



Original Article

A direct gene transferring system for *Oncidium* orchids, a difficult crop for genetic transformationKrittiya Niyomtham,^a Kisana Bhinija,^b Pattana S. Huehne^{b, c, *}^a Genetic Engineering and Bioinformatics, Interdisciplinary Graduate Programs, The Graduate School, Kasetsart University, Bangkok 10900, Thailand^b Laboratory of Biotechnology, Chulabhorn Research Institute, Bangkok 10210, Thailand^c Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

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ABSTRACT

Oncidium orchids are susceptible to damage through viral infection caused by the Cymbidium mosaic virus (CymMV). Generating a virus-resistant plant is a major challenge. Unfortunately, gene transformation in *Oncidium* orchids is not easy. This study developed selection steps for a direct gene transferring system through particle bombardment. Optimized hygromycin screening was reported in the transformed protoplast-like bodies (PLBs) of two commercial *Oncidium* hybrids (*Onc. Gower Ramsey* and *Onc. Sweet Sugar*) with the RNAi construction of the coat protein gene of CymMV. The transgenic *Onc. Sweet Sugar* PLBs were regenerated on a modified Murashige and Skoog medium containing hygromycin. The effective selection system in exterminating non-transformed PLBs and the functional transgene in the transgenic lines was analyzed using reverse transcription polymerase chain reaction and quantitative polymerase chain reaction. The application of this study may help the gene transformation system of other orchid plant species.

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Introduction

Oncidium, a sympodial epiphyte orchid, is commonly known as the dancing lady orchid because of its fascinating array of flowers, and it is predominance in the tropical regions of East Asia (Hieber et al., 2006). The *Oncidium* orchid was initially imported to Thailand in 1976 and then popularized as a cut flower (Chang et al., 2011). In recent decades, new floral character traits have been produced by hybridizing with intergeneric and relative varieties, such as *Onc. Gower Ramsey* (Goldiana × Guinea Gold), which has become one of the most sought-after varieties in the Asian flower industry (Hieber et al., 2006). *Onc. Sweet Sugar*, an almost identical hybrid to *Onc. Gower Ramsey*, is the cross-pollination hybrid between *Onc. Aloha Iwanaga* and *Onc. Gomesa Varicose* (White, 2009).

Unfortunately, orchid plants are particularly vulnerable to virus infections. Thus far, two viruses, namely the Odontoglossum ring-spot virus and the Cymbidium mosaic virus (CymMV), have been known to infect orchids (Jensen and Gold, 1951). These two viruses are a worldwide issue of a co-infectious disease in orchids (Tanaka

et al., 1997). Some virus-infected orchids appear with either asymptomatic lesions or severe disease symptoms. However, their synergy greatly harms flower production quality and quantity, particularly the breakdown of coloring through chlorosis and necrosis (Jensen and Gold, 1951). The inability to prevent CymMV infections has caused significant financial losses to the orchid industry because of the costly extermination of infected plants before the infection spreads to neighboring plants (Hu et al., 1994). Incidentally, the rapid development of biotechnology for orchids has produced genetically modified traits to create robust transgenic orchids. Two decades ago, the RNA-mediated gene silencing of the post-transcriptional gene known as RNA interference (RNAi) was developed by inserting an RNAi construct of the target gene into the host chromosome for sequence-specific RNA degradation (Baulcombe, 2004). The challenges of the RNAi technology were successfully overcome to degrade the RNA transcripts of the CymMV coat protein gene, which reduced the virus infection in *Dendrobium* (Liao et al., 2004; Chang et al., 2005; Petchthai et al., 2015) and *Phalaenopsis* orchids (Chan and Chan, 2005).

In the last 15 years, both agrobacterium transformation system and direct gene transformation systems through particle bombardment have been established for genetically modified *Oncidium* orchids (Liau et al., 2003b; Li et al., 2005; You et al., 2003).

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For example, the following have been described in *Oncidium* orchid biotechnology: transforming *Oncidium* with the sweet pepper ferredoxin-like protein gene, which confers resistance against soft rot disease, through the agrobacterium system (Liau et al., 2003b; You et al., 2003), suppressing the flower color gene by particle bombardment (Yee et al., 2008), and transforming the ethylene receptor gene through the agrobacterium system (Raffeiner et al., 2009). More challenging still, the *Oncidium* transformation was developed by using the phosphomannose isomerase gene as a selectable marker for the agrobacterium transformation system (Thiruvengadam et al., 2011). However, previous reports on the development of the genetic transformation in the *Oncidium* species are limited because of their sensitivity to hygromycin antibiotics and long-time regeneration. The present study used the direct gene transformation system to mediate two transgenic *Oncidium* orchids by introducing green fluorescent protein (*gfp*), hygromycin phosphotransferase (*hptII*) and *CymMV-CP* genes into the protocorm-like bodies (PLBs) of *Onc. Gower Ramsey* and *Onc. Sweet Sugar*. Several transgenic *Oncidium* orchids were investigated by molecular analysis to confirm the heritability of the transgenes.

Materials and methods

Plant materials

The young flower stalks of two commercial *Oncidium* hybrid orchids, *Onc. Gower Ramsey* and *Onc. Sweet Sugar* (Fig. 1), were used as explants for tissue culture. The *Oncidium* tissues were maintained by sub-culturing them as PLBs in a mixture of an equal volume of two media, namely, half-strength MS medium (Murashige and Skoog, 1962) and half-strength VW medium (Vacin and Went, 1949), supplemented with 10 g/L sucrose and 75 ml/L coconut water. The pH of the mixture media was adjusted to 5.6 with 1 N KOH or HCl prior to autoclaving for 15 min at 121 °C. The *Oncidium* tissue cultures were shaken at 90 revolutions per minute, incubated under a 16 h:8 h daylight:dark photoperiod of 2000 lx at 25 ± 2 °C and sub-cultured every 3 wk.

Plasmid DNA vectors

The recombinant vector called pCB199 contained two copies of the *CymMV-CP* gene of the RNAi structure (Srifah et al., 1996), which was ligated into the pCB2 plasmid. The cassette of the RNAi structure was driven by the 35S promoter and terminated by the nopaline synthase (NOS) terminator (35S pro:*CymMV-CP*:*CymMV-CP*:nos ter) (Fig. 2A) (Petchthai et al., 2015). The second vector used for co-transformation was the pMNK1005 plasmid (Upadhyaya et al., 1998), which contained the selectable marker of the hygromycin phosphotransferase (*hptII*) gene. The reporter gene of

the modified green fluorescent protein (*S65Tgfp*) gene was driven by the Ubi-1 promoter and terminated by the NOS terminator (Ubi pro:*hptII*::*S65Tgfp*: nos ter) (Fig. 2B). These marker genes were used to evaluate the genetic transformation in *Oncidium* orchids.

Gene transformation

The new actively produced *Oncidium* PLBs were pre-cultured overnight on a solid MS medium containing 8 g/L mannitol. A mixture of pCB199 and pMNK1005 at a ratio of 1:1 was coated onto 1.0 µm gold particles (Bio-Rad; Hercules, CA, USA) as described by Christou (1997). Then, the gold-coated particles were bombarded into the mannitol pre-cultured *Oncidium* PLBs using the PDS-1000/He system (Bio-Rad; Hercules, CA, USA) according to Suwanaketchanati et al. (2007). After bombardment, the transformed PLBs were maintained in darkness for a few days before transferring them to be maintained under a 16 h:8 h daylight:dark photoperiod (2000 lx) at 25 ± 2 °C. They were sub-cultured on a half-strength MS medium containing 0.5 mg/L benzylaminopurine (BA), 0.25 mg/L α-naphthalene acetic acid (NAA), and hygromycin at concentrations of 5, 10, 15, and 5 mg/L for 1 mth, 2 mth, 3 mth and 4 mth, respectively. Afterward, the hygromycin-resistant PLBs were transferred to a half-strength MS medium containing 2.5 mg/L hygromycin for growth and regeneration to plantlets.

Polymerase chain reaction

The transgenes located in the DNA vectors were amplified to determine the transgenic lines from the genomic DNA (gDNA). The gDNAs were extracted from the leaves of the putative transgenic plantlets using a modified CTAB method (Porebski et al., 1997). The target genes were confirmed by using the *hptII* primers, 5'-GCGAAGAATCTCGTCTTTC-3' and 5'-TCGCTGAATCCCCAATGTC-3', and the *gfp* primers 5'-GACGTAACGGCCACAAGT-3' and 5'-GACGTAAACGGCCACAAGT-3', with the expected size of the amplified DNA fragments of 163 bp and 188 bp, respectively. Then, the *CymMV-CP* transgene was investigated using a pair of primers 5'-GCTACCCTGCTCGGTTTCTG-3' and 5'-GGCATCGAAGAAGTCAAAGG-3' with a 219 bp expected fragment size. The 10 µL of polymerase chain reaction (PCR) reaction consisted of 0.1 µg DNA, 5 µL DreamTaq PCR Master Mix (2X) (ThermoFisher Scientific; Waltham, MA, USA), and 3 µM of each primer. The PCR amplifications used the following conditions: 95 °C for 3 min (1 cycle), 95 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min (40 cycles) and a final extension at 72 °C for 10 min in a Thermocycler (Mastercycler® Pro; Eppendorf; Wesseling-Berzdorf, Germany).

Reverse transcription polymerase chain reaction and quantitative polymerase chain reaction

The total RNAs from the leaves of transgenic lines for reverse transcription polymerase chain reaction (RT-PCR) analysis and the leaves of *CymMV*-inoculated transgenic lines *CymMV* for quantitative polymerase chain reaction (qPCR) analysis were immediately extracted using Trizol (TRIzol®; Invitrogen Corp.; Carlsbad, CA, USA) and then treated with 1 unit of RNase-free DNaseI at 37 °C for 30 min to remove the contaminated DNA. The first-stranded cDNA synthesis was transcribed using a reverse transcriptase enzyme (RevertAid First Strand cDNA Synthesis Kit; ThermoFisher Scientific, Waltham, MA, USA) and the cDNA product was used as a template for the RT-PCR analysis with specific primers. Four pairs of specific primers previously mentioned in genomic PCR, a pair of internal control genes of constitutively ribosomal RNA, 5.8S rRNA primers (5'-ATGACTCTCGACAATGGATTT-3' and 5'-GCTTGAAGCCCAGGCAGACG-3'), and a pair of *CymMV-RdRp* primers (5'-CATCAACAGGCCAAGGAT



Fig. 1. Orchid flowers of: (A) *Oncidium Gower Ramsey*; (B) *Onc. Sweet Sugar*.

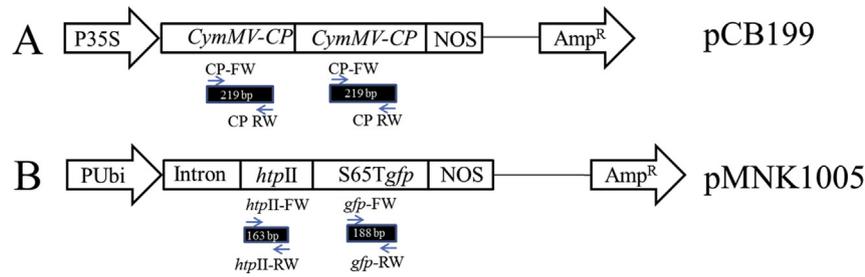


Fig. 2. Schematic representation of: (A) pCB199; (B) pMNK1005, where *CymMV-CP* = gene encoding *CymMV* coat protein; *htpII* = gene conferring resistance to hygromycin phosphotransferase; *gfp* = gene encoding green fluorescent protein; PUbi = maize ubiquitin 1 promoter; P35S = cauliflower mosaic virus 35S promoter; NOS = nopaline synthase terminator sequencer; Amp^R = gene conferring resistance to ampicillin. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

GAAA-3' and 5'-AGCATGGCCGCATCTGTGACTGG-3') (Liao et al., 2004) were used to amplify the expected fragment sizes of 161 bp and 584 bp, respectively. RT-PCR and qPCR reactions were performed in accordance with the instructions of the KAPA SYBR FAST qPCR kit (Kapa Biosystems; Wilmington, MA, USA) on an ABI 7500 Fast Real-Time PCR system (Applied Biosystems; Foster City, CA, USA). The amplification protocol was: 95 °C for 3 min, 40 cycles at 95 °C for 30 s, 58 °C for 30 s and a final extension at 72 °C for 5 min. All reactions were repeated three times.

Results and discussion

Transgenic selection and regeneration

The pCB199 (35S pro:*CymMV-CP*::*CymMV-CP*: nos ter) plasmid was co-transformed with pMNK1005 (Ubi pro:*htpII*::*S65Tgfp*: nos ter) into PLBs of the *Onc. Gower Ramsey* and *Onc. Sweet Sugar* orchids using particle bombardment. To select the putative transformants, the hygromycin selection system was used to discard the non-transformed *Oncidium* PLBs. It was assumed that the putative transgenic *Oncidium* orchids resistant to hygromycin should carry pMNK1005 alone or together with the pCB199 vector. Thus, the surviving and regenerating *Oncidium* plantlets were used for further study. After conducting selection for 1 mth, 5 mg/L of hygromycin in the selective medium completely inhibited cell growth and the development of the non-transformed PLBs of *Onc. Gower Ramsey* and *Onc. Sweet Sugar* orchids (control), which entirely turned brown or had white necrotic PLBs after 1 mth of culturing on a half-strength MS medium containing 0.5 mg/L BA, 0.25 mg/L NAA, and 5 mg/L of hygromycin (Fig. 3), particularly *Onc. Gower Ramsey*. Hygromycin is a common effective selective agent used in most reports on *Oncidium* orchid transformation (Liao et al., 2003a, b; Yee et al., 2008). The use of the hygromycin selection system produced more *Oncidium* transformants and was much less concentrated than using the kanamycin selection system (Thiruvengadam et al., 2011; Raffener et al., 2009).

The minimal lethal concentration of the hygromycin selective agent was 4 mg/L based on evaluation of the survival of non-transformed *Oncidium* Sharry Baby PLBs in various concentrations of hygromycin (Yee et al., 2008). To avoid the evasion of potential non-transformed *Oncidium* cells in the selection process, a concentration of 5 mg/L of hygromycin was commonly used for the lethal dosage in the first step of the hygromycin selection system in most reports (Li et al., 2005; Liau et al., 2003a, b; Yee et al., 2008) and in the current study. Other research groups that analyzed the transformation of *Oncidium* used hygromycin, not kanamycin, for selection (Li et al., 2005; Liau et al., 2003a, b; You et al., 2003).

To establish the stable transformation of *Oncidium* orchids, several steps of the hygromycin selection were applied to discard

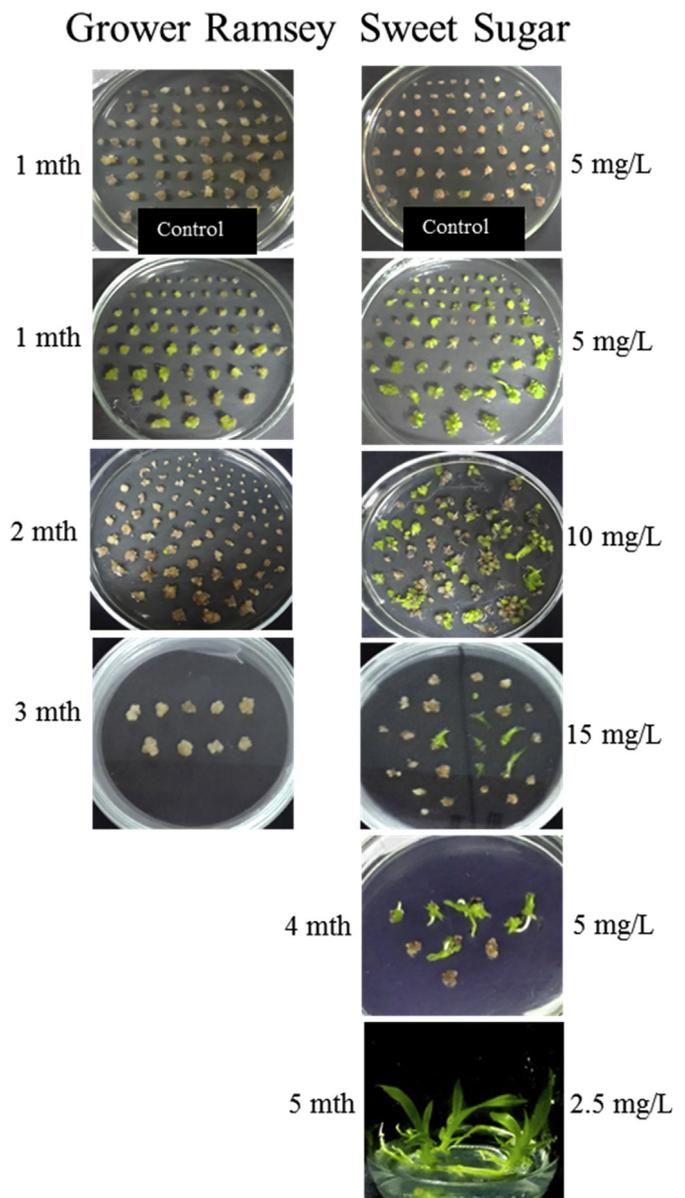


Fig. 3. Generation of putative transgenic *Onc. Gower Ramsey* and *Onc. Sweet Sugar* in a half-strength Murashige and Skoog medium containing 0.5 mg/L benzylaminopurine, 0.25 mg/L α -naphthalene acetic acid and hygromycin at various concentrations during 1–5 mth of the selection steps.

the non-transformed PLBs (*Onc. Sweet Sugar* orchid in particular). After monitoring for 2 mth, most of the transformed *Onc. Gower Ramsey* PLBs stopped growing and turned brown within 1.5 mth, finally dying in the second month of the hygromycin treatment. By contrast, *Onc. Sweet Sugar* PLBs remained green and fast growing (Fig. 3 and Table 1). These results indicated that *Onc. Gower Ramsey* PLBs were more sensitive to hygromycin antibiotics than the *Onc. Sweet Sugar* PLBs used in this study. Thus, hygromycin concentration was increased to treat *Onc. Sweet Sugar* PLBs at concentrations of 10 mg/L, 15 mg/L or 5 mg/L in the second, third and fourth months, respectively, of hygromycin treatment in several selection steps, and the surviving transformed *Onc. Sweet Sugar* PLBs showed normal growth at 11.9%, 2.4% and 2.5%, respectively, for the surviving PLBs of the total transformed PLBs (Table 1). In this study, gene transformation was not easy to achieve in *Onc. Gower Ramsey*, and thus only 2.5% of the surviving PLBs of *Onc. Sweet Sugar* regenerated and developed into plantlets. These results suggested that 5 mg/L hygromycin would be an effective concentration to use for selecting *Onc. Sweet Sugar* PLB orchids at the beginning of the selection step. Fast-growing transformed *Onc. Sweet Sugar* PLBs needed to be selected under the stringency of a higher concentration of 10–15 mg/L hygromycin in the selection process at the second and third steps of hygromycin selection system, unlike the transformation in the hygromycin-sensitive young *Onc. Gower Ramsey* PLBs and other *Oncidium* cultivars, which commonly use hygromycin at one concentration of 5 mg/L for the lethal selection system in most reports (Li et al., 2005; Liao et al., 2003a, b; Yee et al., 2008). However, the efficiency of genetic transformations, which depends on the genetic background of each plant species (Jia et al., 2015) and the choice of newly divided cells (Liao et al., 2003b), plays a major role in the plant transformation system.

Confirmation of gene transformation

The preliminary screening of the putative transgenic *Oncidium* orchids was confirmed using PCR amplification to detect the presence of the *gfp* and *hptII* transgenes in the transgenic lines. The surviving 11 individual hygromycin-resistant transgenic lines were observed for stable transgene integration in the orchid genomes. Genomic DNA extracted from the leaves of the putative transgenic lines was used to perform PCR analysis. The PCR products of *gfp* and *hptII* were amplified from the pMNK1005 vector, and *CymMV-CP* was amplified from the pCB199 vector. Both vectors were used as a positive control (P). An amplified DNA fragment of the integrated *gfp* and *hptII* genes was observed in almost all transgenic lines at 188 bp and 163 bp, respectively (Fig. 4). However, the detection of all *gfp*, *hptII*, and *CymMV-CP* transgenes in the healthy *Oncidium* orchid (negative control, N) presented no amplified DNA fragment. Four transgenic lines (O2, O5, O8 and O9) produced only one intensive band of amplified PCR products (either *gfp* or *hptII*) on the gel

Table 1

Selection of putative transgenic *Oncidium* protocorm-like bodies (PLBs) on half-strength Murashige and Skoog medium containing hygromycin 5 mg/L, 10 mg/L, 15 mg/L and 5 mg/L in four successive months of selection steps.

Experiment	Total number of examined PLBs per % surviving PLBs				
	0 mth	1 mth	2 mth	3 mth	4 mth
<i>Onc. Gower Ramsey</i>					
Non-transformed (Control)	106/100	0/0	0/0	0/0	0/0
pCB199 + pMNK1005	424/100	105/24.7	0/0	0/0	0/0
<i>Onc. Sweet Sugar</i>					
Non-transformed (Control)	115/100	3/2.6	0/0	0/0	0/0
pCB199 + pMNK1005	502/100	80/15.9	60/11.9	12/2.4	13/2.5

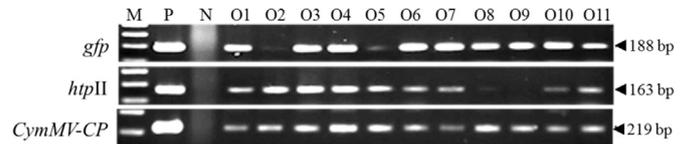


Fig. 4. Amplification of DNA fragments from the polymerase chain reaction (PCR) analysis of the *hptII*, *gfp*, and *CymMV-CP* genes in the genome of putatively transformed *Onc. Sweet Sugar*, where lane M = DNA 100 bp marker; lane P = pMNK1005 and pCB199 plasmid (positive control); N = non-transformed healthy orchid (negative control); and arrows represent the expected sizes of the PCR products.

electrophoresis. These results implied that the integration of these two foreign genes in the transgenic lines was at an inappropriate position because they were placed next to each other in the same pMNK1005 vector as shown in Fig. 2B. Nevertheless, the presence of *CymMV-CP* DNA in the 11 transgenic lines was clearly detected by the 219 bp amplified fragments. These results revealed that the multiple steps of hygromycin selection of *Onc. Sweet Sugar* orchids at a high concentration of 10–15 mg/L hygromycin could eliminate the cells with the non-insertion of the *hptII* transgene. This suggested that both vectors were co-transformed and integrated into the genome of the 11 transgenic lines at random positions. In the current study, the use of a high concentration of hygromycin selection was not consistent with the concentration used for *Oncidium* selection in previous reports. The most suitable concentration of hygromycin in the selection step of *Oncidium* transformation was 5 mg/L hygromycin (Li et al., 2005; Liao et al., 2003a, b; Yee et al., 2008), although, the minimal lethal dose for *Oncidium* was determined as 4 mg/L hygromycin only (Yee et al., 2008). Similar to the *hptII* transgene result, the *gfp* transgene was detected in most *Oncidium* transgenic lines, as both genes were constructed adjacent to each other. In addition, the *gfp* gene is a suitable reporter gene for easily detecting and transferring to new actively dividing PLBs, consisting of high-density young tissues, as previously described in tobacco transformation by Moliner et al. (2000).

Validation of the transgene expression

To confirm the transgene function at the transcriptional level, three transgenic lines (O1, O3, and O11) were selected featuring the presence of the insertion of the *gfp*, *hptII*, and *CymMV-CP* transgenes into the genome of *Oncidium* orchids. Total RNA samples were isolated from the O1, O3 and O11 transgenic lines, healthy *Oncidium* orchid (negative control, N), and long-term *CymMV* naturally infected *Oncidium* orchids (positive control, P). When RT-PCR was performed with the total RNAs of these samples, the result showed that *CymMV-CP* transcripts accumulated in the transgenic lines (O1, O3, and O11) and the *CymMV* naturally infected *Oncidium*

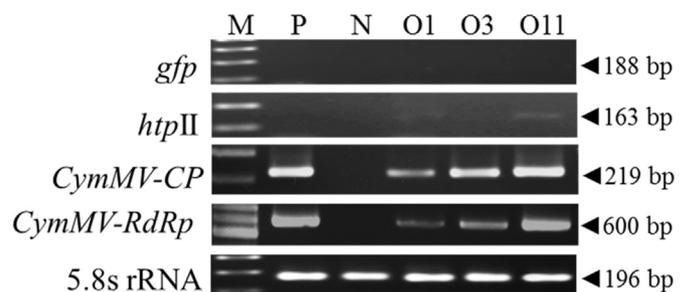


Fig. 5. Reverse transcription polymerase chain reaction analysis of the functional *hptII*, *gfp*, *CymMV-CP*, and *CymMV-RdRp* transgenes in putative transformed *Oncidium* orchid O1, O3 and O11, where lane M = DNA 100 bp marker; lane P = long-term naturally infected orchid (positive control); N = non-transgenic healthy orchid (negative control); and arrows present the polymerase chain reaction expected sizes.

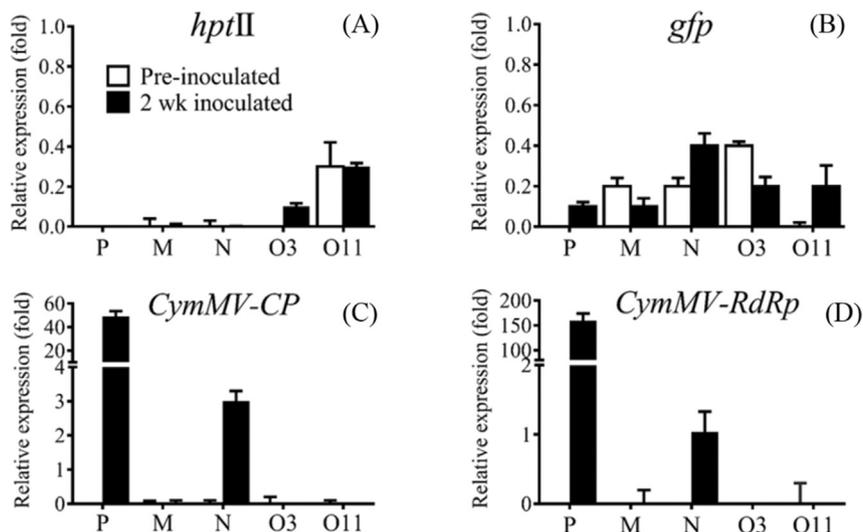


Fig. 6. Quantitative polymerase chain reaction analyses of the functional *hptII* (A), *gfp* (B), *CymMV-CP* (C) and *CymMV-RdRp* (D) transgenes in putative transformed *Oncidium* orchid O3 and O11 inoculated with CymMV, where P = long-term naturally infected orchid (positive control); M = healthy orchid injected with a buffer (mock); N = non-transgenic healthy orchid (negative control) inoculated with CymMV; and each value represents the mean of three replicates, with error bars indicating SD of the independent biological replicates; significant ($p < 0.05$) differences were observed in all mean expression levels among the times in each tissue using one-way analysis of variance.

(P), but they were not detected in the healthy *Oncidium* (N) (Fig. 5). The gene expression of the *hptII* transgene was low in all the transgenic lines, and almost no detectable *gfp* transcripts were found.

To further evaluate the reliability of the *CymMV-CP* transgene to CymMV infection, two transgenic lines (O3 and O11) and a healthy *Oncidium* orchid used in the RT-PCR analysis were inoculated with the purified CymMV and then extracted for total RNAs at 2 wk post-inoculation. A healthy *Oncidium* orchid inoculated with a buffer was used as the mock infection control (M). The transcript levels of two viral genes (*CymMV-CP* and *CymMV-RdRp*) and two marker transgenes (*hptII* and *gfp*) were evaluated using qPCR analysis. As shown in Fig. 6, the qPCR analysis results revealed that the viral *CymMV-CP* and *CymMV-RdRp* transcripts highly accumulated in the long-term naturally infected *Oncidium* (P), which contained the highest level (159-fold) for the *CymMV-RdRp* transcripts, followed by 49-fold for the *CymMV-CP* relative to the gene expression in a healthy *Oncidium* orchid. After 2 wk post-inoculation, the transcripts of *CymMV-CP* and *CymMV-RdRp* in the CymMV-infected healthy *Oncidium* (N) were up-regulated to 3.0- and 1.0-fold, respectively, relative to the gene expression in the healthy *Oncidium* orchid. However, both viral genes expressions in the O3 and O11 transgenic lines were undetectable and very low levels (0.0–0.4-fold) of the *hptII* and *gfp* transcripts were observed. As expected, the overall results of the qPCR analysis were very consistent with those of the RT-PCR analysis.

Although the surviving percentage of transgenic *Onc. Sweet Sugar* was quite low (2.5%), the functional *CymMV-CP* transgene was detected in the selected transgenic lines. At least two transgenic *Oncidium* (O3 and O11) were obtained carrying pCB199 (35S pro:*CymMV-CP*::*CymMV-CP*:nos ter) and pMNK1005 (Ubi pro:*hptII*::*S65Tgfp*: nos ter) plasmids. The *CymMV-CP* transcripts were generated from two copies of *CymMV-CP* transgenes in pCB199 presenting in two transgenic *Oncidium* (O3 and O11). Although this study did not observe and identify the interaction between the position and the number of copies of insertions for the variation in the post-transcriptional gene silencing, the relative quantification analysis showed a significant down-regulation of *CymMV-CP* expression in the transgenic *Oncidium* (O3 and O11) at 2 wk post-inoculation (Fig. 6). The current study was consistent with a report on the effect of *CymMV-CP* construction in pCB199 on

CymMV-CP degradation in a *Dendrobium* orchid infected with CymMV (Petchthai et al., 2015).

The current study applied several critical parameters proposed by Yee et al. (2008) that are capable of increasing stable transformation efficiency. A target PLB size was used of 3–5 mm for *Onc. Gower Ramsey* and *Onc. Sweet Sugar* transformations, and the actively dividing *Oncidium* PLBs of both orchid cultivars were routinely produced by sub-culturing every 3 wk in half-MS-modified medium before subjecting them to bombardment. These parameters were expected to enhance the percentage of survival and the proliferation of transgenic *Oncidium* PLBs. However, the transgenic *Onc. Gower Ramsey* turned brown in the first month of selection on 5 $\mu\text{g}/\text{mL}$ hygromycin. In general, the transgenes mostly integrate into the host chromosome in the early stages of cell division (Yu et al., 1999). Therefore, selection with 5 $\mu\text{g}/\text{mL}$ hygromycin in the first month may not be suitable for the *Onc. Gower Ramsey* transformation system.

Regarding the difficulty of gene transfer in the *Oncidium* orchid species, this study established a selection system for the gene transformation of *Onc. Sweet Sugar* in comparison with the genetic transformation of *Onc. Gower Ramsey* through particle bombardment. Future study should determine the integration position and effect of transgenes on CymMV infection after the propagation and growth of the transgenic plantlets.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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