以多重反轉錄聚合酶連鎖反應同時檢測三種蘭花病毒

Simultaneous detection of three orchid viruses by multiplex reverse transcription-polymerase chain reaction

吳尚均[1]、陳福旗[2]、江主惠[1]*

Hu, Shang-Jun[1], Chen, Fure-Chyi[2], and Chiang, Chu-Hui[1]*

[1] 國立屏東科技大學植物醫學系及 [2]農園生產系

[1] Department of Plant Medicine and [2] Department of Plant Industry, National Pingtung University of Science and Technology, Pingtung

蘭花植物受到許多病毒感染，嚴重影響花卉品質，台灣的蝴蝶蘭目前已發現六種病毒的感染，包括齒舌蘭輪斑病毒(*Odontoglossum ringspot virus*, ORSV)、東亞蘭嵌紋病毒(*Cymbidium mosaic virus*, CymMV)、番椒黃化病毒(*Capsicum chlorosis virus*, CaCV)、胡瓜嵌紋病毒(*Cucumber mosaic virus* , CMV) 、康乃馨斑駁病毒(*Carnation mottle virus*, CarMV)以及落葵皺葉嵌紋病毒(*Basella rugose mosaic virus*, BaRMV，同義於蝴蝶蘭黃化斑點病毒 *Phalaenopsis chlorotic spot virus*, PhCSV)。由於國際貿易之蘭花種苗生產需經過病毒檢測，因此建立可靠又有效的病毒檢測技術成為重要課題。本研究利用RT-PCR方式，分別以對應六種蘭花病毒之專一引子對檢測30個疑似染病之蘭花樣本，發現以ORSV感染比例最高，其次為CaCV之感染。本研究進一步建立多重反轉錄聚合酶連鎖反應(Multiplex RT-PCR) 條件，以同時檢測ORSV、CaCV及CymMV三種病毒，結果發現引子總濃度顯著影響檢測靈敏度，本方法之建立將使蘭花病毒病害檢測更有效率。

Orchid plants are infected by many viruses, which seriously affect the quality of the flowers. Six viruses have been found to infect *Phalaenopsis* orchids in Taiwan, including *Odontoglossum ringspot virus* (ORSV), *Cymbidium mosaic virus* (CymMV), *Capsicum chlorosis virus* (CaCV), *Cucumber mosaic virus*, *Carnation mottle virus*, and *Basella rugose mosaic virus* (synonymous with *Phalaenopsis chlorotic spot virus*). Since the production of orchid seedlings for international trade needs virus testing, establishing a reliable virus detection technique is an important issue. In this study, 30 suspected orchid samples were tested by RT-PCR using 6 specific primer pairs corresponding to each viruses, and ORSV was found to have the highest proportion of infection, followed by the infections of CaCV. Multiplex RT-PCR was further established to detect ORSV, CymMV and CaCV simultaneously. Our results suggest that the total concentration of primers significantly affects the sensitivity for virus detection, and the establishment of this method will make orchid virus disease detection more efficient.

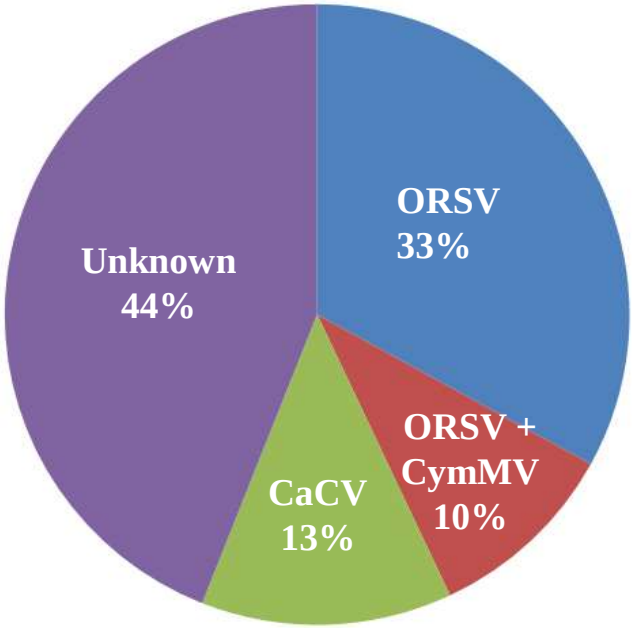

Fig. 1 分別以對應六種蝴蝶蘭病毒之專一引子對，檢測30個疑似染病之蘭花樣本，其中以ORSV感染比例最高，其次為CaCV及ORSV與CymMV之複合感染，Unknown為六種病毒皆未檢測到。

Fig. 1 Thirty suspected orchid samples were detected with specific primer pairs corresponding to six viruses of *Phalaenopsis*, respectively. ORSV was found to have the highest proportion of infection, followed by the infections of CaCV. Unknown represented the undetected for all six viruses.

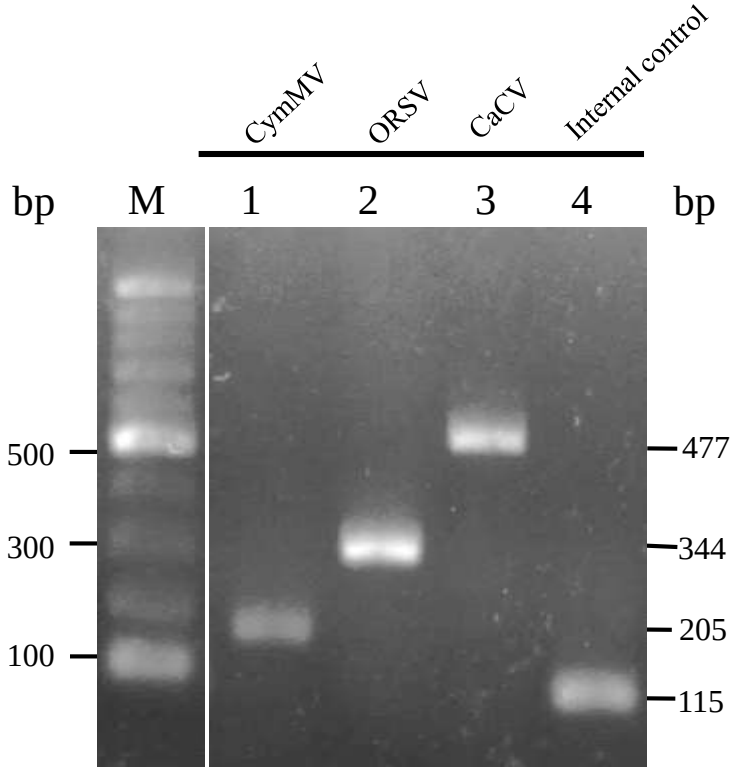

Fig. 2 以RT-PCR評估引子之專一性。首先混合每種病毒的3‘引子進行反轉錄，再混合四對引子進行PCR。Lane 4為針對18S rRNA之片段。

Fig. 2 The specificity of primers was evaluated by RT-PCR. The 3’ primers of each viruses were first mixed to perform reverse transcription, and PCR were carried out by using 4 primer pairs of each. Lane 4 is a DNA fragment amplified from 18S rRNA.

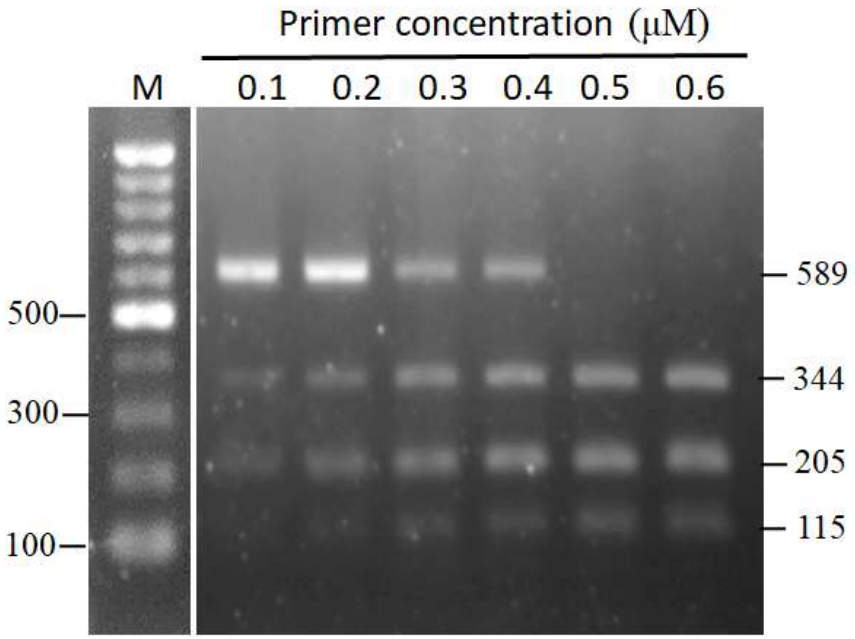

Fig. 3 比較引子總濃度對多重反轉錄聚合酶連鎖反應專一性的影響。

Fig. 3 Compare the effect of total primer concentration on the specificity of Multiplex RT-PCR.

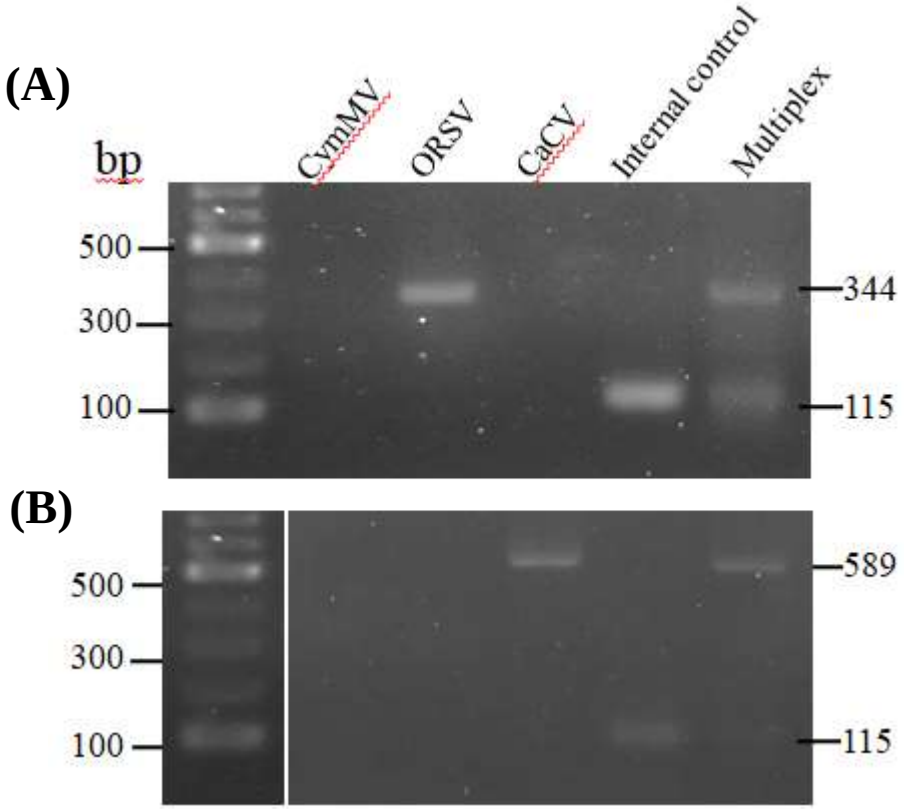

Fig. 4 分別以單一及多重反轉錄聚合酶連鎖反應檢測田間樣本(A)及(B)。

Fig. 4 Detection of field samples (A) and (B) by both single and multiple RT-PCR.