J. Trop. Agric. and Fd. Sc. 34(1)(2006): 139–148

Cymbidium mosaic virus and odontoglossum ringspot tobamovirus genes cloned from infected *Oncidium* orchids

(Gen-gen cymbidium mosaic virus dan odontoglossum ringspot tobamovirus yang diklon daripada orkid *Oncidium* yang terinfeksi)

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Key words: coat protein gene, cymbidium mosaic virus, odontoglossum ringspot tobamovirus, *Oncidium* Goldiana x *Oncidium* Flexuosum, genetic engineering

Abstract

Several recombinant phages were picked at random from the cDNA library of *Oncidium* (*Oncidium* Goldiana x *Oncidium* Flexuosum) flowers, converted into plasmids by in vivo excision and sequenced. Two of the clones named CyMV1 and CyMV2, showed very high DNA and protein sequence homology to those of the cymbidium mosaic virus (CyMV) in the genebank database. CyMV1, 1,186 bp in size, contained within it the entire sequence for coat protein (CP) gene, movement protein (MP)3 gene and an almost complete sequence for MP2 gene. CyMV2, which is 626 bp in size, only contained the extreme 3' end sequence of the RNA polymerase gene. The percentage of homology of the isolated CyMV1 gene was 97% to the Taiwanese strain (AY571289), 96% to the Korean type 2 CyMV complete genome (AF016914) and to the Singaporean CyMV complete genome (CMU62963) in the CP and MP regions of the genome. CyMV2 showed 95% homology to the Korean type 2 CyMV complete genome (AF016914) and to the Singaporean CyMV complete genome (CMU62963) but in the RNA polymerase region. Another clone named ORSV1, 728 bp in size, isolated by RT-PCR method was a partial fragment of odontoglossum ringspot virus (ORSV) RNA replicase gene. This partial gene sequence of ORSV1 showed 98% homology to the ORSV gene isolated from United States (Accession nos. ORU89894), Taiwan (Accession nos. AY571290) and Korea (Accession nos. X82130). All of these genes could be used in developing *Oncidium* orchids resistant to CyMV or ORSV through the transgenic approach.

Introduction

Orchids have more viral disease problems than most other crops (Zettler et al. 1990). The problems are particularly important in the orchid industry to this day because the infection affects flower quality and production. The commercial value of an orchid can be considerably reduced when the quality of the flowers are low.

Much information about viruses of orchid has been recorded since 1947, when the first orchid virus was described (Nobrega 1947). At least 25 viruses of orchids have been listed (Chang 1985;

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Lawson and Brannigan 1986; Zettler et al. 1987). Of these, only cymbidium mosaic virus (CyMV) and odontoglossum ringspot tobamovirus (ORSV) are the most prevalent and economically important viruses infecting orchids worldwide (Zettler et al. 1990). Both have been extensively studied (Francki and Randles 1970).

CyMV, a member of the potexvirus family, and ORSV, a member of tobamovirus, were described over 40 years ago (Lawson and Brannigan 1986). Both CyMV and ORSV apparently infect only cultivated orchids. Surveys have shown that these two viruses are rare or absent in wild orchids (Zettler et al. 1990). Since there are no confirmed reports of natural infection by either CyMV or ORSV in wild orchids, the origins of these viruses remain unknown.

Orchids infected with CyMV and/or ORSV exhibit various symptoms, or can be symptomless (Wong et al. 1989). CyMV induces floral (Yuen et al. 1979) and foliar (Dunn 1980) necrosis, while ORSV causes blossom brown necrotic streak (Thornberry and Phillippe 1964). Tanaka et al. (1997) reported that in Thailand, CyMV was often detected not only from *Oncidium* plants with symptoms but also from healthy-looking plants, whereas, ORSV was detected only from symptomatic plants. Similar results were also seen in Singapore (Wong et al. 1989) and in Indonesia (Inoue 1993). Zettler et al. (1990) reported that symptoms on orchid plants, such as *Oncidium,* become obvious after the double infection by these two viruses. However, it is unknown whether CyMV and ORSV induce these symptoms independently or by a mixed infection of the two viruses.

It is known that CyMV and ORSV are very stable and easily spread among plants by contaminated tools and pots during handling and harvesting. Good cultural practices may help reduce the spread of these viruses. It has been suggested that the viruses can be controlled using virus free orchid materials and good sanitation practices (Wisler 1989). The use of virus resistant varieties may be very effective but these varieties could not be easily developed through conventional breeding. Developing virus resistant orchid plants through the transgenic approach is a promising alternative.

In transgenic approach, virus resistance is conferred by incorporating the virus gene sequence(s) into the genome of the plant. This approach is commonly known as virus mediated resistance and was first shown for tobacco mosaic virus resistance in tobacco (Powell-Abel et al. 1986). Virus gene for coat protein (CP) has been successfully used to achieve resistance (Roger et al. 1990). Several major crop plants have been engineered to resist important viral pathogens. This includes CP mediated resistance against papaya ringspot virus (PRSV) in papaya (Maureen et al. 1992). The production of transgenic orchids with virus mediated resistance in orchids has not been reported yet although there are many reports on cloning of genes from orchid viruses for use in developing transgenic orchid plants resistant or immune to the virus (Gourdel and Leclercq-Le Quillec 2001; Khairun et al. 2001).

This paper describes the CyMV and ORSV partial genes sequences cloned from *Oncidium* orchids. These virus genes were cloned unintentionally while cloning other important genes from orchids. This came about when unknowingly the orchid flowers used in the cloning of targeted genes were infected with the viruses as they were symptomless. Nevertheless, these genes are useful in the future for developing transgenic orchid varieties resistant to the most common virus diseases.

Materials and methods

Plant material

Oncidium (*Oncidium* Goldiana x *Oncidium* Flexuosum) flowers were used in this experiment. Orchid plants were purchased from a local commercial orchid farm and maintained under normal conditions in a glasshouse at MARDI, Serdang, Malaysia.

Fully opened flowers were harvested, cleaned by rinsing in distilled water, frozen in liquid nitrogen and immediately stored at –80 °C until used.

RNA isolation

Isolation of total RNA from harvested *Oncidium* flowers was as described in Matsumura et al. (1999). Briefly, 2 g frozen tissue was homogenized in NTES buffer [0.25 M NaCl, 0.05 M Tris-HCl, 1% (v/v) SDS, 20 mM EDTA] using a mortar and pestle. After centrifugation to remove the insoluble materials, total RNA was precipitated with 6 M LiCl, kept overnight at 4 °C, pelleted, and washed. The final RNA pellet was resuspended in DEPC treated water. Poly (A) RNA was then isolated using the PolyA Tract System (Promega) according to the manufacturer's instructions.

cDNA library construction

The cDNA library was constructed using 5 µg of the isolated mRNA. It was constructed in the λ lUNI-ZAP XR vectors using the ZAP cDNA Synthesis kit according to the manufacturer's instructions (Strategene).

cDNA clone isolation

About 2000 plaque-forming unit (pfu) were plated on a large petri dish (137 mm). Several single isolated plaques were selected and in vivo excised following Strategene's protocol.

Reverse transcriptase-polymerase chain reaction (RT-PCR) cloning

First-strand cDNAs were synthesized using the Reverse Transcription System (Promega). The final concentration in 20 µl of reaction components were 5 mM $MgCl_2$, 1X Reverse Transcription Buffer, 1 mM dNTP mix, 1 U/µl Recombinant RNasin, 15 U/µg AMV Reverse Transcriptase, 0.5 µg $Oligo(dT)_{15}$ Primer and 5 µg of total RNA. The cDNA was then used as a template for PCR using the oligonucleotide, ETR1F: 5' GCAATAATCGCATGCATCGGTG 3' as the forward primer and ETR1R: 5' GATGGGTACTTGTTCAGTTTGGTG 3' as the reverse primer. The primers were originally designed specific for ethylene receptor (ETR1) gene. Another PCR was performed by using oligonucleotide, ORSVF: 5' GTGAAATTAACACCGTACAC 3' as the forward primer and ORSVR: 5' CTCCTGCTTAGCCATCCA 3' as the reverse primer designed specific for the ORSV RNA replicase gene. The PCR conditions were 2 min denaturation cycle at 94 °C, followed by 30 cycles with each cycle consisting of 1 min at 94 °C, 2 min at 57 °C and 1 min at 74 °C. The final extension cycle was 7 min at 74 °C. 10 µl of the PCR products was separated by electrophoresis on a 1% agarose gel. The amplified fragments were then excised and cloned into Topo2.1 vector (Invitrogen) using manufacturer's protocol.

Plasmid isolation and sequencing

Plasmid DNA was isolated using the Miniprep Isolation Kit (QIAGEN) following manufacturer's protocol. Each plasmid was completely sequenced using either M13 forward or M13 reverse as sequencing primers. Sequencing was carried out by the commercial sequencing service at the Laboratory of Cell and Molecular Biology, Institute of Bioscience, Universiti Putra Malaysia. The sequence data was analysed using the computer DNAStar program from the DNAStar Inc. USA. Database sequence homology searches were carried out using the BLAST programs.

Results and discussion

The *Oncidium* flower cDNA library was constructed in order to isolate important genes from orchid flower, especially genes for flower colour. In the initial process of isolating important genes, several recombinant plaques were picked from the library at random and were auto-excised as recombinant Bluescript (SK-) plasmids.

Sequencing in both directions was then performed and the identity of each clone was determined by homology to known sequences from the genebank database. Two clones showed significant homology to sequences from the genome of the cymbidium mosaic virus in the regions of the coat protein (CP), movement proteins (MP) and the RNA replicase gene.

The first clone was named CyMV1 and has an insert size of 1,186 bp *(Figure 1)*. CyMV1 contained within it the entire open reading frame (ORF) for the CP gene situated at the 3' end region of the clone from nucleotide 443 to nucleotide 1114, encoding a polypeptide of 223 amino acids. CyMV1 also contained sequences for MP genes situated just upstream of the CP gene. However, only MP3 and MP2 sequences are found in CyMV1. The MP3 was from nucleotides 165 to 437, encoding a polypeptide of 91 amino acids, whereas the

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CTCCAAGTCACTCTTCGTCCTTGCTATTGGCATAACTGTGGTCTCCGCATTGTTTGTGCTAAAGTCTCACACTTTTCCAATTGCAGTCGA    90
  S  K  S  L  F  V  L  A  I  G  I  T  V  V  S  A  L  F  V  L  K  S  H  T  F  P  I  A  V  D    30

CAATATTCACCGCTTCCCCTCCGGCGGCCAATATAAAGACGGTACTAAGCAGATAAACTACTGTCCACCTACTCATGCTAGGTACCCGAA   180
  N  I  H  R  F  P  S  G  G  Q  Y  K  D  G  T  K  Q  I  N  Y  C  P  P  T  H  A  R  Y  P  K    60
                                                                            M  L  G  T  R      5

ATATCCTGACTACAAGTGGCTTGCCGCTACCGCCGCCATCGTCATCCCTCTCTGCCTATATATTTCCTACCATCCTGGCAATAATATTCG   270
 Y  P  D  Y  K  W  L  A  A  T  A  A  I  V  I  P  L  C  L  Y  I  S  Y  H  P  G  N  N  I  R    90
N  I  L  T  T  S  G  L  P  L  P  P  P  S  S  S  L  S  A  Y  I  F  P  T  I  L  A  I  I  F      35

CCGTATTTGCCCTAGTTGCAATACACATCACCACCCCTGAGCCTTTCTGCACCATACACATAGACGGGGCGTCTATTACTATCACTAACT   360
  R  I  C  P  S  L  N  T  H  H  H  P  *                                                       102
A  V  F  A  L  V  A  I  H  I  T  T  P  E  P  F  C  T  I  H  I  D  G  A  S  I  T  I  T  N      65

GCCCCGATCCTGCAGCTATATTAAATAAAGTAGCCATAGGCCCCTGGCGAGGGTTAAGTTATCAAAATAATTTGAAATAATCATGGGGAG   450
C  P  D  P  A  A  I  L  N  K  V  A  I  G  P  W  R  G  L  S  Y  Q  N  N  L  K  *               91
                                                                                 M  G  R       3

AGGCCACTCCAACTCCAGCTGGCACTTACTCCGCTGCCGAACCCACTTCTGGACCAAGTTGGGCGACCTGGCTGGCATTAAGTACTCACC   540
  G  H  S  N  S  S  W  H  L  L  R  C  R  T  H  F  W  T  K  L  G  D  L  A  G  I  K  Y  S  P    33

TGTCACCTCCTCTATTGCCACCCCCGAAGAAATCAAGGGCATAACCCAATTGTGGGTTAACAACCTTGGCCTCCCCGCTGACACCGTAGG   630
  V  T  S  S  I  A  T  P  E  E  I  K  G  I  T  Q  L  W  V  N  N  L  G  L  P  A  D  T  V  G    63

TACCGCGGCCATTGACTTGGCCCGCGCCTACGCTGACGTCGGGGCGTCCAAGAGTGCTACCCTGCTCGGTTTCTGCCCTACGAAACCTGA   720
  T  A  A  I  D  L  A  R  A  Y  A  D  V  G  A  S  K  S  A  T  L  L  G  F  C  P  T  K  P  D    93

TGTCCGTCGCGCCGCTCTTGCCGCGCAGATCTTCGTGGCCAACGTCACCCCCCGCCAGTTTTGCGCTTACTACGCAAAAGTGGTGTGGAA   810
  V  R  R  A  A  L  A  A  Q  I  F  V  A  N  V  T  P  R  Q  F  C  A  Y  Y  A  K  V  V  W  N   123

TCTGATGCTGGCCACTAACGATCCGCCCGCCAACTGGGCCAAGGCTGGTTTCCAGGAGGATACCCGGTTTGCCGCCTTTGACTTCTTCGA   900
  L  M  L  A  T  N  D  P  P  A  N  W  A  K  A  G  F  Q  E  D  T  R  F  A  A  F  D  F  F  D   153

TGCCGTCGATTCCACTGCTGCGCTGGAGCCTGCTGAATGGCAGCGCCGCCCGACTGACCGTGAACGTGCTGCGCACTCGATCGGGAAGTA   990
  A  V  D  S  T  A  A  L  E  P  A  E  W  Q  R  R  P  T  D  R  E  R  A  A  H  S  I  G  K  Y   183

CGGCGCCCTTGCCCGTCAGCGTATCCAAAACGGCAACCTCATCACCAACATTGCCGAGGTCACCAAGGGCCATCTTGGCTCCACCAACAC  1080
  G  A  L  A  R  Q  R  I  Q  N  G  N  L  I  T  N  I  A  E  V  T  K  G  H  L  G  S  T  N  T   213

TCTCTATGCTCTGCCTGCACCCCCTACTGAATAACGCCAAACTTAATAAGGCGTGTGGTTTTCTAAAGTTTGTTTCCACTACTGGCATAA  1170
  L  Y  A  L  P  A  P  P  T  E  *                                                            223
TACGTTTAGCCAGATA                                                                            1186
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Figure 1. Nucleotide sequence and deduced amino acid sequence of the clone CyMV1. Bold and underline indicate the start codons. Asterisk (*) indicates the stop codons

MP2 gene, from nucleotides 1 to 307 was slightly truncated at the 5' end, missing only 10 amino acids from the start codon *(Figure 1)*. The MP1 sequence, which is normally situated upstream of MP2, was completely absent from CyMV1. It is interesting to note that there were only two nucleotides between the stop codon in the MP3 gene sequence to the start codon of the CP gene sequence. Comparison of the nucleotide sequence revealed that CyMV1 shared 97% homology to the CyMV Taiwanese strain (Accession no. AY571289), 96% to the Korean type 2 CyMV complete genome (Accession no. AF016914) and the Singaporean CyMV complete genome in the CP and MP regions (Accession no. CMU62963) *(Figure 2)*.

The other clone isolated, CyMV2, contained DNA sequence for the extreme 3' region of the RNA polymerase gene. The entire CyMV polymerase gene in the database (Accession no. AF016914) is 4,253 bp encoding a protein (RNA replicase) of 1,417 amino acids. The CyMV2 clone only contained the last 620 bp of the gene from nucleotide 3758 to 4382, encoding the last 189 amino acids of the protein *(Figure 3)*. The percentage of homology of CyMV2 was 95% to both of the Korean type 2 CyMV complete genome (Accession no. AF016914) and the Singaporean CyMV complete genome in the RNA polymerase region (Accession no. CMU62963) *(Figure 4)*.

In another experiment, a partial sequence of ORSV gene from the flowers of the same *Oncidium* hybrids was isolated by RT-PCR technique. The ORSV gene sequence was first cloned unexpectedly when a set of primers (ETR1F and ETR1R) designed to amplify an ethylene receptor gene, ETR1 was used. The wrong gene amplification is quite common during PCR and it is usually caused by several factors such as carry-over contamination, competitive PCR, temperature errors, bad primers and buffer problems. Therefore, in order to confirm the presence of the virus gene in the RNA sample, the experiment was repeated using another set of primers (ORSVF and ORSVR) designed based on the sequence of the ORSV gene obtained. A fragment specific to ORSV gene was also amplified and then subsequently cloned. The clone was named ORSV1 and was 728 bp in size. It encoded a partial fragment of a readthrough product in which helicase and RNA replicase motifs are found *(Figure 5)*. Interesting to note that the clone also contained a stop codon readthrough in the middle of the gene sequence at amino acid 83 *(Figure 5)*. Readthrough is an unusual process in which the stop codon is misread or skipped resulting in the production of a readthrough protein (Sato et al. 2003). Many protein viruses use stop codon readthrough as a strategy to produce extended coat or replicase protein (Brown et al. 1996). This partial gene sequence of ORSV1 showed 98% homology to the ORSV gene isolated from United States (Accession nos. ORU89894), Taiwan (Accession nos. AY571290) and Korea (Accession nos. X82130) *(Figure 6)*.

Conclusion

The authors have isolated a fragment of complete sequences for CP gene, MP3 gene and partial sequence for MP2 gene of CyMV. Another fragment isolated was a partial sequence for replicase gene of CyMV. They have also managed to isolate a partial sequence for replicase gene for ORSV. Their future plan is to construct the CP gene in a suitable vector for use in the transformation of orchid to produce virus resistant plants. As for the MP2 gene, they will try to generate the missing 30 bp by PCR before using it for plant transformation. Due to the large size of the complete RNA polymerase gene (about 4kb) for both viruses, they will try to isolate the missing regions by rescreening the existing cDNA library. The full sequence once obtained will be used to generate constructs for the use in orchid transformation.

The results also indicate that the *Oncidium* hybrid used in the library

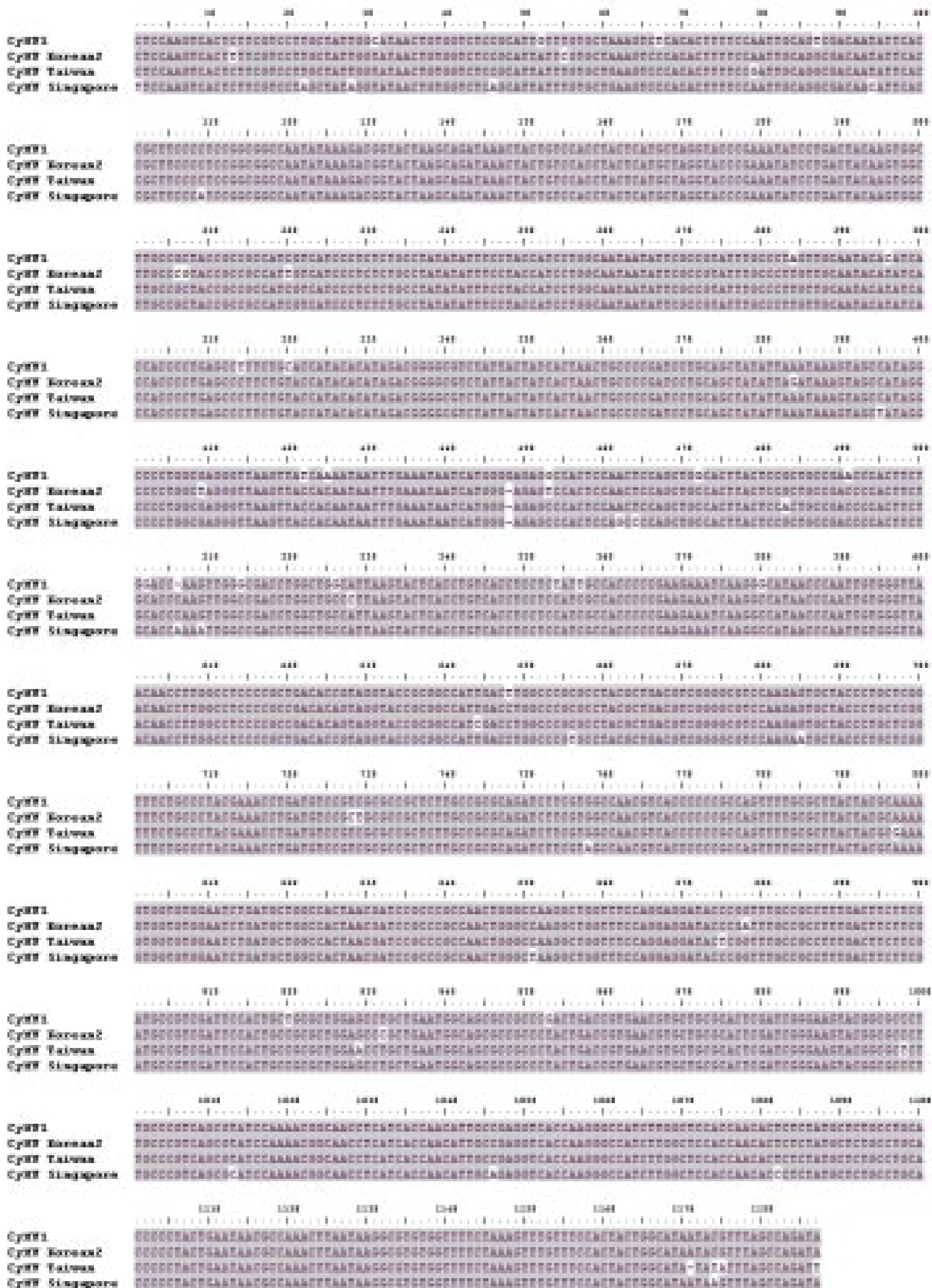

Figure 2. Sequence homology of CyMV1 to the other related Cymbidium Mosaic Virus (Korean type 2 [accession no. AF016914], Taiwan [accession no. AY571289] and Singapore [accession no. CMU62963]) in the GenBank. Highly conserved regions are shaded

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ACCTTCGACGCCAATACTGAATGTTCTATTGCCTACAACGCCACCAGATCCCATATTAATAAAGACGTCACCCAAGTATACGCCGGTGAC   90
 T  F  D  A  N  T  E  C  S  I  A  Y  N  A  T  R  S  H  I  N  K  D  V  T  Q  V  Y  A  G  D   30

GACATGGCTATGGCCCATGTGTGCCCTAAGAAGAAGAGCTTTAAAGCTTTGGAAAAGAAACTGAAACTAACCTCAAACCCTCTCTATCCA  180
 D  M  A  M  A  H  V  C  P  K  K  K  S  F  K  A  L  E  K  K  L  K  L  T  S  N  P  L  Y  P   60

AAGCAAAAACCCGGAGACTGGGCCGATTTCTGCGGCTGGACCATAACGCCTTATGGCATCATCAAGAATCCTAAGAAACTTGATGCATGC  270
 K  Q  K  P  G  D  W  A  D  F  C  G  W  T  I  T  P  Y  G  I  I  K  N  P  K  K  L  D  A  C   90

CTACAATTGCACACCCAACTGGGCGATGCCGATAAAGTCGCTAGATCATACGCACTCGATGCTAAATACGCTTATGATCTGGGCGATCGC  360
 L  Q  L  H  T  Q  L  G  D  A  D  K  V  A  R  S  Y  A  L  D  A  K  Y  A  Y  D  L  G  D  R  120

ATTTATGAGGTTCTGAATGCTGATGAAATGGCCAGCCACTTCAATGTTATAAGACAGTTGCACAAACTGCATCAACAAGATGTGCTGGTC  450
 I  Y  E  V  L  N  A  D  E  M  A  S  H  F  N  V  I  R  Q  L  H  K  L  H  Q  Q  D  V  L  V  150

CCACCCGAGACTACCGTAGCCACAGCGGTAAAGTCTCAACCTGATGTGGAGGATCTGTGGCTCCGTGCGCTTAGCTTCCCTGACTGGACG  540
 P  P  E  T  T  V  A  T  A  V  K  S  Q  P  D  V  E  D  L  W  L  R  A  L  S  F  P  D  W  T  180

GACCGCGCCCAACTTTTAAAACGGGGTTAATTTTTGATGGAGCTAGCGTACTTAGTTAGATTACTAGAGCACAATAAATTC           621
 D  R  A  Q  L  L  K  R  G  *                                                                189
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Figure 3. Nucleotide sequence and deduced amino acid sequence of the clone CyMV2. Asterisk (*) indicates the stop codons

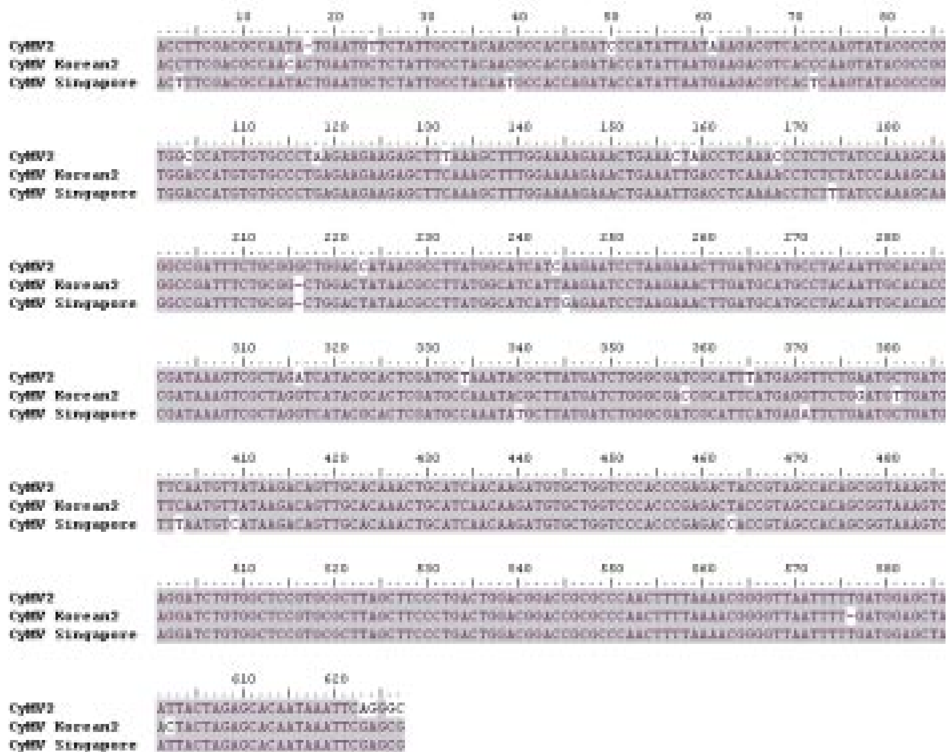

Figure 4. Sequence homology of CyMV2 to the other related Cymbidium Mosaic Virus (Korean type 2 [accession no. AF016914] and Singapore [accession no. CMU62963]) in the GenBank. Highly conserved regions are shaded

GTGAAATTAACACCGTACACGAAATCCAAGGTGAGACCTTTGAAGACGTATCGTTGTTCGTTGACACCAACTCCATTGGAGCTAATATCA 90
V K L T P Y T K S K V R P L K T Y R C S L T P T P L E L I S 30

AAGAGCTCACCGCACGTTCTTGTTGCATTGACAAGACATACTAAAAGCTTCAAATATTACTCTGTGGTTCTCGATCCTCTCGTCAAAGTT 180
K S S P H V L V A L T R H T K S F K Y Y S V V L D P L V K V 60

TGCTCTGATTTAAGCAAGGTATCAGACTTTATACTGAATATGTATAAGGTCGATGCCGGGATCTTA***TAG***CAATTACAGTTAGGGAGTATC 270
C S D L S K V S D F I L N M Y K V D A G I L ***X*** Q L Q L G S I 90

TTCAAGGGCGAAAATCTATTCGTACCATGTCCTAAATCTGGTTATATTTCTGATATGCAATTTTATTATGACACTTTGTTACCTGGAAAC 360
F K G E N L F V P C P K S G Y I S D M Q F Y Y D T L L P G N 120

AGCACTATTTTAAACGAGTATGATGCAGTGACTATGAATCTTCGTGAAAATAATCTTAATGTCAAGGATTGCACGATAGATTTTTCTAAA 450
S T I L N E Y D A V T M N L R E N N L N V K D C T I D F S K 150

TCTGTTAGTGTCCCGAGACAACAACAAGAGTTTTTCACACCAGTTATTCGAACTGCTGCTGAACGTCCACGTAGTGCTGGTCTCCTTGAA 540
S V S V P R Q Q Q E F F T P V I R T A A E R P R S A G L L E 180

AACCTTGTGGCAATGATAAAAAGAAATTCTAACTCTCCAGATTTAACGGGTATATTAGATATTGAAGATACTGCCGAACTTGTAGTTAAT 630
N L V A M I K R N S N S P D L T G I L D I E D T A E L V V N 210

AAGTTTTGGGATGCTTACATTATTGACGAACTTTCTGGTGGAAATGTTACACCGATGACTTCAGATGCCTTTCACAGGTGGATGGCTAAG 720
K F W D A Y I I D E L S G G N V T P M T S D A F H R W M A K 240
CAGGAGAA 728
K Q E 243

Figure 5. Nucleotide sequence of ORSV1 obtained from RT-PCR technique. The deduced amino acid sequence (single-letter code) is shown below the corresponding nucleotide triplets. Bold and italic indicates the stop codons readthrough

construction and RT-PCR technique was severely infected with CyMV and ORSV although symptoms of viral infection were not obvious on the plants and flowers used. This shows that the local orchid hybrids available for commercial productions are commonly infected with these viruses. Hence, there is an urgent need to develop new orchid hybrid that is resistant to viruses as to improve the quality of local orchid.

Acknowledgement

The authors would like to thank Ms Norizwati Amdan at the Biotechnology Research Centre, MARDI for her excellent technical assistance throughout this work, and Dr Habibuddin Hashim of Biotechnology Research Centre, MARDI for valuable comments and suggestion on the manuscript. This project was funded by MOSTI through the National Biotechnology Directorate (BIOTEK), (Research Grant No.01-03-03-007/BTK/ER/014).

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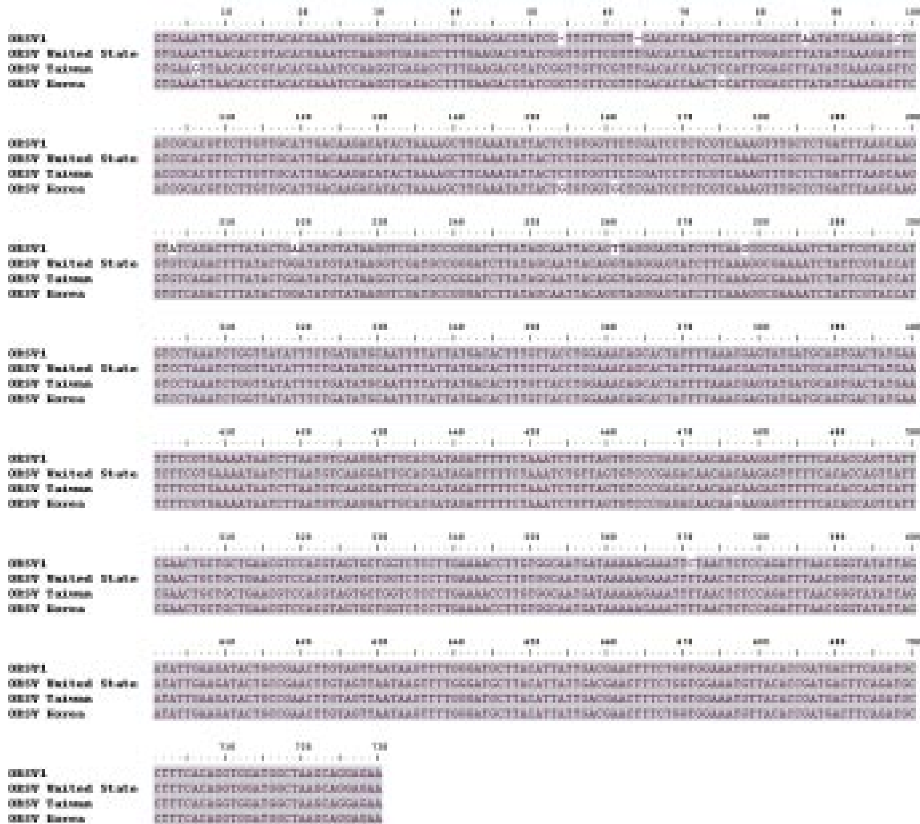

Figure 6. Sequence homology of ORSV1 to the other related Odontoglossum *Ringspot Virus (isolated from United States [accession no. ORU89894], Taiwan [accession no. AY571290] and Korea [accession no. X82130]) in the GenBank. Highly conserved regions are shaded*

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Abstrak

Beberapa faj rekombinan dipilih secara rawak daripada perpustakaan cDNA *Oncidium* (*Oncidium* Goldiana x *Oncidium* Flexuosum). Ia kemudian ditukar ke dalam bentuk plasmid secara pemotongan in vivo dan dijujukkan. Dua klon, iaitu CyMV1 dan CyMV2, menunjukkan kadar persamaan yang tinggi terhadap jujukan DNA dan protein cymbidium mosaic virus (CyMV) dalam pangkalan data genebank. CyMV1, bersaiz 1,186 bp mengandungi jujukan gen protein sarung (CP), protein pergerakan (MP)3 dan hampir keseluruhan jujukan MP2. CyMV2, bersaiz 626 bp hanya mengandungi sebahagian hujung 3’ jujukan gen RNA polimerase. Peratus persamaan gen CyMV1 adalah sebanyak 97% kepada strain Taiwan (AY571289), 96% kepada genom CyMV Korean jenis 2 (AF016914) dan juga kepada genom CyMV Singaporean (CMU62963) pada bahagian protein sarung dan protein pergerakan. CyMV2 pula menunjukkan 95% persamaan kepada genom CyMV Korean jenis 2 (AF016914) dan juga kepada genom CyMV Singaporean (CMU62963) tetapi pada bahagian RNA polimerase. Suatu klon lain bernama ORSV1, bersaiz 728 bp, hasil kaedah RT-PCR adalah sebahagian daripada gen RNA replikase daripada odontoglossum ringspot virus (ORSV). Cebisan gen ORSV ini menunjukkan 98% persamaan kepada gen ORSV dari Amerika Syarikat (Accession nos. ORU89894), Taiwan (Accession nos. AY571290) dan Korea (Accession nos. X82130). Kesemua gen ini boleh digunakan untuk menghasilkan pokok orkid *Oncidium* yang rintang terhadap CyMV atau ORSV melalui pendekatan transgenik.

Accepted for publication on 7 June 2005