# XVI. EUCARPIA

## Capsicum and Eggplant Meeting

KECSKEMÉT • HUNGARY • 12-14.SEPT.2016

*in memoriam*
*Dr. Alain Palloix*

# PROGRAM

# PROGRAM

## Sunday, 11 September

| | |
|---|---|
| 16:00 | **Registration and Poster set up** |
| 19:00-21:30 | **Welcome reception** |

## Monday, 12 September

| | |
|---|---|
| 7:30- | **Registration** |
| 8:30-9:30 | **Opening Session**<br>**Chair:** Sergio Lanteri<br><br>**Lajos Helyes, Katalin Ertsey-Peregi**<br>Welcome on behalf of the Hungarian Local Organizers<br><br>**Jaime Prohens**<br>Welcome by the EUCARPIA Representative<br><br>**Véronique Lefebvre**<br>Alain Palloix commemorative lecture<br><br>**Miklós Fári**<br>**Szent-Györgyi Memorial Lecture**<br>Father of vitamin C, Albert Szent-Györgyi (1893-1986) and his time. Science, creativity and society behind a Nobel Prize winner (1937) |
| 9:30-10:45 | **Session 1 – Breeding Strategies**<br>**Chairs:** Paul Bosland, Giuseppe L. Rotino<br><br>**9:30-9:45**<br>**Jaime Prohens**<br>Utilization of crop wild relatives in eggplant pre-breeding for adaptation to climate change<br><br>**9:45-10:00**<br>**Gábor Palotás**<br>New hot interspecific hybrid variety between *Capsicum annuum* L. and *Capsicum chinense* Jacq. |

**10:00-10:15**
**Sanjeet Kumar**
Male sterility research in peppers at AVRDC – The World Vegetable Center

**10:15- 10:30**
**Dario Danojevic**
Sweet pepper breeding against bacterial spot *(Xanthomonas euvesicatoria)* in Serbia

**10:30-10:45**
**Miklós Fári**
What kind of root should a pepper plant have?

10:45-11:10 **Coffee break and Poster viewing** *sponsored by:*

11:10-11:55 **Session 1 – Breeding Strategies (ctd.)**
**Chairs:** Paul Bosland, Giuseppe L. Rotino

**11:10-11:25**
**Roeland E. Voorrips**
Aphid resistance in a *Capsicum* collection

**11:25-11:40**
**Pál Salamon**
Symptoms caused by *Tomato spotted wilt virus* (TSWV) in Pepper (*Capsicum* spp.) and marker assisted selection of TSWV resistant pepper lines for hybrid constructions

**11:40-11:55**
**István Tóbiás**
Evergreen question: Whether *Tobamoviruses* are transmitted via pepper seeds or not?

12:00-12:45 **Session 2 – Growing and Seed Production**
**Chair:** Zsuzsanna Füstös

**12:00-12:15**
**Katalin Ertsey-Peregi**
Sweet pepper (*Capsicum annuum* L.) growing on a basis of thermal water with respect of protection the natural environment

**12:15- 12:30**
**John Damicone**
Biology and management of bacterial spot of pepper in Oklahoma, United States

**12:30- 12:45**
**András Kovács**
Short evaluation of eggplant production and variety usage in Romania



12:45-14:30 **Lunch**

14:30-14:50 **Gathering in the lobby for the technical visit**

15:00-19:30 **Technical visits**
- Pepper and eggplant trials (ZKI)
- Pepper processing plant (UNIVER)

**Open field trip**

19:30-22:30 **Traditional folklore dinner**

## Tuesday, 13 September

8:30-10:00 **Session 3 – Genetic Resources**
**Chairs:** Jaime Prohens, Marie-Christine Daunay

**8:30-8:45**
**Marie-Christine Daunay**
Eggplant resistance to bacterial wilt and to *Fusarium* wilt: Is there a link?

**8:45-9:00**
**Zsuzsanna Füstös**
Study of morphological characteristics of eggplant *(Solanum melongena* L.*)* varieties

**9:00-9:15**
**Claudio Dal Zovo**
Wild *Capsicum* in the area of the Amboró National Park in Bolivia

**9:15-9:30**
**John Samuels**
*Solanum insanum* L. (Solanaceae): Linnaean species or introgressed hybrid?

**9:30-9:45**
**Olga Babak**
Development of DNA-markers to fruit quality genes of sweet pepper *(Capsicum annuum* L.*)*

**9:45-10:00**
**Rosana Rodrigues**
A breeding program for resistance to anthracnose in sweet and chili pepper

**10:00-10:15**
**François Villeneuve**
Screening of solanaceous wild relatives for graft affinity with eggplant *(Solanum melongena* L.*)*

10:15-10:40 **Coffee break and Poster viewing** *sponsored by:*

10:40-11:40 **Session 3 – Genetic Resources (ctd.)**
**Chairs:** Jaime Prohens, Marie-Christine Daunay

**10:40-10:55**
**Awang Maharijaya**
Antixenosis and antibiosis based resistance of chilli pepper to melon aphid

**10:55-11:10**
**Orarat Mongkolporn**
Genetic diversity of Thai native chili using diversity arrays technology

**11:10-11:25**
**Lucie Tamisier**
Quantitative trait loci in pepper genome control the effective population size of two RNA viruses at inoculation

**11:25-11:40**
**Helena Stavělíková**
Germplasm of pepper *(Capsicum annuum* L.*)* in Czech Republic

11:40-12:55 **Session 4 – Physiology and Nutritional Value**
**Chair:** Lajos Helyes

**11:40-11:55**
**Zsuzsanna Füstös**
The nutrition value and storage of eggplant *(Solanum melongena* L.*)* varieties

**11:55-12:10**
**I. Kutalmis Kutsal**
Effects of mychorriza on pepper plant growth parameters and nutrient uptake under salinity stress

**12:10-12:25**
**Kietsuda Luengwilai**
Does anthracnose resistance associate with cuticle characteristics and spore attachment?

**12:25-12:40**
**Ozlem Altuntas**
Effects of michorriza on alleviating salt stress of *Capsicum annuum* L. by ion regulation

**12:40-12:55**
**Lajos Helyes**
Correlation between carotenoid components of chili pepper fruits and VIS/NIR reflectance





13:00-14:45 **Lunch and Poster viewing**

14:45-16:15 **Session 5 – Molecular Genetics and Biotechnologies**
**Chairs:** Sergio Lanteri, Anikó Gémes Juhász

**14:45-15:00**
**Ezio Portis**
A high quality eggplant *(Solanum melongena* L.*)* genome sequence

**15:00-15:15**
**Zoltán Kristóf**
Ultrastructural study of *in vitro* and *in situ* pepper embryo development

**15:15-15:30**
**Giuseppe L. Rotino**
MicroRNA156/7-Mediated control of anthocyanin pigment accumulation in eggplant fruit skin

**15:30-15:45**
**Rodrigo A. VALVERDE**
Interactions between *Bell pepper endornavirus*, bell pepper, and acute plant viruses

**15:45-16:00**
**Santiago Vilanova**
The transcriptomes of *Solanum incanum* and *S. aethiopicum* provide information of relevance for common eggplant breeding

**16:00-16:15**
**Sylvia E. Salgon**
Genetic mapping of broad-spectrum QTLs and strain-specific major QTL for resistance to *Ralstonia solanacearum* in eggplant using GBS

**16:15-16:30**
**Yoshiyuki Tanaka**
Multiple mutated putative aminotransferase alleles contribute to low pungency and capsinoid biosynthesis in *Capsicum chinense*

16:30-16.50 **Coffee break** *sponsored by:*

16:50-19:30 **Optional cultural program**

20:00-23:00 **Gala dinner**

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## Wednesday, 14 September

8:30-10:30 **Poster session**
**Chair:** Katalin Ertsey-Peregi

10:30-10:45 **Coffee break** *sponsored by:* Dél-Kerti SZENTES

10:45-11:45 **Session 5 – Molecular Genetics and Biotechnologies (ctd.)**
**Chairs:** Sergio Lanteri, Anikó Gémes Juhász

**10:45-11:00**
**Laura Toppino**
QTLs mapping for *Fusarium oxysporum* and *Verticillium dahliae* resistance in eggplant *(Solanum melongena* L.*)*

**11:00-11:15**
**Pasquale Tripodi**
Genotyping by sequencing for population structure and genome-wide association analysis for fruit shape and size in pepper (*C. annuum* L.) germplasm

**11:15-11:30**
**Jana Leide**
Cutin deficiency of bell pepper *(Capsicum annuum* L.*)* results in gluey berries

**11:30-11:45**
**Hatira Taskin**
Comparison of pepper genotypes originated from Turkey and the other countries for anther culture response

11:45-12:15 **Conclusion and closing**

12:15-14:00 **Lunch**

14:30- **Optional post-conference tours**





# LIST OF POSTERS

## Session 1 – Breeding Strategies

| Poster number | Presenting author | Title of poster |
|---|---|---|
| P1-01 | **András Andrásfalvy** | István Túri – The innovative pepper breeder |
| P1-02 | **Andrea Moór** | Lambert Angeli, pioneering breeder of the first white, sweet variety of bell pepper was born a hundred years ago |
| P1-03 | **Zoltán Timár** | The life and work of a paprika breeder Ferenc Márkus |
| ~~P1-04~~ WITHDRAWN | ~~**Saadet Büyükalaca**~~ | ~~Investigation of obtaining fertile *S. melongena x S. torvum* hybrid populations~~ |
| P1-05 | **Ros Caridad** | Could quantitative resistance increase the durability of major genes conferring nematode resistance in pepper? |
| P1-06 | **Dilek Kandemir** | Determination of reaction of *Solanum aethiopium* and *Solanum incanum* genotypes against *Fusarium oxysporum f. sp. melongenae* |
| P1-07 | **Gábor Palotás** | 20 years of non-hypersensitive, non-specific, recessive resistance in pepper – review |
| P1-08 | **Mariola Plazas** | Screening for drought tolerance in eggplant relatives and interspecific hybrids |
| P1-09 | **Claudia Ribeiro** | Breeding Calabrian pepper lines (*Capsicum annuum* L.) for Brazilian agriculture from *sui generis* introduction of germplasm |
| P1-10 | **Claudia Ribeiro** | Synthesis of a base population of Habanero chile pepper and initial assessment of derived $F_3$ lines *(Capsicum chinense)* |
| P1-11 | **Attila Rózsás** | Conservation, landscape and home garden varieties in South part of Hungary |
| P1-12 | **Zsolt Sági** | Higher quality traits – breeding strategies in pepper |
| P1-13 | **Csaba Sebesi** | High quality apple peppers for the canning industry |
| P1-14 | **Olga Timina** | Breeding use of *Capsicum annuum* L. mutant genepool |
| P1-15 | **Péter Varró** | Breeding of a high yielding white waxy hibrid ZKI 113485 for the Mediterranean region |
| P1-16 | **Lajos Zatykó** | The role of general and specific combining abilities in pepper hybrid breeding |

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## Capsicum and Eggplant Meeting

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# PROCEEDINGS


**Editors:**

Katalin Ertsey-Peregi
Zsuzsanna Füstös
Gábor Palotás
Gábor Csilléry

# P5-05

# Isolation and characterization of pepper genes involved in CMV-P1 infection

Yeaseong Ha, Joung-Ho Lee, Yoomi Choi, Min-Young Kang, JeeNa Hwang, Won-Hee Kang, Jin-Kyung Kwon and Byoung-Cheorl Kang

*Department of Plant Science, Plant Genomics and Breeding Institute and Vegetable Breeding Research Center, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea*

**Abstract**

*Capsicum annuum* 'Bukang' is aresistant variety to *Cucumber mosaic virus* isolate-P0 (CMV-P0), CMV-P1 can overcome the CMV resistance of 'Bukang' due to mutations in Helicase (Hel) domain of CMV RNA1. To identify host factors involved in CMV-P1 infection, a yeast two-hybrid system derived from *C. annuum* 'Bukang' cDNA library was used. A total of 156 potential clones interacting with the CMV-P1 RNA helicase domain were isolated. These clones were confirmed by β-galactosidase filter lift assay, PCR screening and sequence analysis. Then, we narrowed the ten candidate host genes which are related to virus infection, replication or virus movement. To elucidate functions of these candidate genes, each gene was silenced by virus induced gene silencing in *Nicotiana benthamiana*. The silenced plants were then inoculated with green fluorescent protein (GFP) tagged CMV-P1. Virus accumulations in silenced plants were assessed by monitoring GFP fluorescence and enzyme-linked immunosorbent assay (ELISA). Among ten genes, silencing of *formate dehydrogenase* (FDH) or *calreticulin-3* (CRT3) resulted in weak GFP signals of CMV-P1 in the inoculated or upper leaves. These results suggested that FDH and CRT3 are essential for CMV infection in plants. The importance of FDH and CRT3 in CMV-P1 accumulation was also validated by the accumulation level of CMV coat protein confirmed by ELISA.

Altogether, these results demonstrate that FDHand CRT3are required for CMV-P1 infectionin plants.

**Keywords:** *Capsicum annuum*, *Cucumber mosaic virus*, host factor, virus resistance, *formate dehydrogenase*, *calreticulin-3*

# Isolation and Characterization of Pepper Genes Involved in CMV-P1 Infection

Yeaseong Ha[1], Joung-Ho Lee[1], Yoomi Choi[1], Min-Young Kang[1], JeeNa Hwang[1], Won-Hee Kang[1], Jin-Kyung Kwon[1] and Byoung-Cheorl Kang[1*]

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## ABSTRACT

*Capsicum annuum* 'Bukang' is a resistant variety to *Cucumber mosaic virus* isolate-P0 (CMV-P0), CMV-P1 can overcome the CMV resistance of 'Bukang' due to mutations in Helicase (Hel) domain of CMV RNA1. To identify host factors involved in CMV-P1 infection, a yeast two-hybrid system derived from *C. annuum* 'Bukang' cDNA library was used. A total of 156 potential clones interacting with the CMV-P1 RNA helicase domain were isolated. These clones were confirmed by β-galactosidase filter lift assay, PCR screening and sequence analysis. Then, we narrowed the ten candidate host genes which are related to virus infection, replication or virus movement. To elucidate functions of these candidate genes, each gene was silenced by virus induced gene silencing in *Nicotiana benthamiana*. The silenced plants were then inoculated with green fluorescent protein (GFP) tagged CMV-P1. Virus accumulations in silenced plants were assessed by monitoring GFP fluorescence and enzyme-linked immunosorbent assay (ELISA). Among ten genes, silencing of *formate dehydrogenase* (FDH) or *calreticulin-3* (CRT3) resulted in weak GFP signals of CMV-P1 in the inoculated or upper leaves. These results suggested that FDH and CRT3 are essential for CMV infection in plants. The importance of FDH and CRT3 in CMV-P1 accumulation was also validated by the accumulation level of CMV coat protein confirmed by ELISA. Altogether, these results demonstrate that FDH and CRT3 are required for CMV-P1 infection in plants.

## INTRODUCTION

Viruses are intimately associated with and completely dependent on their host, and interaction with host factor requires for infection cycles. Mutations in host factors may abolish the interaction (Whitham et al., 2004). For this reason, host factors have been studied in various plants and viruses. For example, Tsip1 which interacts with the CMV 1a and 2a controls CMV multiplication in tobacco plant (Huh et al., 2011). And NtTLP1 which directly interacts with CMV 1a protein plays an important role in the regulation of CMV replication and/or movement (Kim et al., 2005).

CMV has the broadest host range among plant viruses. One of the CMV resistance sources C. annuum 'Bukang', which is resistance to CMV-P0 strains (CMV-Kor and CMV-Fny). C. annuum 'Bukang' contains a single dominant resistance gene, Cmr1 (Kang et al., 2010) which suppresses the systemic infection of CMV-P0 strains. Recently, a new strain CMV-P1 breaking the Cmr1 resistance was identified in South Korea.

To clarify which RNA genome is involved in breaking the Cmr1, chimeric CMV viruses were constructed by combining CMV-Fny and CMV-P1 cDNA clones (Kang et al., 2011). 3' region of CMV-P1 RNA1 helicase domain has been implicated to play a role in viral replication and systemic infection (Kang et al., 2011).

In this study, we tried to identify host genes that interact with CMV-P1 helicase domain using a yeast two-hybrid system. To validate requirement of selected host genes in CMV-P1 infection, selected genes were silenced via VIGS and silenced plants were challenged with CMV-P1 harboring the green fluorescent protein (GFP). Later, virus accumulation in silenced plants was assessed by monitoring GFP fluorescence and enzyme-linked immunosorbet assay (ELISA). Through these steps it was revealed that *formate dehydrogenase* and *calreticulin-3 precursor* are essential host genes required for CMV-P1 infection

## OBJECTIVES

- To isolate and characterize host factors interacting with CMV-P1 RNA1 helicase domain from C. annuum 'Bukang'
- To identify of CMV-P1 infection mechanism to C. annuum 'Bukang'
- To engineer of CMV resistance using the host factors

## MATERIALS & METHODS

- **Yeast two-hybrid screening**

Primer was designed based on CMV-P1 RNA1 helicase domain sequence. To construct the bait vector, CMV-P1 RNA1 helicase domain fragment was prepared by PCR from CMV-P1 cDNA clone. CMV CMV-P1 RNA1 helicase domain fragment was cloned into pBD-GAL4 Cam vector (Agilent Technologies, Santa Clara, CA, USA) as a bait. The bait vector (pBD-GAL4 Cam vector) was transformed into *Saccharomyces cerevisiae* strain YRG-2, and was incubated in the selection media (SD-Trp, Leu). *C. annuum* 'Bukang' cDNA library which was cloned into pAD-GAL4-2.1 vector (Agilent Technologies, Santa Clara, CA, USA) as a prey was provided by professor Doil Choi. *C. annuum* 'Bukang' cDNA library screening was conducted as described in the manufacturer's instruction (Agilent Technologies, Santa Clara, CA, USA). The prey vector (pAD-GAL4-2.1 vector) was transformed into YRG-2 which contains the bait vector (pBD-GAL4 Cam vector), and was incubated in the selection media (SD-Trp, Leu, His).

- **β-Galactosidase Filter lift assay**

Candidate clones were incubated for 4 days at 30℃ and 3MM paper (Whatman, Maidstone, Kent, UK) was contacted with all of the clones. The 3MM paper was dipped in the liquid nitrogen for 15 seconds and thawed for 1 minute. After this step was repeated three times, the 3MM paper soaked in the Z buffer with X-gal. It was then incubated at 25℃ for 5 hours in the dark.

- **Cloning & sequencing**

To isolate the candidate cDNA, primer was designed based on pAD-GAL4-2.1 vector sequence (Agilent Technologies, Santa Clara, CA, USA). T-Blunt PCR cloning system (Solgent, Daejeon, South Korea) was used for candidate gene TA cloning. Candidate genes were amplified by colony PCR from yeast clones containing candidate cDNA. PCR product sequencing was performed at NICEM (Seoul national university, Seoul, South Korea).

- **Sequence analysis of candidate genes**

DNA and protein sequence analysis were performed with the BLAST algorithm at the National Center for Biotechnology Information (NCBI, Bethesda, MA, USA). Using *Capsicum annuum* BAC Database Pepper ver. 0.83_contig CLC-Gapfilling (Molecular Genetics Laboratory in Seoul national university, Seoul, South Korea), the candidate genes was identified copy number and full length sequence in *C. annuum*.

- **Virus-induced gene silencing**

*Agrobacterium* containing TRV1 and 10 TRV2::candidate gene (or TRV2::PDS) were incubated in liquid LB media containing antibiotics (50 mg/L kanamycin and 50 mg/L rifampicin) for 20 hours at 30℃. The *Agrobacterium* cells were harvested and resuspended in infiltration media (10 mM $MgCl_2$, 10 mM MES, 200 mM acetosyringone). *Agrobacterium* cells containing TRV2::PDS or TRV2::Candidate gene were adjusted to 0.4 $OD_{600}$ and incubated at room temperature with shaking for 4 hours. *Agrobacterium* cells containing TRV1 was adjusted to 0.3 $OD_{600}$ and incubated as described previously. TRV1 and TRV2 were mixed in a 1:1 ratio and infiltrated into N. benthamiana at the four-leaf stage by a 1ml syringe needle.

- **Inoculum preparation and virus inoculation**

*Agrobacterium tumefaciens* strain GV2260 containing CMV-P1-GFP constructs were incubated in 3 ml LB media containing antibiotics (50 mg/L kanamycin and 50 mg/L rifampicin) at 30℃. After harvest, each cell culture was adjusted to 0.4 $OD_{600}$, and incubated for 4 hours. Then cell cultures were mixed in a 1:1:1 ratio and infiltrated into *N. benthamiana*. CMV-P1-GFP inoculum was prepared from infected *N. benthamiana* leaves after 14 days of inoculation. One gram of leaves was ground in 10 ml of 0.1 M potassium phosphate buffer (pH 7.5). Plants were inoculated by rub-inoculation with light carborundum dusting. Plants were kept in a growth chamber at 25℃ until symptom observation. Virus accumulation was tested at 5 and 10 days post inoculation (dpi) using DAS-ELISA according to the manufacturer's instructions (Agdia, Santa Fe Springs, CA, USA). Each sample was measured at 405 nm absorbance value.

## RESULTS & DISCUSSION

- **Identification of avirulence determinant of CMV-P1**

To identify the infection aspect of CMV-P1, CMV-Fny and CMV-P1, they were inoculated in *C. annuum* 'Bukang'. And the plant which was inoculated with CMV-P1 showed leaf distortion symptoms and systemic infection. To identify the avirulence determinant in CMV-P1, chimeric CMV viruses were constructed by substituting the genomic regions of CMV-P1 with CMV-Fny. Chimeric viruses containing CMV-P1 RNA1 genome able to induce systemic infection, and 3' region of CMV-P1 RNA1 helicase domain is especially related to systemic infection (Kang et al., 2011).

- **Isolation of candidate genes interaction with CMV helicase domain**

To identify host proteins interacting with CMV-P1 RNA1 helicase domain, CMV-P1 RNA1 helicase domain was fused to bait vector and *C. annuum* 'Bukang' cDNA library was fused to prey vector. 100,080 transformants were screened on the selection media (-Trp, Leu, His) for interaction identification, and 156 candidate clones were identified. To confirm the interactions, we performed β-galactosidase filter lift assay. The color of 82 candidate clones was changed to blue (Table 1).

Then, we performed colony PCR for 82 candidate clones. 80 candidate genes were amplified, and were used for sequence analysis. The sequence analysis revealed that 78 candidate genes encoded different kinds of genes in plant. 10 genes were non-relation with pathogens, 13 genes had low coverage and identity with NCBI DB,17 genes were related to pathogens and 38 genes were false or unknown function. 10 candidate genes were selected based on high identity and coverage from pathogens related group for gene characterization (Table 2). Each genes are encoded acireductone dioxygenase, ADP-glucose pyrophosphorylase, ADP-ribosylation factor 1, ADP-ribosylation factor, calreticulin-3 precursor, cysteine synthase, formate dehydrogenase, histone-H3, phosphomannomutase and polyubiqutin 6PU11.

**Table 1**. Summary of screening of host genes interacting with the CMV-P1 helicase domain

| Total number Of clones | Number of selected clones | | | |
|---|---|---|---|---|
| | Y2H screening | β-galactosidase filter lift assay | PCR screening | Sequence analysis |
| 100,080 | 156 | 82 | 80 | 78 |

**Table 2**. List of candidate genes interacting with the CMV-P1 helicase domain.

| Candidate gene | Putative Function | Sequence ID in *C. annuum* |
|---|---|---|
| *PPM* | Phosphomannomutase | CA08g10130 |
| *FDH* | Formate dehydrogenase | CA02g29530 |
| *CRT3* | Calreticulin-3 | CA00g87370 |
| *UBI11* | Hexameric polyubiquitin 6PU11 | CA00g79660 |
| *Cysk* | Cysteine synthase | CA08g04930 |
| *AGP-S2* | ADP-glucose pyrophosphorylase | CA07g05920 |
| *ARF1* | ADP-ribosylation factor 1 | CA08g00830 |
| *ARF* | ADP-ribosylation factor | CA01g22680 |
| *H3* | Histone-H3 | CA04g15130 |
| *ARD* | Acireductone dioxygenase | CA03g06820 |

- **Functions of candidate genes in CMV infection**

To test the requirement of selected genes for CMV infection, the selected genes were silenced by a TRV-based VIGS system. Ten selected genes were cloned into the TRV2-LIC vector. Each gene was silenced in *N. benthamiana*. *Phytoene desaturase* (PDS)-silenced plants were used as a silencing control. As expected, the silencing of PDS resulted in photo-bleaching at 10 to 12 dpi. To check the level of silenced gene expression, real-time PCR using gene-specific primers was performed. Infiltrated TRV2::00 plant was used for comparison with candidate gene-silenced plants. Real-time PCR analysis showed that expression levels of 2 candidate genes (*formate dehydrogenase*, *calreticulin-3 precursor*) were significantly reduced (Figure 1).

To investigate whether selected genes play a key role in CMV-P1 infection, gene-silenced plants were challenged with CMV-P1. After infection, virus spreads in plants were observed under a confocal laser-scanning microscope. GFP fluorescence in inoculated and systemic leaves of silenced plants were monitored at 5 and 10 days post inoculation (dpi). TRV::00 plant was used as a positive control and showed GFP signals in the whole inoculated leaves at 10 dpi as well as upper leaves. At 10 dpi, *Formate dehydrogenase* silenced plants showed local infection in inoculated leaves and no CMV-P1-GFP signal in upper leaves. In the case of *calreticulin-3 precursor* silenced plants, there was no CMV-P1-GFP signal in both inoculated and upper leaves (Figure 2).

To confirm the virus infection in the candidate gene-silenced plants, CMV-P1 coat protein (CP) was detected by ELISA at 10 dpi. As was observed in confocal microscopy analysis, in the leaves of *formate dehydrogenase*-silenced and *calreticulin-3 precursor*–silenced plants, CMV-P1 coat protein accumulation were greatly reduced and the same results were obtained in the upper leaves. (Figure 3). Taken together, these results strongly suggest that *formate dehydrogenase* and *Calreticulin-3 precursor* play a essential role in CMV-P1 replication.

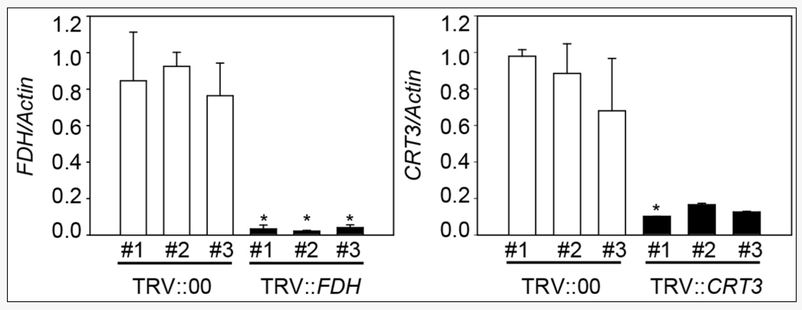


**Figure 1. Real-time PCR analysis of host factor gene expression of in silenced plants.** The mRNA expression level of each candidate gene was evaluated by Real-Time PCR analysis. The words which are located left side of images are candidate genes. Each candidate gene cDNA was amplified at 40 cycles. Asterisks indicate statistically significant differences relative to empty vector (*p < 0.05)

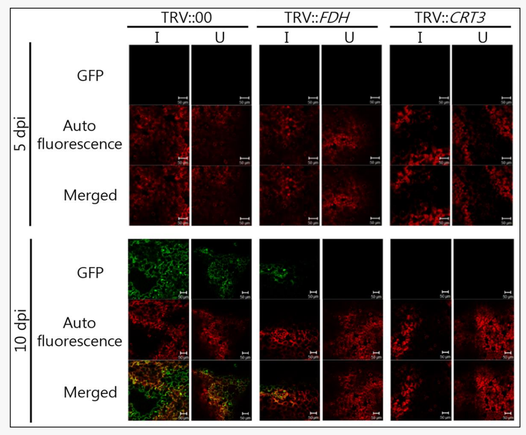


**Figure 2. Monitoring CMV replication and movement by GFP fluorescence in silenced plants inoculated with CMV-P1-GFP.** GFP fluorescence was observed at 5 dpi and 10 dpi in VIGS plants, and TRV::00 plants were used as a positive control. Images on the left are optical sections of the inoculated leaves, and those on the right are optical sections in the upper leaves. Images top to bottom are GFP, autofluorescence, and merged images, respectively. The green fluorescence signal indicates CMV-P1 expressing GFP, and red fluorescence signal indicates chloroplast. Scale bars = 50 μm.

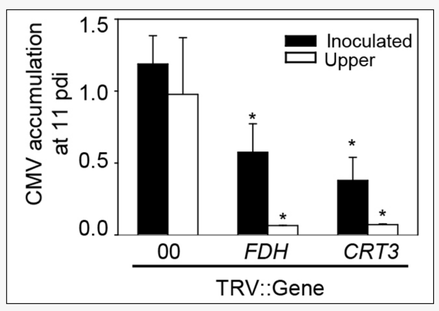


**Figure 3. CMV accumulation in plants silenced with candidate host factors.** Virus accumulation was detected by ELISA. Two leaf discs of the inoculated and the upper leaves of the inoculated plants were sampled at 10 dpi.

## REFERENCE

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