A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Identification and Inheritance of New Sources of Resistance against *Cucumber mosaic virus* Isolate P1 in *Capsicum* and Development of a *Tomato yellow leaf curl Kanchanaburi virus* Infectious Clone

고추에서의 오이모자이크병 P1분리균주에 대한 새로운 저항성 자원의 발견과 유전양상 및 토마토황화잎말림 Kanchanaburi 바이러스의 감염 클론 개발

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ABSTRACT

Peppers (*Capsicum* spp.) are important vegetable and spice crop worldwide. It possesses rich accumulations of secondary metabolites, colors and certain essential vitamins and minerals. Viral diseases are severe threat to both hot and sweet peppers resulting in significant yield loss. In the present study, two pepper viruses causing such excessive yield and quality loss of pepper were studied. In the first chapter, *Capsicum* germplasm collection was screened to identify resistance sources of CMV-P1, a new variant of *Cucumber mosaic virus* (CMV) strain. No accumulation of the virus in resistant pepper accessions was verified by enzyme linked immunosorbent assay (ELISA). Among the 4,197 accessions, seven accessions of *C. annuum* have been screened out as new resistant sources. Pepper accessions exhibiting strong

resistance against CMV-P1 were used to develop F_1 population and each of them was crossed with a susceptible line *C. annuum* 'Jeju' and a resistance line *C. annuum* 'Lam32', containing *cmr2*. The developed populations were further utilized to identify the inheritance of genetic resistance. The results suggest that the identified seven resistant accessions showed an identical pattern of genetic inheritance as the resistance gene, *cmr2*. The resistant resources identified in this study are expected to be applied in pepper breeding to develop CMV-P1 resistant varieties. In the second chapter, the genome of *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV), a bipartite *Begomovirus* first reported to infect tomato and eggplant in Kanchanaburi (Thailand), was sequenced and confirmed. A TYLCKaV infectious clone was developed using the golden gate cloning method and was agroinfiltrated in tobacco, tomato and pepper plants. The virus inoculated tobacco and tomato plants displayed viral symptoms. However, no obvious TYLCKaV symptoms were observed in pepper. Accumulation of the virus was also confirmed using polymerase chain reaction (PCR). The developed TYLCKaV infectious clone will be a useful tool for studies on TYLCKaV resistance.

Keywords: *Cucumber mosaic virus* (CMV), *Tomato yellow leaf curl Kanchanaburk virus* (TYLCKaV), infectious clone, *Capsicum*, Pepper virus

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LIST OF ABBREVIATIONS

Avr	Avirulence factor
Cmr1	*Cucumber mosaic resistance 1*
CMV	*Cucumber mosaic virus*
CP	Coat protein
DAS	Double antibody sandwich
dpi	Day post inoculation
eLRR	Extracellular LRR
ISR	Induced systemic resistance
KASP	Kompetitive Allele Specific PCR
NB-LRR	Nucleotide binding/leucine-rich repeat
NHR	Non-host resistance
ORFs	Open reading frames
PVY	*Potato virus* Y
QTL	Quantitative trait loci
RdRp	RNA-dependent RNA polymerase
Ren	Replication enhancer protein
Rep	Replication-associated protein
RNAi	RNA interfering
SAR	Systemic acquired resistance
TYLCD	Tomato yellow leaf curl disease
TYLCKaV	*Tomato yellow leaf curl Kanchanaburi virus*
TYLCV	*Tomato yellow leaf curl virus*

GENERAL INTRODUCTION

Peppers (*Capsicum* spp.) are one of the major vegetable crops grown worldwide. Peppers are enriched nutritional components, such as vitamin-C, vitamin-A, and antioxidants such as flavonoids. With the accumulation of these essential metabolites, it is used as an ingredient in health and medicinal products (Pérez-López et al., 2007). Pepper production has been steadily increased due to the increase of demand and breeding elite cultivars. Among the various pathogens, viral diseases are a major constraint in pepper production. To overcome this limitation, it is important to develop the virus resistance varieties of pepper.

To develop virus resistant cultivars, the sources of viral resistance within the plant species should be identified first. To screen a large number of germplasm accessions, accurate and rapid viral screening methods is crucial. In this study, two major pepper viral diseases, *Cucumber mosaic virus* (CMV) and *Tomato yellow leaf curl virus* (TYLCKaV), are studied and discussed.

It is difficult to measure the stability of resistance, because it requires to test the identified resistance genes in the field conditions under the changing environments. CMV, a member of the *Cucumovirus* genus in the *Bromoviridae* family, is one of the most common diseases affecting pepper production. The typical symptoms of CMV infection are severe mosaic and mottling (Palukaitis et al., 1992). CMV has a large host range and is transmitted by more than 80 aphid species. For this reasons,

preventing CMV infection by environmental control has been extremely difficult. Many breeders and researchers have identified several CMV resistance peppers. However, the reported quantitative trait loci (QTL) genes for disease resistance, remain difficult to be implemented in breeding procedures. *Cmr1*, a dominant resistance gene against CMV-P0 strains (CMV-K and CMV-Fny) has been previously reported (Kang et al., 2012). The resistance source was identified by Korean breeders in a Chinese pepper called 'Likeumjo' (Kang, 2011). It was used as a commercial pepper breeding material for the development of CMV resistant commercial F_1 hybrid *Capsicum annuum* 'Bukang'. However, a new CMV isolate, CMV-P1 was reported to overcome the *Cmr1* mediated resistance in 'Bukang' (Kang et al., 2012). In chapter I, screening of pepper germplasms for genetic position of resistance against CMV-P1 was performed. With the newly identified target for resistance, inheritance study and allelism test were performed.

TYLCV is a geminivirus that is prevalent in the tropical and subtropical regions. There are many isolates of TYLCV and TYLCV-like viruses around the world. As the symptoms are very similar among these viruses, TYLCD is been used as a general term for all these viral infections (Abhary et al., 2007). TYLCV has mono or bipartite DNA genome encapsidated in an icosahedral particle (Lazarowitz, 1992; Mansoor et al., 2006). The whitefly (*Bemisia tabaci*), a well-known insect vector, feeds on plant sap from a broad range of vegetable crops and is responsible for transmission of TYLCV particles in this process.

Tomato is one of the major crops affected by TYLCD infection. Numerous studies on identification of resistance genes in tomato have been conducted. However, yield loss by TYLCD problem in pepper has been obscure (Koeda et al., 2016). Unlike the TYLCV studies in tomato, there are limited information and researches on pepper resistance against TYLCV. Therefore, in chapter II, I developed the TYLCKaV infectious clone and established a method to infect pepper plants for screening purpose.

LITERATURE REVIEW

1. Plant resistance to pathogen

Plants are an important renewable natural resources, and human beings rely on plants for their food, feed, fiber, therapeutics etc. Plants are sessile organisms and are more prone to environmental changes and pathogen attacks. Furthermore, unlike animals, plants lack somatic adaptive immune system (Jones and Dangl, 2006). However, plants have adapted with sophisticated mechanisms to prevent or repair the damage caused by pathogens and environmental stresses.

Based on plant-pathogen interactions, plant resistance can be separated into two broad categories; non-host resistance and host resistance (Kang et al., 2005). In non-host resistance (NHR), all individuals of a species are generally no infected by a given pathogen (Fraser, 1987). The most pre-dominant type of NHR relies on physical barrier preventing pathogen invasion hence causing no symptoms or detectable multiplication of pathogen. When pathogens overcome these barriers, NHR can induce programed cell death at the site of infection (de Ronde et al., 2014). The resistance mechanism of NHR is known to be complicated due to the involvement of multiple pathways. Because of the complexity of NHR, this response is considered as durable defense against invasive pathogens and are continuously investigated (Jones and Dangl, 2006; Gill et al., 2015).

The second category of resistance to pathogen is called host resistance, which is displayed when a host plant can resist invasion of a specific pathogenic strain (Király et al. 2007). In this case, not all genotypes of a host species may be susceptible, and some may exhibit complete resistance. The host resistance can be further classified in two types. The first type is cultivar resistance, expressing resistance towards a pathogen induced by a single or multiple genes. This is the type of resistance mostly availed by the plant breeder because of the heritability. Second type is induced resistance which is switched on after the first inoculation in a susceptible plant. Induced resistance includes systemic acquired resistance (SAR) and induced systemic resistance (ISR). Unlike cultivar resistance, induced resistance is not heritable, and must be conferred fresh on each generation (Sequeira, 1984; Fraser, 1987; Moore et al., 2001).

2. Genetics of plant virus resistance

Viruses are an important group of pathogens that causes significant losses to crops, such as reducing crop yields and quality deterioration (Kang et al., 2005). The use of resistant cultivars is considered to be the most effective, and in many cases the only strategy to limit viral plant diseases (Gray and Moyer, 1993). Numerous researches have been carried out to find virus resistance genes and dissect their resistance mechanisms in order to introduce strong and durable resistance into plants.

Heritable resistance can be regulated by dominant, incomplete dominant or

recessive genes. The most widely studied are single dominant resistance (R) genes, which trigger programed cell death responses such as rapid appearance of necrotic lesions (a hypersensitive response, HR) and invisible necrosis (extreme resistance, ER) (de Ronde et al., 2014). Recent evidence suggests that the *R*-gene products indirectly sense the presence of avirulence factors (Avr). The guard hypothesis says R protein 'guard' and cellular proteins 'guardees' detect Avr effector, and the resistance evoked when it detects interference with the guardee protein (Soosaar et al., 2005).

Recessive resistance is more common for viruses than any other pathogens. Most of the well known examples of recessive resistance are for *potyvirus* resistance. The resistance genes were cloned from crop species and their role in disease resistance has been studied in detail. It has been identified that the eukaryotic translation initiation factor 4E (eIF4E) and 4G (eIF4G) are required for multiplication and cell to cell movement of viral particles. Mutation in these genes results in interference in viral multiplication and its movement (Diaz-Pendon et al., 2004; Truniger and Aranda, 2009).

3. Cucumber mosaic virus

Cucumber mosaic virus (CMV), member of the *Cucumovirus* genus, *Bromoviride* family, is one of the widely distributed plant viral disease. CMV virus was first reported in 1916 (Palukaitis et al., 1992). The genome of CMV is composed

of three single-stranded RNAs (RNA1, RNA2, and RNA3). The size and composition of the satellite RNAs can differ slightly based on the strain. CMV genome contain five open reading frames (ORFs) coding for protein 1a, protein 2a, protein 2b, 3a protein and coat protein (CP). Protein 1a, encoded by RNA1, is involved in virus replication from the tonoplast. RNA2 encodes the protein 2a and protein 2b. The large 2a protein contains typical motifs for RNA-dependent RNA polymerases (RdRp), interacting with proteins 1a. The small 2b protein is involved in inhibition of the host RNA interfering (RNAi) pathway and some direct role in virus movement. Both 3a and CP encoded by RNA3 are involved in cell-to-cell and plant-to-plant virus movement. CP also has the role of protecting virus RNAs from degradation (Palukaitis et al., 1992; Palukaitis and Garcia-Arenal, 2003; Scholthof et al., 2011; Jacquemond, 2012). CMV particles are icosahedral in shape and are carried by more than 80 aphid species. CMV host range is very broad encompassing more than 1,200 plant species including fruit crops, vegetables and ornamentals. The typical symptoms of CMV infection include leaf mosaic and distortion, stunting, yellowing, chlorotic or necrotic local lesion and ring spot (Palukaitis et al., 1992; Palukaitis and Garcia-Arenal, 2003).

4. Tomato yellow leaf curl-like virus

TYLCD is a devastating plant disease in many tropical and subtropical regions around the world. The disease is caused by several viruses belonging to *Begomovirus*

genus of the *Geminiviridae* family. Because of the common symptoms including leaf curling, yellowing, dwarfing and flower abortion, the viruses were referred to as "Tomato yellow leaf curl viruses" (TYLCV) (Cohen and Harpaz, 1964; Cohen and Antignus, 1994; Czosnek and Laterrot, 1997; Moriones and Navas-Castillo, 2000; Abhary et al., 2007).

The first TYLCD originated in Israel in 1930s and the outbreak of disease resulted in big economic crisis in Middle East countries until 1970s. From 1980s, the TYLCV-like diseases spread to the Southeast Asia and recent reports of TYLCD identifications confirmed the prevalence of TYLCD all around the world, including Western Europe, China and Caribbean Islands (Lefeuvre et al., 2010).

Begomoviruses, which include all the TYLC-like viruses, have small genomes of covalently closed, circular ssDNA. The viral genomes are encapsidated in the paired-icosahedral particles (García-Andrés et al., 2006). Their propagation mainly relies on host encoded DNA and RNA polymerase unlike RNA viruses, which replicate via RNA intermediates using virus-encoded replicases (Hanley-Bowdoin et al., 1999). The single genomic component is approximately 2.5 to 3 x 10^3 nucleotides. Most of the begomoviruses consist of two (bipartite) and some with single (monopartite) DNA components with or without the DNA satellites (α-satellite and β-satellite). In a bipartite TYLCV, DNA-A encodes functional proteins such as a replication-associated protein (Rep), replication enhancer protein (REn), the coat protein (CP), and transcription activator protein (TrAP). The DNA-B encodes

movement protein (MP) and nuclear shuttle protein (NSP) essential for virus movement in plants. ORFs are organized bi-directionally and are separated by an intergenic region (IR), which forms a hairpin like structure and serve as a key element for the virus replication and transcription (Lazarowitz, 1992; Nawaz-ul-Rehman and Fauquet, 2009; Hanley-Bowdoin et al., 2013). TYLCD is naturally transmitted by the whitefly (*Bemisia tabaci*) and therefore, to prevent the virus infection and subsequent crop losses, stringent control of whitefly population and breeding of TYLCD resistant crop varieties need to be contemplated (Cohen and Harpaz, 1964; Abhary et al., 2007).

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CHATER 1

Identification and Inheritance of New sources of Resistance against *Cucumber mosaic virus* Isolate P1 in *Capsicum*

ABSTRACT

Cucumber mosaic virus (CMV) belonging to genus *Cucumovirus* in the family *Bromoviridae* is the most destructive virus infecting in pepper production. Several resistance genes against CMV have been identified in pepper. *Cmr1* is a single dominant gene known to confer resistance against CMV isolate P0 (CMV-P0). However, a new isolate CMV-P1 identified in Korean field has the potential to overcome the resistance conferred by the *Cmr1* gene. Only a few resistance sources to the CMV-P1 isolate have been found. More and better resistance sources to the CMV-P1 is necessary to prepare an endemic break out of CMV-P1. In this study, a pepper germplasm collection consisting of 4,197 accessions was screened for resistance against CMV-P1. CMV-P1 inoculated plants were evaluated three weeks post inoculation. *C. annuum* 'Jeju' and 'Bukang' were used as a susceptibility control and *C. annuum* 'Lam32' (*cmr2*) was used as a resistance control. A total of 21

symptomless peppers were selected as potential source of resistance. The CMV accumulation levels in these plants were also evaluated with a double antibody sandwich (DAS)-enzyme-linked immunosorbent assay (ELISA). Finally, seven accessions of *C. annuum* have been selected with resistance against CMV-P1. These resistant plants were further crossed with 'Jeju' and 'Lam32', and the developed populations were undergone genetic analysis to test the allelism. The results revealed that all the resistant accessions have the same recessive resistance gene as that of 'Lam32'.

INTRODUCTION

Cucumber Mosaic Virus (CMV), a member of the genus *Cucumovirus* in the family *Bromoviridae*, is one of the most destructive viruses in temperate regions. It is responsible for important economic losses in many crops, including peppers (Palukaitis et al., 1992). Therefore, development of CMV resistant varieties has been an important goal for pepper breeding. With an effort of many breeders and researchers, various sources of resistance to CMV have been identified. However, most sources display polygenic resistance controlled by multiple genes. These include *C. annuum* 'Perennial' (Caranta et al., 1997; Lapidot et al., 1997; Grube et al., 2000), *C. annuum* 'Vania' (Caranta et al., 2002), *C. annuum* 'Sapporo-oonaga' and 'Nanbu-oonaga' (Suzuki et al., 2003), *C. frutescens* 'BG2814-6' (Grube et al., 2000), *C. frutescens* 'LS1839-2-4', and *C. baccatum* 'PI439381-1-3' (Suzuki et al., 2003; Kang et al., 2010). Diverse mechanisms behind the resistance of these varieties have been reported, ranging from inhibition of viral replication, inhibition of cell-to-cell movement or long-distance movement of viral particles. Genetic analysis were also carried out to identify genomic regions controlling the resistance and showed that the resistance was controlled by several QTLs in some resistant varieties (Caranta et al., 1997; Chaim et al., 2001; Caranta et al., 2002).

Unlike other resistant sources, in the commercial F_1 cultivar, *C. annuum* 'Bukang', the resistance is controlled by a single dominant gene, *Cmr1* (resistant to

CMV-P0) (Kang et al., 2010). Several molecular markers linked to *Cmr1* were developed, and these markers have been used for developing CMV resistance cultivars (Kang et al., 2010). However, a new isolate, CMV-P1 identified in Korea was found to evade the CMV resistance by *Cmr1* gene (Lee et al., 2006). Therefore, it is highly crucial to identify new resistant varieties of pepper and to identify the responsible genetic element.

Recently, a new resistance source against CMV-P1 was identified in *C. annuum* 'Lam32'. According to a previous study, 'Lam32' has a single recessive resistance gene, *cmr2,* against CMV-P1 (unpublished data). The objectives of the present study was to identify additional resistance sources against CMV-P1 and to further investigate the nature of genetic inheritance of the resistance gene using traditional and molecular breeding tools.

MATERIALS AND METHODS

Plant materials

C. annuum 'Lam32' was used as a resistance control, and *C. annuum* 'Jeju' and the commercial F_1 hybrid *C. annuum* 'Bukang' were used as susceptible controls in CMV-P1 resistance screening studies. To identify CMV resistant cultivars, 4,197 pepper accessions were kindly provided by the National Agricultural Plant Genebank of RDA (Jeonju, Republic of Korea). Pepper accessions showing resistance to CMV-P1 were identified and crossed with 'Jeju' to study the inheritance pattern of the resistance gene. For the inheritance study, all the accessions exhibiting CMV-P1 resistance were crossed with 'Jeju'. Further, and F_2 population, and reciprocal backcross populations were developed for the *C. annuum* 'IT221660'. For allelism test, all the accessions exhibiting CMV-P1 resistance were crossed with 'Lam32'. All the procedures from plant cultivation to seed harvesting were performed in greenhouse at Suwon Farm (Seoul National University, South Korea).

Virus inoculation

The virus inoculums were kindly provided by 'Nongwoo-Bio' Co., Ltd. (Yeoju, South Korea). The inocula of the CMV-P1 strain were innoculated and multiplied in *Nicotiana rustica*. One gram of freshly harvested infected *N. rustica* leaves was

ground in 10 mL of 0.1 M phosphate buffer pH 7.0. The inoculum preparation was carried out on ice. The test plants were used at two foliage leaves stage and the CMV inoculum was manually rubbed on the two cotyledons after dusting with Carborumdum #400 (Hayashi Pure Chemical Co., Ltd. Japan). The inoculated plants were then rinsed with fresh tap water post 10 min after inoculation. After one week, inoculation process was repeated on the first foliage leaves to prevent virus-escape. The inoculated plants were kept in the growth chamber with 16 hrs light/ 8 hrs dark cycle at 23 ℃. For each accessions, at least 4 plants were inoculated.

Detection of CMV accumulation using DAS-ELISA

Double antibody sandwich (DAS) -ELISA was used for CMV detection according to the manufacture's protocol (Agdia, Elkhart, IN, USA). At 25 days post inoculation (dpi), two leaf discs from systemic leaves were collected and used for ELISA analysis. The samples were read at an absorbance of 405 nm in a Zenith 200 ELISA reader (Anthos, Eugendorf, Austria) (Kang et al., 2012).

Testing Affy4 marker

Affy4 marker is a Kompetitive Allele Specific PCR (KASP) marker closely linked to the *cmr2* of 'Lam32'. It was developed from the F_2 populations of cross between 'Lam32' and 'Jeju' (Kang, Unpublished data). Affy4 marker was applied on the resistant and susceptible lines and the F_1 individuals. Thermocycling and

endpoint genotyping for the KASP assays were performed in Roche LC480 (Roche Applied Science Indianapolis, IN). To genotype segregating plant populations, 1 µL plant DNA (100 ng) was mixed with 5 µL KASP reaction mix and 0.14 µL gene-specific KASP primer mix in a total reaction volume of 10 µL. The entire setup was run in 96-well qPCR a plate and, the thermal cycling condition was 94℃ hot start for 15 min; followed by 9 cycles of 94 ℃ for 20 s and 61 ℃ for 60 s, the annealing temperature was dropped at the rate of 0.6 ℃/cycle, followed by 27 cycles at 94 ℃ for 20 s and 55 ℃ for 60 s and 3 cycles of 94 ℃ for 20 s and 57 ℃ for 60 s. Post-melt cycles were at 57 ℃ for 1 s and cooling to 20 ℃ during plate reading. For signals with weak separation, five additional cycles of 94 ℃ for 20 s and 57 ℃ for 60 s were performed.

RESULTS

Germplasm screening

In the span of two years, 4,197 accessions of *Capsicum* germplasm were screened for CMV-P1 resistance, along with the susceptible controls 'Jeju' and 'Bukang', and the resistant control 'Lam32'. The accessions were germinated and the cotyledons from the 8-10 old seedlings were gently inoculated with CMV-P1 and the symptoms were scored every alternate days. Susceptible controls 'Jeju' and 'Bukang' showed the viral symptoms at 7 dpi with yellowing and distorted leaf. The symptoms were clearly visible, complicated by mosaics, leaf distortion and necrosis spot by about 25 dpi. Development of viral symptoms in 'Bukang' further confirms that CMV-P1 can combat the *Cmr1* mediated resistance as shown in Figure 1A. Recent research found that the helicase domain of RNA1 permit CMV-P1 to overcome the *Cmr1* resistance (Kang et al., 2012) and thus explains appearance of CMV symptoms in the resistant variety 'Bukang'. However, 'Lam32' did not show any symptoms, providing an evidence of carrying an alternate gene distinct from *Cmr1* for resistance against CMV-P1.

From the first screening of CMV-P1 inoculated *Capsicum* germplasm, 21 accessions were selected as potential resistant plants. They included 18 accessions of *C. annuum* and three accessions of *C. frutescens* (**Table 1**). For further validation, inoculated and systemic leaves form these 21 resistant candidates were harvested,

and subjected to ELISA assay. It was confirmed that seven (IT221660, IT221661, IT221885, IT236359, IT236402, IT248570, and IT264081) out of 21 accessions were 100 percent symptomless with multiplication and movement of virus. Accumulation of viral components was also negligible (<1 value) in the systemic leaves (**Figure 1. B**). Significantly lower levels of virus accumulation in the systemic leaves of the resistant accessions compared to the susceptible controls suggest the presence of a strong resistance gene.

Table 1. Summary of *Capsicum* germplasm screening against the CMV-P1 isolate.

Capsicum species	2015			2016		
	Total number of accessions	Number of accessions		Total number of accessions	Number of accessions	
		At 25 dpi			At 25 dpi	
		Susceptible	Resistant		Susceptible	Resistant
C. annuum	840	826	14	1754	1750	4
C. baccatum	56	56	0	192	192	0
C. chinense	43	43	0	165	165	0
C. frutescens	60	58	2	86	85	1
C. pubescens	2	2	0	0	0	0
Total	2000	1984	16	2197	2192	5

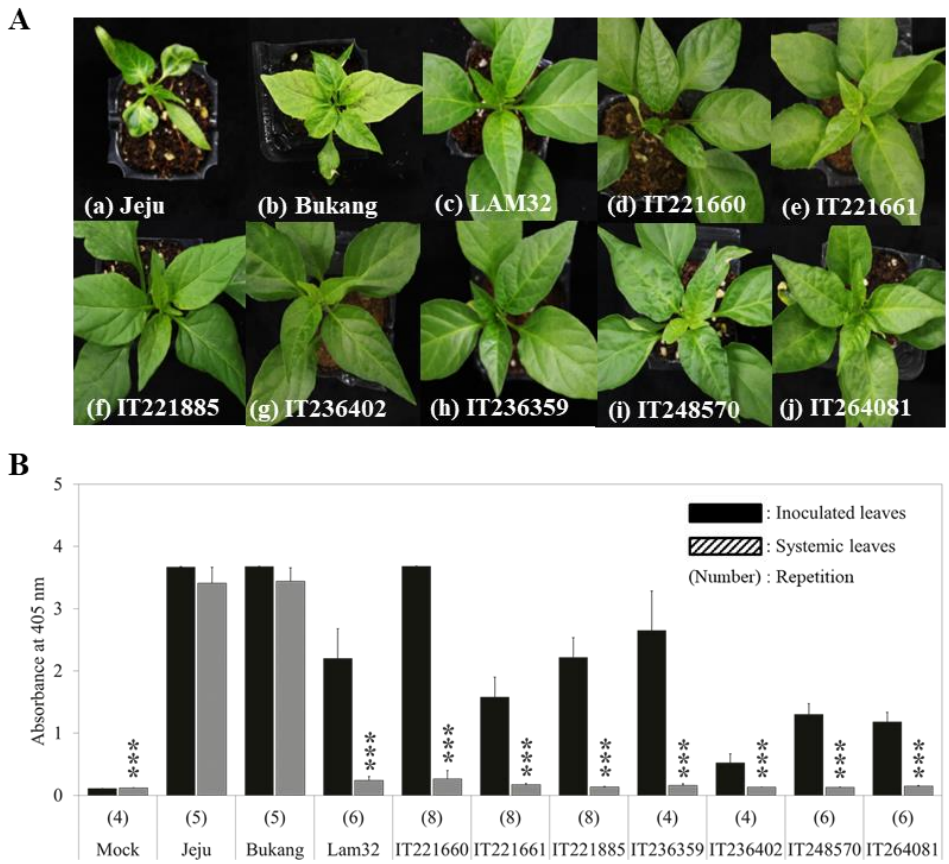

Figure 1. Disease responses to CMV-P1 infection at 25 dpi. (A) (a) and (b) are susceptible control, (c) is a resistant control. Accessions (d) to (j) were observed to have no clear symptoms. (B) Detection of CMV coat protein accumulation in inoculated and systemic leaves of CMV-P1 resistant accessions. Error bars indicate for standard deviation of the mean absorbance value in each replicates of accessions and asterisks mean significant difference between resistance and susceptible lines [analysis of variance (ANOVA) $P \geq 0.05$].

Inheritance study and allelism test of the new sources of resistance against CMV-P1

Seven selected accessions were crossed with 'Jeju' to study the inheritance of resistant trait. About 10 to 20 F_1 plants from each cross were inoculated with CMV-P1 and scored at 25 dpi. The results showed that all of the F_1 hybrids were found to be susceptible demonstrating that the resistance is controlled by either a single or multiple recessive genes (**Table. 2**). Since the positive control 'Lam32' is known for a recessive resistance gene, additional allelism test was performed between 'Lam32' and the seven resistance lines. To reveal the allelic relationship of new resistance gene with the *cmr2* gene, the developed F_1 hybrids were inoculated by CMV-P1. The accumulation of viral coat protein was quantified and all the F_1 plants were confirmed to exhibit resistance to CMV-P1 (**Table. 3**). Further, the F_2 and BC population of 'IT221660' were analyzed to see the segregation ratio of phenotype (**Table. 4**). In the F_2 population, resistance and susceptible phenotypes were found to segregate in a 1:3 ratio. The segregation of backcross population BC_1F_1 ('Jeju'×'IT221660')×'Jeju' was found to be 0R:1S, whereas in ('Jeju'×'IT221660')×'IT221660' BC_1F_1 population, the resistant and susceptible phenotypes segregated in 1R:1S. These results is consistent with the Mendelian segregation of a single recessive gene. Collectively, these results suggest that the newly found resistant pepper accessions are likely to carry the same resistance gene in *cmr2*.

Table 2. Inheritance study of the new source of resistance against the CMV-P1.

Parent lines and populations	Number of plants			Expected ratio (R : S)
	Total	Resistant (R)	Susceptible (S)	
Jeju	10	0	10	0:1
IT221660	7	7	0	1:0
IT221661	6	6	0	1:0
IT221885	10	10	0	1:0
IT236359	7	7	0	1:0
IT236402	6	6	0	1:0
IT248570	10	10	0	1:0
IT264081	10	10	0	1:0
F_1 'IT221660×Jeju'	10	0	10	0:1
F_1 'IT221661×Jeju'	10	0	10	0:1
F_1 'IT221885×Jeju'	10	0	10	0:1
F_1 'IT236359×Jeju'	5	0	5	0:1
F_1 'IT236402×Jeju'	18	0	18	0:1
F_1 'IT248570×Jeju'	19	0	19	0:1
F_1 'IT264081×Jeju'	20	0	20	0:1

Table 3. Allelism test between the new resistance sources and 'Lam32'.

Parent lines and populations	Number of plants			Expected ratio
	Total	Resistant (R)	Susceptible (S)	(R : S)
Lam32	10	10	0	1:0
IT221660	7	7	0	1:0
IT221661	6	6	0	1:0
IT221885	10	10	0	1:0
IT236359	7	7	0	1:0
IT236402	6	6	0	1:0
IT248570	10	10	0	1:0
IT264081	10	10	0	1:0
F_1 'IT221660×Lam32'	10	10	0	1:0
F_1 'IT221661×Lam32'	10	10	0	1:0
F_1 'IT221885×Lam32'	10	10	0	1:0
F_1 'IT236359×Lam32'	5	5	0	1:0
F_1 'IT236402×Lam32'	19	19	0	1:0
F_1 'IT248570×Lam32'	20	20	0	1:0
F_1 'IT264081×Lam32'	18	18	0	1:0

Table 4. Inheritance study of ‘IT221660’.

Parent lines and populations	Number of plants			Expected ration (R:S)	χ^2	*P*-
	Total	Resistant	Susceptible			
‘Jeju’	9	0	9	0:1	-	-
‘IT221660’	15	15	0	1:0	-	-
F_1 ‘Jeju’×’IT221660’	10	0	10	0:1	-	-
F_2 ‘Jeju’×’IT221660’	135	36	99	1:3	0.2	0.6547
BC_1F_1 (‘Jeju’×’IT221660’)×‘Jeju’	24	0	24	0:1	-	-
BC_1F_1 (‘Jeju’×’IT221660’)×‘IT221660’	22	11	11	1:1	0	1

cmr2-linked Affy4 marker analysis

As the seven accessions were confirmed to have same resistance gene, the closest marker 'Affy4' to c*mr2* was applied for their analysis. Affy4 marker was designed based on SNP between 'Lam32' and 'Jeju' using Affymetrix genechip array. It was mapped 2.3 cM away from the *cmr2* gene in the 'Jeju×Lam32' F_2 population (Unpublished data). This marker was subject to analyze the F_1 plants derived from the resistant plants crossed with 'Jeju'. The marker was applied on three to five plants of each accession. As a result, all the resistant accessions showed polymorphism against susceptible plant 'Jeju'. It was the same genotype as 'Lam32' (**Figure 2**). This represents that the seven CMV resistant accessions can be used for fine mapping of *cmr2*, and Affy4 marker will be a useful tool in breeding CMV resistant cultivars.

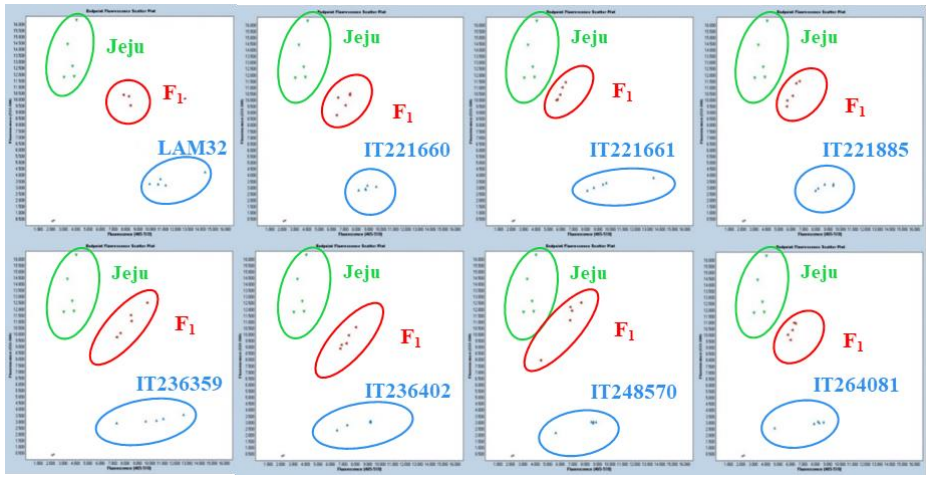

Figure 2. Kompetitive Allele Specific PCR (KASP) analysis of the *cmr2* marker (Affy4).

DISCUSSION

CMV have been constantly resulting in significant damage of pepper and a huge loss in its production. Since the chemical control of viruses is impossible, there have been several approaches being performed to prevent the yield loss: environmental control, developing transgenic resistance, and traditional breeding approaches. As for the environmental control, removing the virus infected host plants and insects from the cultivation area is the main key. However, CMV has a broad range of host plants including symptomless plants and furthermore CMV transmitting aphids are extremely difficult to control. Therefore, the most effective approach is to develop virus resistant pepper varieties. There are several reports on development of virus resistant transgenic plants in tobacco, cucumber, melon, tomato and pepper (Anderson et al., 1992; Morroni et al., 2008). However, pepper is recalcitrant to be transformed. Thus, resistance sources from the natural germplasm is essential.

In this study, seven different accessions of *C. annuum* were identified to be resistant to CMV-P1. All the developed F_1 hybrids from crosses between each of the seven resistant accession and 'Jeju' were found to be susceptible to CMV-P1 isolate. Whereas the F_1 hybrids developed from crosses with 'Lam32' containing *cmr2* were resistant to CMV-P1. In addition, the segregation ratio of F_2 and backcross populations of 'IT221660' satisfied the Mendelian inheritance pattern of a single recessive gene. These results suggest that the identified seven accessions like 'Lam32', carried CMV resistance gene *cmr2*.

The discovery of single resistance gene is one of the key factors for a successful

breeding of resistance cultivars. This is because it is manageable to introgress a single gene to susceptible elite breeding lines. To date, most of identified CMV resistance genes in *Capsicum* spp. are controlled by QTLs, while *Cmr1* is the only single dominant gene. The first studied resistance gene was a QTL from *C. annuum* 'Perenial' against CMV-N. It restricts the virus movement by forming localized necrotic lesions (Caranta et al., 1997). The virus multiplication is restricted and the disease resistance is probably achieved by a monogenic recessive or partially dominant *R* gene (Pochard and Daubeze, 1989). However, when 'Perenial' was inoculated with an alternative strain CMV-MES, the resistance mechanism and the associated resistance genes were reported to be different. In 2002, Caranta et al. identified resistant accessions *C. annuum* 'Vania' and *C.annuum* 'Milord' against CMV-MES, and *C. annuum* 'Avelar' and *C. baccatum* 'Pen 3-4' against CMV-KM. The following year, hypersensitive response (HR) assisted resistance was found in *C. baccatum* 'PI439381-1-3', *C. frutescens* 'LS 1839-2-4', and *C. frutescens* 'Tabasco' (Caranta et al., 2002; Suzuki et al., 2003). Resistance associated with QTL has a greater advantage of combating multiple virus strains. However, in terms of breeding, there are limitations to introduce resistance genes into cross varieties. Recently, *Cmr1* has been found as a single dominant resistance gene against CMV-FNY in Korea. It has been found that *Cmr1* controls the virus movement from epidermal cell to deeper cell layer (Kang et al., 2010). However, CMV-P1, with mutated helicase domain of RNA1 was found to overcome *Cmr1* resistance. Supporting this, a recent study indicates that CMV resistance can be easily broken

by several amino acid modification in the virus (Choi et al., 2016).

The resistance gene found in this study is *cmr2,* a single recessive resistance gene, which can be easily to be implemented in breeding programs. Recessive resistance genes can be used to identify essential host factors and understand the virus multiplication mechanism. The present study showed lower levels of virus accumulation in the systemic leaves compared to the inoculated leaves in the identified resistant accessions, suggesting that the resistance mechanism is inhibition of the systemic movement of the virus. Since the systemic movement is more difficult to study than cell-to-cell movement, there are only few potential candidate *R* genes identified till date. There is another report about CMV accumulation in the external but not internal phloem (Dufour et al., 1989). The resistance mechanism in the present study might be associated with the insufficient supply of components required for the viral movement. However, the functional validation that *cmr2* is the responsible *R* gene to restrict systemic movement of the viral components is yet to be done (Kang et al., 2005).

In future, these seven resistant accessions are to be tested against other CMV strains as well. Although *cmr2* gene is postulated to be responsible for CMV-P1 resistance in this study, there are high chances of additional favorable alleles to exist in the identified resistant peppers. For example, in pepper *potato virus Y* (PVY) resistance gene, *pvr2*, has been found associated with two different susceptible alleles and two different resistance sequences with different PVY resistance spectrum (Ruffel et al., 2002).

In conclusion, we found seven accessions of *C. annuum* as CMV-P1 resistance genetic sources. From the inheritance study, it was suggested that all of them were likely to have single recessive gene. Further allelism tests with 'Lam32' confirmed that the nature of genetic resistance was similar to *cmr2* of 'Lam32'. This demonstrates that *cmr2* is a common CMV recessive resistance gene that can be found in *C. annuum*. Resistant genetic resources identified in the present study will further be used as breeding materials for constructing population in the process of *cmr2* fine mapping.

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CHATER 2

Development of *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV) infectious clone for searching resistance source in *Capsicum* spp

ABSTRACT

Tomato yellow leaf curl disease (TYLCD) are plant viral diseases mainly caused by TYLCV like viruses belonging to the *Begomovirus* group in the *Geminiviridae* family. Begomoviruses are circular single-stranded DNA viruses. Severe crop losses due to TYLCV infection have been documented, particularly in tomato. TYLCD was reported to cause similar losses in pepper production in tropical countries. TYLCKaV is one of the well known virus strains infecting pepper. In this study, TYLCKaV was isolated from the TYLCD symptomatic leaves of pepper, the viral genome was sequenced and an infectious TYLCKaV clone was developed. Both DNA-A and DNA-B were designed to have partial tandem repeats and was inserted into the pICH86988 binary vector using golden gate cloning method. The constructed TYLCKaV binary clones were co-infiltrated into tobacco, tomato, and

pepper to test its infectivity. One hundred percent infectivity was observed in the tobacco plants with typical TYLCV symptoms. Virus inoculated tomato plants also exhibited similar viral symptoms, however, the symptoms were less severe in tomato compared to those of the tobacco plants. TYLCKaV infection was successfully confirmed in all the symptomatic plants of tobacco and tomato using PCR. Additionally, it was also found that the TYLCKaV strain can overcome the TYLCV resistance carried by *Ty-3* and *ty-5* genes. Unlike the tobacco and tomato, TYLCKaV infected pepper did not show any viral symptoms and no virus accumulation was observed. These results indicate that Agrobacteroium-mediated TYLCKaV infection is difficult and an alternate approach has to be considered for successful TYLCKaV infection in pepper.

INTRODUCTION

TYLCD is a plant disease caused by several virus species, collectively called "Tomato Yellow Leaf Curl Virus" (TYLCV). TYLCD is caused by TYLCV-like viruses (genus *Begomovirus*, family *Geminiviridae*). The viruses were first observed in the infected tomato plants from the eastern Mediterranean more than 70 years ago (Cohen and Antignus, 1994; Lapidot and Friedmann, 2002). TYLCV is characterized by a covalently closed circular single-stranded DNA genome (css-DNA) enclosed in twinned icosahedral geminate particles and is transmitted by whiteflies (Goodman, 1977). All the TYLCV-like viruses show similar pattern of symptoms on infecting host plant such as leaf curling, yellow mosaics and stunted growth (Polston and Anderson, 1997; Abhary et al., 2007).

Numerous studies are conducted to identify resistance genes against TYLCV infection in tomato. To date, several resistance sources have been uncovered against TYLCV in tomato, most of them with high levels of tolerance. *Ty-1* from *S. chilense* accession 'LA1969' and *Ty-3* from *S. chilense* accession 'LA2779' are resistance alleles on the same gene located on chromosome 6, coding for a RNA-dependent RNA polymerase (RDR) (Verlaan et al., 2013). Single dominant gene, *Ty-2* from *S. habrochiates* f. glasbratum accession 'B6013' is located on chromosome 11 (Banerjee, 1990; Hanson et al., 2006). *Ty-4* from *S. chilense* accession 'LA1932' was mapped to chromosome 3 (Ji et al., 2009). *ty-5* of *S. peruvianum* accession 'TY172'

was found to be linked to the marker SINAC1 on chromosome 4 (Anbinder et al., 2009). *Ty-6* from *S. chilense* accession 'LA1938' was mapped to chromosome 10 (Scott et al., 2015). The introgression of these resistance genes into TYLCV susceptible species of tomatoes were carried out in many tomato breeding programs.

TYLCD is not only common in tomato, but has a broad host range including other dicot species such as pepper, potato and tobacco. Unlike tomato, there have been limited studies on TYLCD resistance in pepper. In 2005, Morilla and her colleagues infected pepper plants with TYLCV infectious viral clone. No significant damage was observed in the infected peppers suggesting that pepper is a dead-end host for TYLCV (Morilla et al., 2005). However, peppers with leaf crumple and yellow mosaic, caused by TYLCV-like viruses were reported from pepper fields worldwide. *Ageratum yellow vein virus* (AYVV), *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), *Tomato leaf curl Java virus* (ToLCJaV), *Tomato leaf curl Sulawesi virus* (ToLCSuV), *Tomato yellow leaf curl Indonesia virus* (TYLCIDV), and *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV) were detected from tomato or pepper. Coinfection of these viruses results in significant yield loss (Kenyon et al., 2014; Koeda et al., 2016). To minimize the losses caused by the TYLCD infection, it is essential to develop the TYLCD resistance genetic sources and varieties. For such researches, development and establishment of infectious clones of the plant viruses is highly necessary. Therefore, the objective of this study was to develop an infectious clone of TYLCKaV and design an effective method for screening *Capsicum* germplasm for TYLCKaV resistance.

MATERIALS AND METHODS

Plant materials

Tobacco (*N. benthamiana*), tomato (*S. lycopersicum* 'A39', *S. lycopersicum* 'A45', and *L. esculentum* breeding lines 'TY172') and peppers (*C. annuum* 'Jeju', *C. annuum* 'CM334', and *C. annuum* 'Early California Wonder 30R' – ECW 30R) were used to test virus infectivity. Plants were grown in a growth chamber with 16 h light/8 h dark cycle at 23℃.

Virus inoculation

TYLCKaV-infected pepper (*C. annuum* 'CM334') leaves served as sources for viral DNAs. The virus infected leaves were kindly provided by the East-West Seed Company (Indonesia).

Detection and sequencing of the viral genomes

Viral DNA was detected by polymerase chain reaction (PCR) using universal primers, pAL1v1978 (5'GCATCTGCAGGCCCACATYGTCTTYCCNGT3') and pAR1c 715 (5'GATTTCTGCAGTTDATRTTYTCRTCCATCCA3') (Rojas et al. 1993). For viral DNA amplification, the following PCR condition were used: 30 cycles of denaturation at 94 ℃ for 60 s, annealing at 50 ℃ for 60 s and extension, at 72 ℃ for 3 min. The integrity of the PCR products were checked using 1 % agarose

gel. Amplified products of 1.4 kb size were gel eluted using gel and PCR clean-up Kit (Cosmo, Korea) and sequenced at Macrogen (Republic of Korea). Viral DNA sequences were verified by BLAST search at NCBI (www.ncbi.nlm.nih.gov/). Based on the resulting sequence information, additional primer sets were designed to get the full-length viral genome sequence data. Both DNA-A and DNA-B were sequenced using 'WsTY03-F/ WsTY03-R' and 'TY-B001F/ TY-B001R' primer sets (**Table 1**).

Construction of an infectious clone

To construct TYLCKaV infectious clone, golden-gate cloning method was used (**Figure 1**). Based on the virus sequence, primer sets flanked by BsaI sites were designed. Two different 1 mer TYLCK-A fragments (2.7 kb) were amplified using 'Gold-TYLCV01-F/ Gold-TYLCV01-R' and 'Gold-TYLCV02-F/ Gold-TYLCV02-R'. Similarly, 'TY-B-GOLD-01F/ TY-B-GOLD-0R' and 'TY-B-GOLD-02F/ TY-B-GOLD-02R' were used to amplify 1 mer (2.7 kb) and 0.9 mer (2.4 kb) TYLCKaV-B fragments. The thermal cycling conditions followed are initial denaturation for 3 min at 98 ℃, followed by 35 cycles of 98 ℃ for 10 s, 55 ℃ for 15 s, and 68 ℃ for 1 min per 1 kb, followed by a final extension for 5 min at 68 ℃. PCRs were performed using PrimerSTAR® GXL DNA Polymerase (Takara, Shiga, Japan). The amplified PCR products were purified using gel and PCR clean-up Kit (Cosmo, Korea) and cloned in the SmaI site of a pUC19b vector. Ligation was performed at 23-25 ℃ for 12 hours. The ligation volume of 15 μL contained 100-200 ng of PCR

products, 50 ng pUC19b, 10 mM MgCl2, 50 mM Tris-HCl, 1 mM ATP, 10 mM DTT (pH 7.5), 0.5 µL of SmaI , and 0.5 µL of T4 DNA Ligase (New England Biolabs, England). *E. coli* (DH5α) was transformed with 5 µL of ligation products. Selection of positive clones as performed by blue/white screening in LB agar plates supplemented with 45 µL X-Gal and 15 µL IPTG (1 M).

For assembling, pICH86988 (9.1 kb) with kanamycin resistance and a p35S promoter was used. Standard Golden gate reactions for generation of plasmids for gene replacement mutants were set up in 20 µL containing 100 ng each of the respective shuttle vectors, 75 ng destination vector, 1X T4 DNA ligase buffer (NEB), 1 µL BsaI (NEB; 10 U) and 1 µL T4 DNA ligase (NEB; 10 U). The reaction was performed in a PCR cycler using the following program: [37 ℃ 3 min, 16 ℃ 4 min] 25 cycles, 50 ℃ 5 min, and 80 ℃ 5 min. 5 µL of each Golden Gate reaction was transformed into chemically competent *E. coli* (DH5α) and plated on LB plates containing 50 µg/ml kanamycin.

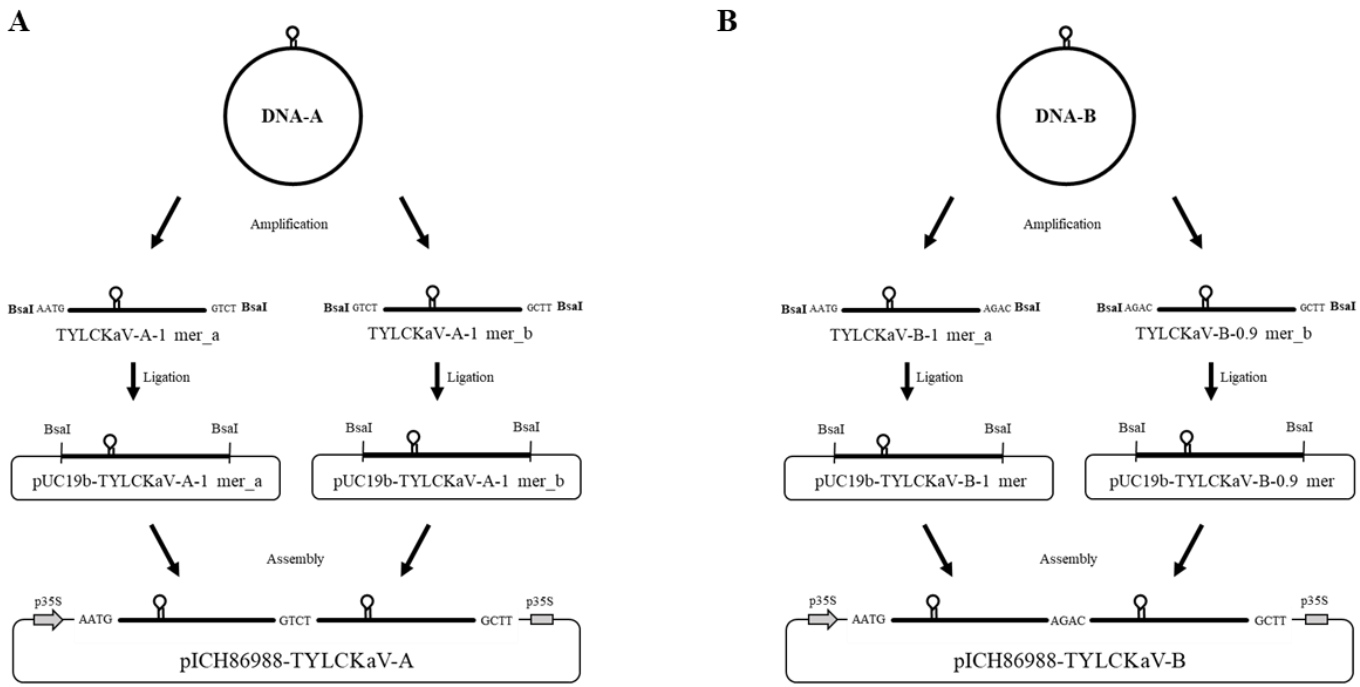

Figure 1. Construction scheme of TYLCKaV infectious clone. TYLCKaV, a bipartite begomovirus, has two components, DNA-A and DNA-B. (A) ppICH86988-TYLCKaV-A-2mer and (B) pICH86988-TYLCKaV-B-1.9mer were developed using golden gate cloning method with BsaI.

Validation of infectious TYLCKaV clone

N. benthamiana were grown to 3-4 weeks old stage in growth chamber and used for validation of TYLCKaV infectious clone using TRV VIGS system (Lu et al., 2003; Chung et al., 2004, Dinesh-Kumar et al., 2007). TRV:PDS construct was used as positive control. For negative controls infiltration buffer (Mock) and pICH86988 (empty vector) were used. Briefly, pICH86988-TYLCKaV-A, pICH86988-TYLCKaV-B, TRV1+TRV:PDS were transformed into *Agrobacterium tumefaciens* strain GV3101. Overnight cultures of *Agrobacterium* were grown at 28 °C in LB medium containing antibiotics (50 mg/L kanamycin and 50 mg/L rifampicin). *Agrobacterium* cells were pelleted and resuspended in infiltration medium (10 mM $MgCl_2$, 10 mM MES, 200 μM acetosyringone). OD600 was adjusted to 0.7 and incubated at room temperature with shaking for 2-3 hrs. *A. tumefaciens* carrying pICH86988-TYLCKaV-A and pICH86988-TYLCKaV-B were mixed 1:1, and similarly TRV1 with TRV:PDS. The second and third true leaves were infiltrated at the four-leaf stage of *N. benthamiana*. *S. lycopersicum, C. annuum* plants were grown to cotyledon stage and the two cotyledons were gently infiltrated with constructs using 1 mL needleless syringe.

DNA extraction and electrophoresis

Total genomic DNA was isolated from the plant tissues using cetyltrimethylammonium bromide (CTAB) method (Hwang et al., 2009). The

extracted DNAs were quantified using Epoch microplate spectrophotometer (BioTek, Vermont, US)

Table 1. List of primers used for the construction of an infectious TYLCV clone and virus detection.

Primer name	Sequence (5'-3')	Reference
Gold-TYLCV01-F	GGTCTCAAATGCCACGGCCGCGCAGCGGCATC	
Gold-TYLCV01-R	GGTCTCAAGACCCATAGGAATGGTGATTG	
Gold-TYLCV02-F	GGTCTCAGTCTCCACGGCCGCGCAGCGGCATC	
Gold-TYLCV02-R	GGTCTCAAAGCAGACCCATAGGAATGGTGATTG	
TY-B-GOLD-01F	GGTCTCAAATGCCATGTCAGTTCTCTGCTGTG	
TY-B-GOLD-0R	GGTCTCAGTCTCTTGTCACGAACAATT	
TY-B-GOLD-02F	GGTCTCAAGACCATGTCAGTTCTCTGC	
TY-B-GOLD-02R	GGTCTCAAAGCCGAATTAAAACCTGAATTCC	
PAL1v1978	GCATCTGCAGGCCCACATYGTCTTYCCNGT	(Rojas et al., 1993)
PAR1c715	GATTTCTGCAGTTDATRTTYTCRTCCATCCA	
TYLCKV-A-detec.F	ATGATCTGGCCCACATTGTTTTGC	
TYLCKV-A-detec.R	TCTTTAATTTAATATTCTCATCCATCCA	
TY-B001F	CTCAAACTGACAAGAGATAAGG	
TY-B001R	GTCAGTTTGAGTACATACGTGG	
NbActin_F	CCAGGTATTGCTGATAGAATGAG	(Choi et al., 2016)
NbActin_R	CTGAGGGAAGCCAAGATAGAG	
Pepper_Actin-F	CTTGTCTGTGATAATGGAACAG	(Tanaka et al., 2010)
Pepper_Actin-R	GGGATACTTCAAGGTGAGAATA	
TOMACTF139	TGAGGATATTCAGCCCCTTG	(Sade et al., 2012)
TOMACT728	TCAGCAGTGGTGGTGAACAT	

RESULTS

Detection and sequence analysis of the TYLCKaV

Total genomic DNA was isolated from TYLCD symptomatic leaf samples and the viral detection was performed using polymerase chain reaction (PCR). Using begomovirus universal primers, PAL1v1978/PAR1c715 (Rojas et al., 1993), the expected size of ~1.4 kb amplicon was detected from TYLCKaV-A as shown in **Figure 2. (A)**. Sequence analysis of the amplicon showed that the virus shared 99.8% of homology with *Tomato yellow leaf curl Kanchanburi virus* (TYLCKaV, GenBank Accession No. KF446675.1). Additional primer sets, WsTY03-F/WsTY03-R and TY-B001F/ TY-B001R were used to amplify viral DNA-A and DNA-B and the resulting 2.7 kb fragments. A 2,752 bp PCR amplicon was produced with a following nucleotide composition: DNA-A: 27.2 % A, 32.1 % T, 21.4 % G, and 19.3 % C, DNA-B: 28.5 % A, 33.0 % T, 20.0% G, 18.5 % C. Both showed a 161 bp similar sequence in the intergenic region (IR). The 9 bp sequence 5'-TAATATTAC-3', common in the genomes of all geminiviruses was also observed in intergenic region of TYLCKaV (Lazarowitz and Shepherd, 1992) (**Figure 3**). Based on the NCBI data, the proteins of TYLCKaV was analyzed. DNA-A encodes 6 proteins, coat protein and replication associated proteins (in the virion-sense AV1 (coat protein), AV2 (pre-coat protein), and in the complementary sense AC1 (replication associated protein), AC2 (transcriptional activator protein), AC3 (replication enhancer protein), and AC4. The DNA-B encodes two viral movement associated proteins, in the virion-sense

BV1 (nuclear shuttle protein) and in the complementary sense BC1 (movement protein) (**Figure 2. B**).

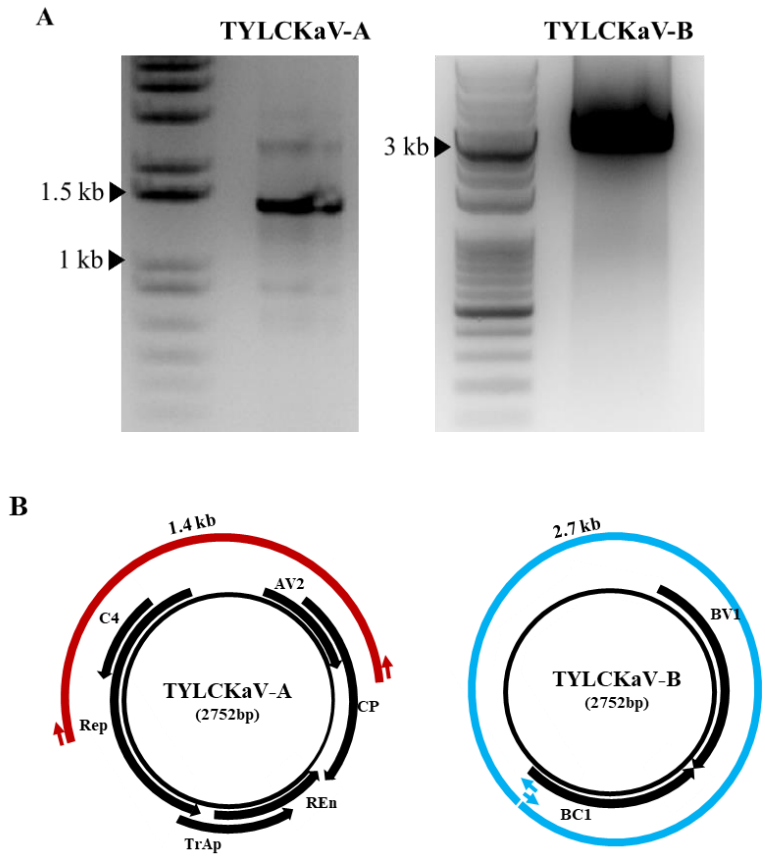

Figure 2. Detection and analysis of viral genome. (A) Using the geminivirus universal primer, ±1.4 kb TYLCKaV fragments were detected. (B) Binding and amplification by the primers in the viral genome.

>**TYLCKaV-A**

a1	**AC**CGAGGGCC	GCGGGGTTTT	GGAGTATGGT	CCCACATGTA	CTGCTGACCA	ATCAGCTTTC
61	AGTTTGAAAG	CTATTTAAGT	GCGTACCGCA	CTACAAAAGC	AGTCATATTT	ACAGCTAATG
121	GAAAACATGT	GGGATCCATT	GCTACACCCT	TTTCCAGAGA	GTTTGCACGG	CTTTAGATGT
181	ATGCTTGCAA	TCAAGTATCT	TCAAAGCTTA	CAGAGTAAGT	ATTCTCCTGA	TACTCTTGGG
241	AGTGAATTCT	TGAAGGATTT	CATCTGCATA	TTGCGGAGTA	GAAATTATGC	CGAAGCGTTC
301	AATAGATACA	GTGACGTCGT	TGCCAATGTC	TATAACACGC	CGGAGGTTAA	ACTTCGGGAG
361	TCAGTACAGT	CTCCCTGCCT	CTGCCCCCAC	TGCCCCAGGC	ATGTCGTACA	AACGAAGGGC
421	TTGGAAAAAC	CGTCCTATGT	ACACGAAGCC	ACGGTTTTAT	CGGTGGCGAA	GGAGCAGTGA
481	TGTTCCACGG	GGTTGTGAGG	GTCCTTGTAA	GGTTCAGTCC	TTTGAGCAGA	GGCATGATAT
541	AACACACACC	GGCAAGGTGT	TGTGTGTGTC	TGATGTTACT	AGAGGCAATG	GTATTACTCA
601	TAGAATAGGT	AAAAGATTTT	GCGTTAAGTC	TGTTTATGTT	ATGGGCAAAA	TCTGGATGGA
661	TGAGAATATT	AAATTAAAGA	ATCACACCAA	CACTGTTATG	TTTTGGCTTG	TTCGCGACAG
721	AAGACCTGTT	ACTACCCCAT	ATGGATTTGG	AGAGTTATTC	AACATGTATG	ACAACGAGCC
781	CAGTACTGCA	ACAATAAAGA	ACGATCTTAG	AGATCGTGTG	CAAGTGCTTC	ATCGTTTCTC
841	AGCATCATTA	ACCGGTGGTC	AATATGCCAG	CAAGGAACAA	GCAGTTATTA	AGAAGTTTTT
901	TAGAGTTAAT	AATTATGTGG	TGTACAATCA	CCAAGAAGCT	GCTAAGTATG	AAAATCATAC
961	TGAGAATGCT	TTGCTATTGT	ATATGGCATG	TACTCATGCC	TCTAATCCTG	TGTATGCAAC
1021	ACTTAAGATT	AGAATTTATT	TTTATGACAA	TGTAACTAAT	TAATAAATGT	TGAATTTTAT
1081	TATATGACAT	TGGTCTACAT	AACTTGTATT	TTCCAATACA	TCCCACAATA	CATAATCAGC
1141	TGCGCGTATT	ACATTGTTAA	TTGAAACTAT	ACCTAAGTTA	TTCAAATATT	TCATACATTG
1201	ATTTTTAAAT	ACTCTTAAGA	AATGCCAGGT	CTGAGGTTGT	AAAGGAGTCC	ATATTCTGAA
1261	TATCAGGAAA	CACTTGTGAA	TCCCCAACGC	CTTCCTGAGG	TTGTGGTTGA	ATTGGATCTG
1321	TATCTCCAAA	TAGTCGTTGT	TGTCGTTGAA	CGGTTTGCTG	TTGTGCTTCA	GCACCTTGAA
1381	ATACAGGGGA	TTTGGAACTT	CCCATATATA	CACGCCATTC	TGCGCTTGAG	CTGCAGTAAT
1441	GGTTTCCCCT	GTGCGTAAAT	CCATATTTGT	GACAATTAAT	GTTTACGTAA	TAGGAACAGC
1501	CACAGTCTAA	GTCTACTCTG	CGTCTTCTTG	GTATTTTTTT	CTTTGCAATT	TTATGTTGTA
1561	CTTTGACTGG	TACAATTGTA	GAGTGGCTGT	TGGAGGGTGA	CGAATTCTGC	ATTTTTCAAA
1621	GCCCAATTTT	TTAATGCCTC	ATTTTTTGGT	TCGTCCAAAA	ACTCCTTAAA	AGAAGAAGAT
1681	GGACCAGGAT	TGCAGAGGAA	GATAGTGGGA	ATTCCACCTT	TAATTTGAAT	TGGTTTACCG
1741	TACTTTGTAT	TGCTTTGCCA	GTCCCTCTGG	GCCCCCATGA	ATTCTTTTAA	GTGCTTTAAA
1801	TAATGCGGAT	CTACGTCATC	AATGACGTTA	TACCAAGCAT	CATTACTGTA	CACTTTTGGG
1861	CTAAGGTCTA	GATGTCCACA	CAAATAATTA	TGAGGGCCCA	ATGATCTGGC	CCACATTGTT
1921	TTGCCGGTTC	TAGATTCACC	TTCAATCACC	ATTCCTATGG	GTCTCCACGG	CCGCGCAGCG
1981	GCATCACATA	CATTTTTCTC	AGCCCAGGAG	GCAAGTTCTT	TTGGAACTTG	ATTGAAAGAC
2041	GACAACTCGT	AAGGATTAAC	AAAAAATTCA	TGGATTGGGG	CAAATATCCG	GTCCAAATTA
2101	GCATTTAGGT	TGTGGTACTG	CAGTACATAG	TCCTTTGGAG	CTAGTTCCTT	AAGGACTGCA
2161	AGAGCCTCCG	ACTTACTTCC	GCAGTTAATT	GCCTTTGCGT	ATGCGTCATT	GGCGGATTGT
2221	TGTCCGCCTC	TAGCAGATCT	TCCATCAATC	TGGAATTCTC	CCCATTCAAT	TGTATCTCCG
2281	TCCTTCTCCA	AATAGGATTT	GACGTCGGAG	CTTGATTTAG	CTCCCTGAAC	ATTTGGATGG
2341	AAATGTGCTG	ATCTGGTTGG	GGAAACCAGG	TCGAAGAATC	TGTTATTTTT	GCACTGGAAT
2401	TTCCCTTCGA	ATTGAATGAG	CACATGGAGA	TGAGGGCTCC	CATCTTCGTG	TAACTCCCTG
2461	CAGATTTTGA	TATATTTTTT	TGAGGTTGGG	GTTTGTAGTT	CCTTCAATTG	AGAGAGAGTT
2521	TCTTCCTTGC	TCAGAGAGCA	CTGAGGATAT	GTGAGAAAAT	AGTTTTTGGC	ATTTATTCTG
2581	AATTTATTTA	GAGAAGGCAT	GCTGACCTGT	CTAATGGTGT	CTCTCCATTT	ATCACATATA
2641	TTGGTGTATA	AGAGTCCTAT	ATATAGTAGA	GACACTAAAT	GGCAATTTTG	GTAATTTTGA
2701	GAGACACCAA	TAGCTTTATT	TTGAATTTAC	AAAGCGGCCC	TCGTA**TAATA**	**TT**

>**TYLCKaV-B**

1	**AC**CGAGGGCC	GCGGGTTTTT	TGGTGTCCCA	ACCCTACTCC	TGACATCATT	GTCCCCTTTT
61	AAATAATCTG	AGCCGTTGCA	AGGTAACACG	TGTTACCATC	CTAGGTCAGG	AGCTTTTCAA
121	TGTTCTGACT	ATATATTGGT	GTTATTTATT	TTTCATCTCA	TTTGTATCTA	AAAATGAGAG
181	TTCCAATCCG	GAGGAATTCA	GGTTTTAATT	CGGACCGTCG	TCCTTCAAAT	GGTTCAATCC
241	ACCGGTGGAA	TTACCCATAT	TCCGGCTATT	TTGGCAGGAG	GATTGGCAAC	CGCGTATATG
301	GCATGCCATT	TGGAAGCCGT	CAAGTCCAAA	GACGTGAATT	TCAGCAGACT	CATCGTCCTA
361	TCAAGTCTTC	TGAGCATGTT	TATTCTCGGA	GGAAGTTTGT	GAAGACTGTA	GAGGAAATTC
421	ACGATGGCAC	TGACTACTTG	CTTTGCAGTA	ACATGTCTAA	GGTGTCGTAC	ATTAGTTACC
481	CTCCTCTATC	CGGTACTGAA	CATGGTAGTA	GAGCTGAATC	CTATCTCAAA	GTCATGGGTT
541	TTAATGTATC	TGGGTCTGTT	GTGCTGAAGC	AGCTGCACAT	GCGTGAAGCA	GATTTGTCTC

601	AAGGAATTCA	TGGCATATTT	ACCACAGTAA	TTGTTCGTGA	CAAGAGACCA	TGTCAGTTCT
661	CTGCTGTGGA	TCCTATCATC	CCATTTGTTG	AGTTATTTGG	ACCAGAAAAG	AGAGCATGCT
721	CCACATTACG	TGTTAGAGAT	TCTTATAAGA	ATCGATTTAG	TGTTGTTTAC	CAAAAGAAAC
781	ATGTTGTAAA	TAGTGCTATG	ACAACACATG	TATTTAGGTA	TAATTTTAAT	GTTAAGTTTT
841	CTAGGTTTCC	TTTCTGGGTG	TCTTTCAAAG	ACACATCCAA	TGCAGAGCCT	ACTGGGCTAT
901	ATTCTAATGT	TTCTAAGAAC	GCATTGGTGG	TGTACTACGT	GTGGCTGTGT	GATTCAAATG
961	TAACAGCCGA	AGTGCACGTA	CAGTATGATT	TGAACTATAT	TGGATAAATA	AAATCATATT
1021	TTTTTTACAT	TTGAAGAGAC	ATTACTCCCT	TCCATACATA	ATTCAACTGT	TTTTTTAATA
1081	ATATTAATTA	CATCATCATT	CACTTTGTTG	CTACATGCAA	CAACTTCCGA	TGCCGAAGGG
1141	CCAGGATCCA	GAGTTGCATC	TTGCAGCTGA	TGCAAATGTC	TGTATGGGTA	TTCCTCTATC
1201	TCGTTATTCC	CCACTGCGCT	GGCTGAAGCC	CAGGTGTGCC	CTTGTGGAAT	GGCATTTGTA
1261	GTGTATCTGG	AAGACTGTGA	CCTAAATTGT	GATAGGGCTT	GTACTGGTTT	CCTTGATACC
1321	TTGGATTGAG	GCACATGCCA	GAAATCTATG	TGGTTCATGT	TATATGCCTT	TGACAGTATT
1381	TCAATTTTTG	GGGACTTAAA	TGTTATTTCA	GAGGACTGTT	TTGCAGAGGA	CATTTTTAAC
1441	TTCCCTTGCA	TCCTACAAAA	GTGAACACCA	TTGACCACGT	TAGTGTCGTC	CACCCTGTAC
1501	AGCACCCTCC	ATGGATTTGG	ATCTTTGGGG	GAGAAGTATG	AGGATGAGTA	GTAGTGGATA
1561	TTGCAGTTGC	ACCCTATGGG	TATTGTGAAC	TCAGCCTGCT	TTGAGTCTCC	CTCATGTAAC
1621	CTTGTGTCGT	GCATTTCTAT	GACCACATGC	CCAGTTGCAT	TGACAGGCAC	TTGATTCCTG
1681	TATTGGAGGA	TGACATGGTC	AATCCTGAGA	CACTTTCCCA	GGAGCATTGA	TAATTTTTGA
1741	TCGACGGTTG	AGGGAAACAA	CAGAGTGACT	TCTGTTTTTT	CATTGGACAG	CTGAAATTCA
1801	GTCCTATCCG	ACGTCGTATA	GGCAATACTG	CTATTGCTGG	ACTCCATTAT	TGCCGATGTC
1861	AATATAATAA	TGATAATTCA	GCTTTTTATA	GACTTTTCAG	AGACTGTCAG	GATAGTGGAA
1921	TGTGACTATT	CCAGTCTATG	CCTTTAAATT	CCACTATCTT	AAAGAGCCAA	TGAGATACGT
1981	CCACGTATGT	ACTTGAACTG	ACAAGATATA	AGGTTATATA	CTTGGTTGTG	GTTCAGCCAC
2041	GTCGCATATT	TAATAGTGGA	ATACAGCTGT	ATTCCACCAC	CACTTCCTTC	CATTGGAAGT
2101	TTTGAAAGAT	ATTATCTTGA	CATATTTTAT	AACAATAATA	ACTTAATTAA	TAATTGAATT
2161	AAGAATTAAA	TTAAGGAACT	AGCTCCAAAG	GACTATGTAC	TGCAGTACCA	CAACCTAAAT
2221	GCTAATTTGG	ACCGGATATT	TGCCCCAATC	CATGAATTTT	TTGTTAATCC	TTACGAGTTG
2281	TCGTGAAATC	GACAATTTAG	AGTAATGTTT	AACTGATCCA	ACAAACTAAT	TATGAGAAAA
2341	CACACACGTT	TGAACATCCT	AATCTTGAGA	AAATCCCGGC	CGCGCAGCGG	CTATGTTCCG
2401	AAAATTAATT	AGAAATTTAG	AAGTGTTAAC	CCCATTTATC	GTATATATTT	AGAAAATAAT
2461	TTAGTGTAAA	TTATTTTTTG	TTAAAGGATA	TTCAGGTGTT	ATATGTATCA	CACTTAAATT
2521	GAAAAATATT	AATGCAATAT	AATTATTGCA	TATTTAAAGG	AAATATTAGA	AATAAATTTT
2581	TAGAGAGAGA	AAGTCTAGAG	AGAAGGCAGA	CTGGTGTCTC	TCCATTTATC	ACATATATTG
2641	GTGTATAAGA	GTCCTATATA	TAGTAGAGAC	ACTAAATGGC	AATTTTGGTA	ATTTTGAGAG
2701	ACACCAATCA	CTTTAATTTG	AATTTGTACA	CGCGGCCCTC	GTATA**TAATA**	**TT**

Figure 3. Nucleotide sequence of the TYLCKaV genome (DNA-A and DNA-B). The hairpin structure sequence 5'-TAATATTAC-3' represented in bold and underlined.

Infectivity test of TYLCKaV infectious clone in tobacco

To validate the infectivity of the developed TYLCKaV infectious clone, *N. benthamiana* plants were coinfiltrated with the developed two constructs of TYLCKaV infectious clones (pICH86988-TYLCKaV-A' and 'pICH86988-TYLCKaV-B') following VIGS protocol (Chung et al., 2004). Initial symptoms of systemic leaf curling was observed within 10 dpi. Severe symptoms were seen in the infected plants compared to the mock infiltrated plants with excessive leaf curling and retarded plant growth by 20 dpi. Finally, at 45 dpi, acute chlorosis and mosaic symptoms were seen (**Figure 4. A**). Viral genome detection by PCR showed that all the infected *N. benthamiana* expressed genomic fragments of TYLCKaV (**Figure 4. B**). Thus, these results confirms and validate the infectivity of the developed TYLCKaV infectious clone.

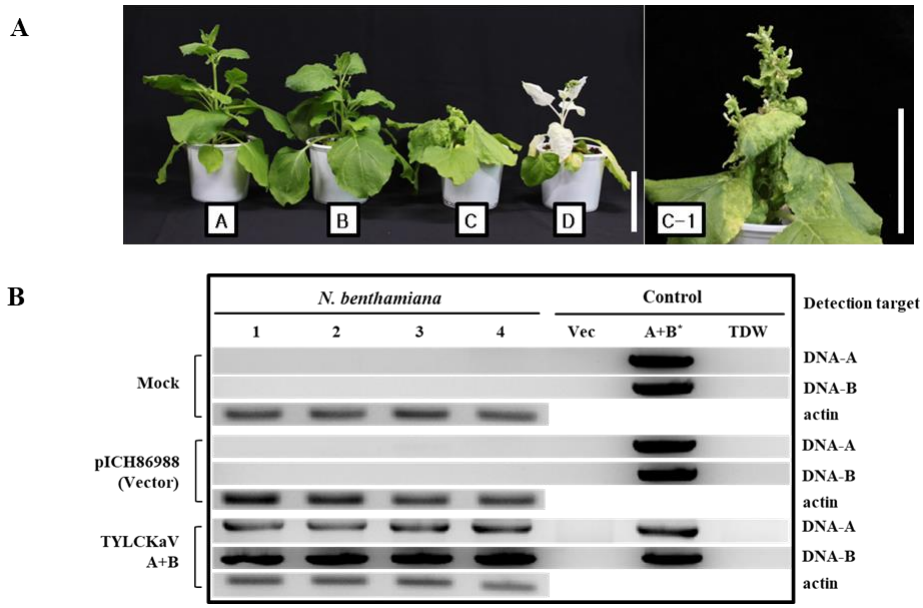

Figure 4. *N. benthamiana* infected with TYLCKaV. (A) A: Mock, B: pICH86988, C: pICH86988-TYLCKaV-A+pICH86988-TYLCKaV-B, D: TRV1+TRV:PDS (at 25 dpi). C-1: pICH86988-TYLCKaV-A+pICH86988-TYLCKaV-B infected tobacco (at 45 dpi). (B) PCR detection of TYLCKaV in the systemic leaves of the four infected *N. benthamiana* plants. TYLCKaV-A detection primer set were used.

Infectivity test of TYLCKaV infectious clone in tomato

TYLCKaV infectivity was tested in three tomato accessions; ‘A39’, ‘A45’ and ‘TY172’. ‘A39’ is TYLCV susceptible tomato, while ‘A45’ (*Ty-3*) and ‘TY172’ (*ty-5*) are TYLCV resistant tomatoes (Dong et al., 2016; Lapidot et al., 2015). The symptoms of TYLCKaV were observed at 20 dpi. The Mock and pICH86988 (empty vector) infected plants were similar with no visible sign of defect or infection. The TYLCKaV (pICH86988-TYLCKaV-A+pICH86988-TYLCKaV-B) infected tomato plants showed prominent visible symptoms such as curling and yellowing of systemic leaves followed by affected growth at 30 dpi (**Figure 5**). The symptoms were observed in all plants including resistant cultivars ‘A45’ and ‘A172’. PCR detection for viral genome showed that TYLCKaV amplification was detected in the systemic leaves of tomatoes (**Table 2; Figure 6**). These results suggest that the developed TYLCKaV infectious clone have the ability to endure the resistance mediated by the resistance genes *Ty-3* and *ty-5*.

Figure 5. Visual symptoms of TYLCKaV in tomato at 30 dpi. Viral symptoms include curling of leaves and, yellow chlorosis on leaves and shoots in TYLCKaV infected plants.

Table 2. Infectivity of TYLCKaV in tomato.

Plant	Number of plants [infected/inoculated]				
	Agro-inoculation construct				PCR detection
	Mock	TRV:PDS	Vector	TYLCKaV	(TYLCKaV)
S. lycopersicum 'A39'	[0/3]	[3/3]	[0/3]	[5/8]	[5/8]
S. lycopersicum 'A45'	[0/3]	[4/4]	[0/3]	[5/11]	[5/11]
S. lycopersicum 'TY172'	[0/4]	[3/4]	[0/3]	[6/9]	[6/9]

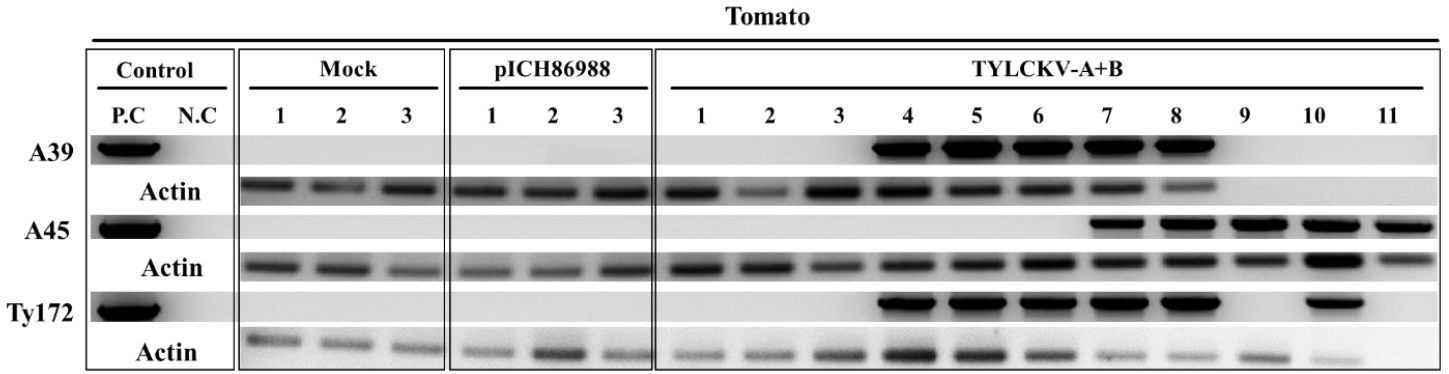

Figure 6. PCR detection of viral DNA in systemically infected leaves of tomato.

Infectivity test of TYLCKaV infectious clone in pepper

Infectivity test were performed in pepper with similar conditions to that of tomato experiments. A commonly used susceptible cultivar 'CM334', ‘Jeju’ and ‘ECW30R’ were included for the study. The TYLCKaV symptoms were scored two weeks after the second inoculation (25 dpi from the first infection). The systemic leaves of VIGS PDS silenced pepper plants showed visible bleaching in cultivars ‘Jeju’ and ‘ECW30R’. In ‘CM334’, PDS silencing was not observed but had visible TRV infectious symptoms. However, no clear TYLCD symptoms were observed in the pepper plants infected with the developed TYLCKaV infectious clone, and were similar to ‘Mock’ and ‘pICH86988’ infiltrated pepper plants (**Figure 7**). Furthermore, no viral genome fragments were detected in the PCR analysis for viral genome detection (**Table 3; Figure 8**).

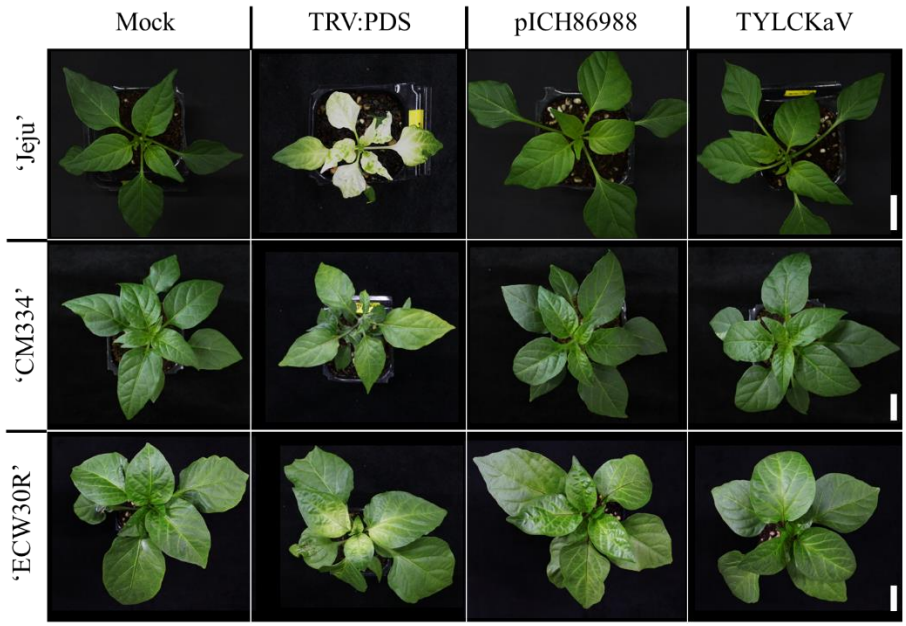

Figure 7. TYLCKaV infectivity assay in pepper at 25 dpi.

Table 3. Infectivity of TYLCKaV in pepper.

Plant	Number of plants [infected/inoculated]				
	Agro-inoculation construct				PCR detection
	Mock	TRV:PDS	Vector	TYLCKaV	(TYLCKaV)
C. annuum 'Jeju'	[0/3]	[4/7]	[0/5]	[0/9]	[0/9]
C. annuum 'CM334'	[0/3]	[0/3]	[0/4]	[0/8]	[0/8]
C. annuum 'ECW30R'	[0/3]	[3/3]	[0/5]	[0/8]	[0/8]

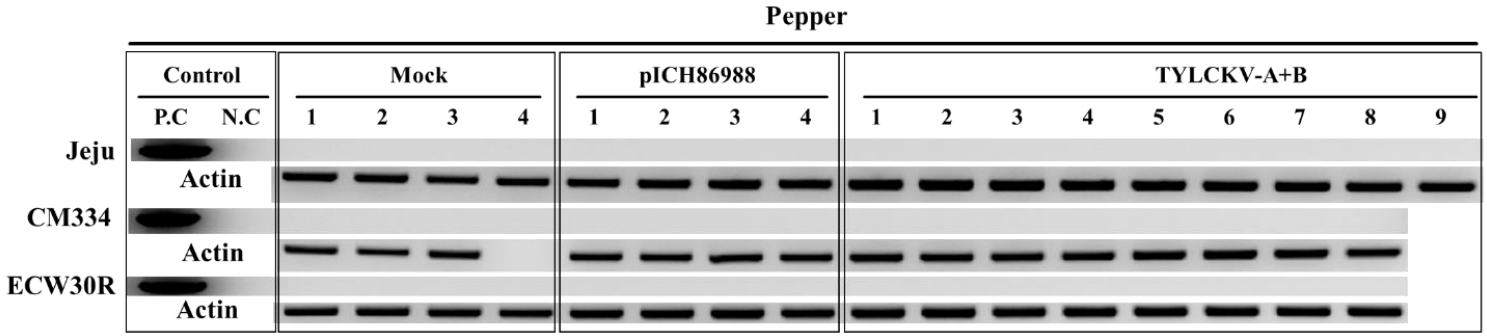

Figure 8. PCR detection of viral DNA in systemically infected leaves of pepper.

DISCUSSION

TYLCKaV strain is one among many TYLCV-like viruses which has started to emerge as a prominent threat to Solanaceae crops in Southeast Asia. TYLCKaV was first reported as a novel bipartite *Begomovirus* species infecting tomato and eggplants in Kanchanaburi Province, Thailand (Green et al., 2003). Recently, TYLCKaV was isolated from the symptomatic pepper and an efficient agroinoculation method was proposed in tomato plants (Koeda et al., 2016). When the TYLCKaV DNA-A genome ssequence was compared to other begomovirus, it showed a high sequence identify (98.0%) with *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) known as pepper-infecting virus (data not shown). This indicates that TYLCKaV is closely associated with pepper infecting TYLCV like viruses. With the rapid spread of the TYLCKaV, research to develop resistant cultivars has to be progressed which requires effective infectious clones and infectivity method in that process.

In this study, TYLCKaV infected pepper leaves were obtained from Thailand and infectious clones were constructed. Major Solanaceae crops, tomato and pepper along with the model plant *N. benthamiana* were chosen for this study. The infectivity tests were performed on the selected plants with the developed TYLCKaV infectious clone. The infectivity was remarkable in *N. benthamiana* with the development of rapid and prominent TYLCD symptoms. Although the infectivity

level was lower in tomato plants compared to tobacco, obvious TYLCD symptoms were seen within 20 dpi. Furthermore, TYLCKaV was detected in the symptomatic leaves from the infected plants supporting the visual observation.

Even though the virus was initially revived from the TYLCKaV infected pepper samples and the TYLCKaV clone had infectivity in tomato and *N. benthamiana*, peppers did not show any symptoms and no virus accumulation upon TYLCKaV inoculation. There are two reasonable possibilities for failure of TYLCKaV infection and no symptom development in pepper. The first possibility is that low efficiency of Agrobacterium-mediated inoculation in pepper. A previous study demonstrated that *Agrobacterium* infiltration method is an appropriate infection tool for many TYLCV (Morilla et al., 2005). In that study, they were able to detect the virus from the inoculated leaf via biolistic bombardment method, whereas agroinfiltration method failed to inoculate none of three monopartite viruses (TYLCSV-ES[2], TYLCV-Mld[ES01/99] and TYLCV-[Alm]) (Morilla et al., 2005). Therefore, for successful TYLCKaV infection in pepper, a different inoculation methods should be tested. Moreover, PDS silencing responses mediated by the TRV vector were different depending on pepper accession. While both the 'Jeju' and 'ECW30R' showed consistent TRV symptoms and PDS silencing, 'CM334' showed only TRV symptoms without PDS silencing.

The second possibility is that TYLCKaV may not have the ability to infect pepper by itself. Successful infection by geminiviruses requires viral DNA

replication and gene expression as well as nuclear, cellular and long distance movement (Morra and Petty, 2000; Tejo-Saavedra et al., 2009). TYLCKaV cannot complete these processes by itself, but can overcome the difficulty by co-infecting with other viruses. Mixed infection is a common phenomenon in viral diseases and there are several mixed infections of begomoviruses reported in tropical and subtropical area (Morilla et al., 2005). Mixed infection of *Pepper huasteco yellow vein virus* (PHYVV) and *Pepper golden mosaic virus* (PepGMV) showed synergistic interaction mainly due to an increased DNA concentration (Rentería-Canett et al., 2011). Synergistic interaction not only provides greater aggression and virulence, but also provide virus a high chance to overcome certain leaf curl resistance and establish property of viruses (Morilla et al., 2005; Kenyon et al., 2014). The first report of TYLCKaV from pepper was also a mixed infection with *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) *and Ageratum yellow vein virus* (AYVV) (Koeda et al., 2016). This suggests that TYLCKaV co-infection with other TYLCV like viruses can be a solution. Additionally, there is another report that co-infection with RNA virus can suppress the resistance by interfering the silencing mechanism of plant defense (Mbanzibwaet al., 2009; Hanley-Bowdoin et al., 2013).

In South Korea, there has been no report on the incidence of TYLCD in pepper. TYLCV incidence in tomato was first reported in 2008 (Ko et al., 2014; Kwak et al., 2008). Due to global warming, the average temperature of South Korea is continuously rising making the crop cultivation environment more favorable for

whitefly multiplication. Under such environment conditions, TYLCV-like viral infection can rapidly spread in Korea similar to TYLCD pandemic in Southeast Asia. This study will be helpful to understand the interaction between TYLCD and pepper and to develop an efficient method of TYLCD viral infection in pepper.

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ABSTRACT IN KOREAN

고추는 매운 맛을 내는 세계적으로 매우 중요한 채소 작물 중 하나이다. 고추는 색소, 필수적인 비타민과 미네랄 등 풍부한 이차대사산물의 소재로 이용된다. 고추 재배에 있어 바이러스 병은 단고추, 매운 고추 모두에서 심각한 생산량 손실을 일으키는 요인이다. 본 연구에서는 고추에 있어서 심각한 수량 손실 및 품질 하락의 원인이 되는 두 가지 바이러스에 대한 연구를 진행하였다.

첫 번째 장에서는 오이 모자이크 바이러스 (CMV) 의 새로운 변이 균주인 CMV-P1을 확인하고 이에 대한 저항성 자원을 찾고자 고추 유전자원에서 바이러스 저항성을 검정하였다. 정확한 저항성을 확인하기 위해서 바이러스는 효소면역정량법 (ELISA) 을 이용하여 식물세포 내에 존재하는 바이러스 축적량을 측정하였다. 강한 저항성을 보이는 고추 계통을 선발한 후, 이를 대상으로 각각을 'Jeju'와 'Lam32'에 교배시켜 받은 F_1에서 저항성 유전자의 유전양식을 확인했다. 그 결과, 7개의 저항성 유전자원들이 발견되었고 저항성 유전자원들이 갖는 저항성 유전자가 기존에 알려진 *cmr2* 저항성 유전자와 공통적인 것임을 확인했다. 본 연구에서 발견한 저항성 유전자원은 CMV-P1 저항성 고추 품종 개발에 활용될 수 있을 것으로 기대된다.

두 번째 장에서는, begomovirus에 속하는 토마토 황화 잎마름 Kanchanavuri 바이러스 (TYLCKaV)의 유전체를 확인 및 분석했다. TYLCKaV 유전체 정보를 바탕으로 Golden-gate 클로닝 기법을 이용하여 바이러스 감염 클론을 개발했다. TYLCKaV 감염클론은 agroinfiltration 법으로 담배, 토마토, 고추에 접종했다. 접종된 담배와 토마토에서는 바이러스의 증상이 확인된 반면, 고추에서는 TYLCKaV의 감염 증상이

나타나지 않았다. 바이러스의 감염 여부는 중합효소 연쇄 반응(PCR)법을 이용하여 재 확인 하였다. 이 연구에서 개발된 TYLCKaV 감염 클론은 앞으로 병저항성 연구에 유용한 도구로 이용될 것이다. 한편, TYLCKaV와 고추간 이병성을 나타내는 요소에 대해서는 추가적인 연구가 필요할 것이다.

주요어: 오이 모자이크 바이러스, 토마토 황화 잎말림 바이러스, 접종 클론, 고추, 고추 바이러스

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