips tabaci*, an insect vector of Iris yellow spot tospovirus, ceased to be active at temperatures <0°C then resumed its activity at temperatures ≥0°C to be the infection source of the weeds in the vicinity; *Lactuca serriola* and *Descurainia sophia*, in the following crop year, it was determined that the high average temperature in 2014-2017 would have affected the activities of insect vectors and the infections of weeds in the vicinity to affect the incidence of CA viral infection in the following crop year.

Types and life cycles of CMV/TSWV-infected weeds around CA fields. Through RT-PCR on the 918 weed samples collected during 5 years, 66 species infected by CMV and 36 species infected by TSWV were detected

Table 5. Correlation analysis on CMV infection in weeds of the previous year and CA of the current year

Virus	2015_Pe_CMV[a]	2016_Pe_CMV	2017_Pe_CMV	2018_Pe_CMV
2014_We_CMV	0.66**	-	-	-
2015_We_CMV	-	0.46**	-	-
2016_We_CMV	-	-	0.41**	-
2017_We_CMV	-	-	-	0.26**

CMV, cucumber mosaic virus; CA, *Capsicum annuum*.
*$P < 0.05$, **$P < 0.01$.
[a]Pe and We mean pepper and weed, respectively.

Table 6. Correlation analysis on TSWV infection in weeds of the previous year and CA of the current year

Virus	2015_Pe_TSWV[a]	2016_Pe_TSWV	2017_Pe_TSWV	2018_Pe_TSWV
2014_We_TSWV	0.00	-	-	-
2015_We_TSWV	-	0.24**	-	-
2016_We_TSWV	-	-	0.93**	-
2017_We_TSWV	-	-	-	0.16*

TSWV, tomato spotted wilt virus; CA, *Capsicum annuum*.
*$P < 0.05$, **$P < 0.01$.
[a]Pe and We mean pepper and weed, respectively.

(Table 3). As shown in Tables 5 and 6, weeds were correlated with the incidence of viral infection as the primary infection source; hence, the pattern of viral spreading was analyzed based on the weed life cycles and infection percents (Table 7). The infection percent for CMV was the highest at 14.0% in summer annual weeds, followed by 11.4% in perennial weeds, and the lowest at 7.8% in winter annual weeds. The high infection percent in summer annual weeds could be attributed to the time of sample collection being the summer season (June and August). Still, considering the overwintering period in combining the infection percents of winter annual and perennial weeds, the percent was 5.2% higher than that of summer annual weeds, which implicated that these weeds were the main habitats of insect vectors during the period without CA. A similar trend was found for TSWV with the infection percent in the following decreasing order: summer annual weeds 10.4%, perennial weeds 6.4%, and winter annual weeds 6.2%. This deviated from a previous study analyzing the life cycles of the TSWV weed hosts that reported winter annual weeds at 42.9%, summer annual weeds at 30.9%, and perennial weeds at 26.2%. However, it may be due to the location and time of sample collection (Kil et al., 2020).

As with CMV, the infection percent for TSWV in winter

Table 7. Infection percent of CMV and TSWV in detected weeds

Life cycle	Family	Species	CMV		TSWV	
			No.S (No.D)[a]	Infection percent (%)	No.S (No.D)	Infection percent (%)
SA	Amaranthaceae	*Amaranthus lividus*	10 (1)	10.0	10 (0)	0.0
	Asteraceae	*Bidens frondose*	14 (1)	7.7	14 (0)	0.0
		Bidens tripartite	3 (0)	0.0	3 (1)	33.3
		Erechtites hieraciifolius	1 (0)	0.0	1 (1)	100.0
		Siegesbeckia glabrescens	2 (0)	0.0	2 (1)	50.0
		Xanthium orientale	4 (3)	75.0	4 (0)	0.0
	Cannabaceae	*Humulus japonicus*	39 (6)	15.4	39 (1)	2.6
	Chenopodiaceae	*Chenopodium album*	28 (4)	14.3	28 (1)	3.6
		Chenopodium ficifolium	16 (1)	6.3	16 (2)	12.5
	Convolvulaceae	*Ipomoea purpurea*	1 (1)	100.0	1 (0)	0.0
		Ipomoea triloba	1 (1)	100.0	1 (0)	0.0
		Quamoclit coccinea	3 (1)	33.3	3 (1)	33.3
	Commelinaceae	*Commelina communis*	43 (4)	9.3	43 (1)	2.3
	Cucurbitaceae	*Lagenaria leucantha*	1 (1)	100.0	1 (0)	0.0
	Euphorbiaceae	Acalypha australis	35 (1)	2.9	35 (0)	0.0
	Fabaceae	*Amphicarpaea trisperma*	3 (1)	33.3	3 (1)	33.3
		Glycine max	47 (4)	8.5	47 (0)	0.0
		Glycine soja	1 (1)	100.0	1 (0)	0.0
		Vigna angularis	4 (0)	0.0	4 (2)	50.0
	Lamiaceae	*Mosla dianthera*	1 (0)	0.0	1 (1)	100.0
		Perilla frutescens	4 (2)	50.0	4 (0)	0.0
	Poaceae	*Digitaria ciliaris*	18 (0)	0.0	18 (3)	16.7
		Echinochloa esculenta	12 (0)	0.0	12 (2)	16.7
	Polygonaceae	*Fallopia dumetora*	2 (1)	50.0	2 (0)	0.0
	Portulacaceae	*Portulaca oleracea*	37 (5)	13.5	37 (2)	5.4
	Subtotal		279 (39)	14.0	192 (20)	10.4
WA	Asteraceae	*Crepidiastrum sonchifolium*	18 (1)	5.6	18 (1)	5.6
		Erigeron annuus	42 (3)	7.1	42 (1)	2.4
		Erigeron canadensis	33 (3)	9.4	33 (3)	9.4
		Lactuca indica	12 (1)	8.3	12 (0)	0.0
		Sonchus asper	11 (1)	9.1	11 (0)	0.0
		Youngia japonica	4 (0)	0.0	4 (1)	25.0
	Subtotal		116 (9)	7.8	97 (6)	6.2

(Continued)

Table 7. Continued

Life cycle	Family	Species	CMV		TSWV	
			No.S (No.D)[a]	Infection percent (%)	No.S (No.D)	Infection percent (%)
P	Apiaceae	*Torilis scabra*	1 (1)	100.0	1 (0)	0.0
	Asclepiadaceae	*Metaplexis japonica*	32 (3)	9.4	32 (1)	3.1
	Asteraceae	*Artemisia princeps*	69 (5)	7.2	69 (2)	2.9
		Helianthus tuberosus	12 (0)	0.0	12 (1)	8.3
		Ixeris dentata	11 (2)	18.2	11 (0)	0.0
		Taraxacum officinale	4 (1)	25.0	4 (0)	0.0
	Caryophyllaceae	*Stellaria media*	1 (1)	100.0	1 (0)	0.0
	Convolvulaceae	*Calystegia davurica*	3 (1)	33.3	3 (0)	0.0
		Calystegia sepium	1 (1)	100.0	1 (0)	0.0
	Cucurbitaceae	*Trichosanthes kirilowii*	1 (0)	0.0	1 (1)	100.0
	Lamiaceae	*Leonurus japonicus*	2 (1)	50.0	2 (1)	50.0
	Menispermaceae	*Cocculus orbiculatus*	6 (0)	0.0	6 (1)	16.7
	Onagraceae	*Oenothera biennis*	19 (0)	0.0	19 (2)	10.5
	Polygonaceae	*Rumex coreanus*	25 (1)	4.0	25 (0)	0.0
	Subtotal		149 (17)	11.4	141 (9)	6.4
Unidentified			21 (1)	4.8	21 (1)	4.8
Total			565 (66)	11.7	451 (36)	8.0

CMV, cucumber mosaic virus; TSWV, tomato spotted wilt virus; SA, summer annual; P, perennial; WA, winter annual.
[a]No.S and No.D mean the number of surveys and the number of detections, respectively.

annual and perennial weeds was higher by 2.2% than in summer annual weeds. Moreover, previous studies have reported that such winter annual and perennial weeds as *Capsella bursa-pastoris* are the key host plants as the habitat of aphids in the absence of crop plants. The population density of *Frankliniella occidentalis* female imago is higher in biennial strawberry flowers than in annual flowers due to the overwintering in winter annual and perennial weeds such as *Stellaria media*, *Senecio vulgaris*, and *Taraxacum officinale*. Based on our findings and the aforementioned studies, it is conjectured that winter annual and perennial weeds play a crucial role in the overwintering of CMV and TSWV (Sampson et al., 2021; Satar et al., 2021).

To determine the life cycles of infected weeds as well as the preferred weed species as host plants and the respective scope, each main infected plant was analyzed regarding species (data not shown). As a result, 46 families and 155 species were identified. The Asteraceae family demonstrated the highest population at $n = 282$, followed by the Fabaceae family at $n = 92$, the Polygonaceae family at $n = 49$, and the Gramineae family at $n = 35$. This agreed with the report by Lee et al. (2015a) that the distribution of weed species in the crop fields of South Korea was Asteraceae 19.5%, Gramineae 11.7%, Polygonaceae 6.7%, Fabaceae 5.3%, and Lamiaceae 4.3%. However, the pattern of infection percent by plant species varied from the distribution of weeds. For summer annual weeds, the highest CMV infection percent of 57.1% was shown by the Convolvulaceae family including *Ipomoea triloba* and *Quamcolit coccinea*, followed by 50.0% in *Perilla frutescens* of the Lamiaceae family and *Fallopia dumetorum* of the Polygonaceae family, and 22.2% in the Asteraceae family including *Bidens frondosa* and *Xanthium canadense* (Table 7). For winter annual weeds, viral infection was detected solely in the Asteraceae family, including *Erigeron annuus* and *Crepidiastrum sonchifolium*. For perennial weeds, the infection percent was 100% in *Stellaria media* of the Caryophyllaceae family and *Torilis scabra* of the Umbelliferae family and 50% in the Convolvulaceae family, including *Calystegia dahurica* and *Calystegia sepium*. The Convolvulaceae family belongs to the order Solanales as with the Solanaceae family. Considering the report by Hobbs et al. (2000) that the weeds of the Solanaceae family are the crucial CMV infection source, it is likely that a correlation exists with the phylogenetic classification of host plants. In addition, the highest TSWV infection percent was shown by *Mosla dianthera* of the Lamiaceae family among summer annual weeds, followed by 50.0% in the Asteraceae family,

including *Bidens tripartita*, 42.9% in the Fabaceae family including *Glycine max*, and 16.7% in the Gramineae family including *Digitaria ciliaris*. *D. ciliaris*, in particular, is the most dominant species in crop fields; hence, it is likely to be the primary infection source of TSWV. Only the weeds of the Asteraceae family were detected among winter annual weeds. Among perennial weeds, the infection percent was 100% in *Trichosanthes kirilowii* of the Cucurbitaceae family, followed by 50.0% in *Leonurus japonicus* of the Lamiaceae family. Meanwhile, Kil et al. (2020) found that the infection percent was high in *Eclipta prostrata* (95.6%) among summer annual weeds, *Stellaria media* (55.0%) among winter annual weeds, and *Stellaria aquatica* (54.5%) among perennial weeds.

The results indicated that 34 species of weeds were infected by CMV and 25 species of weeds were infected by TSWV in the fields close to the CA cultivation fields. The list of natural hosts updated in this study could prove valuable in the control of CMV and TSWV. Various species of the Asteraceae family were found to have been infected. However, the infection percent was low due to the large population of plants, and this should be investigated in further studies.

Our findings highlight the importance of ambient weeds as potent, infectious agents influencing the spread and diversity of viruses that infect CA. In other words, by revealing that surrounding weeds are a significant source of transmission of viruses infecting CA, we highlight the importance of weed management in the surrounding environment of CA fields. It also provides essential information that complements and extends knowledge of CA viral disease control and prevention strategy development. Findings from this study may help advance our understanding of the management and prevention of CA viral disease and help prevent the spread of CA viral disease by: examples include (1) preventing the spread of CA viral diseases such as TSWV and CMV by removing weeds or taking measures to prevent weeds from becoming infected with viruses, and (2) developing biological control methods for CA viral diseases.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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