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### Review

## Virus resistance in orchids

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#### ABSTRACT

Orchid plants, *Phalaenopsis* and *Dendrobium* in particular, are commercially valuable ornamental plants sold worldwide. Unfortunately, orchid plants are highly susceptible to viral infection by *Cymbidium mosaic virus* (CymMV) and *Odotoglossum ringspot virus* (ORSV), posing a major threat and serious economic loss to the orchid industry worldwide. A major challenge is to generate an effective method to overcome plant viral infection. With the development of optimized orchid transformation biotechnological techniques and the establishment of concepts of pathogen-derived resistance (PDR), the generation of plants resistant to viral infection has been achieved. The PDR concept involves introducing genes that is(are) derived from the virus into the host plant to induce RNA- or protein-mediated resistance. We here review the fundamental mechanism of the PDR concept, and illustrate its application in protecting against viral infection of orchid plants.

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### **Contents**

Introduction	00
Modes of transmission	00
Viruses prevalent in orchids	00
Cymbidium mosaic virus	00
Odotoglossum ringspot virus	00
Detection of orchid viruses	00
Transformation in orchids	00
Particle bombardment	00
Agrobacterium-mediated transformation	00
Virus-resistance in plants	00
Pathogen-derived resistance	00
Coat protein gene-mediated resistance	00
RNA silencing-mediated resistance	00
Other viral protein gene-mediated resistance	00
Suppressor of RNA silencing	
Viral suppressor of RNA silencing	00
VSRs targeting RNA components of RNA silencing	00
VSRs targeting AGO proteins	
VSRs targeting proteins associated with RNA silencing	00
VSRs interfering with secondary siRNA amplification	
VSRs interfering with the epigenetic modification of the viral genome	00
Targeting VSRs	00
Pathogen-derived resistance in orchid	00
Generating multiple resistance via gene-stacking approach	00

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## ARTICLE IN PRESS

K.W. Koh et al. / Plant Science xxx (2014) xxx-xxx

VIGS-based approach to study plant resistance to virus	00
Asymptomatic CymMV as an advantage for loss-of-function study	00
Conclusion and future directions.	00
Acknowledgements	00
References	00

#### Introduction

Orchids, members of the family *Orchidaceae*, are highly evolved monocotyledonous flowering plants, comprising 900 genera and 2500–3500 species [1]. They are one of the largest families to exhibit huge diversities in flower size, shape and colors, and are famous for their longevity and enchantingly beautiful appearance. They are, therefore, economically popular ornamental cut-flowers and potted floricultural crops worldwide. Due to their high economic value, new varieties with specific or improved floral traits and desired characteristics, such as flowering time and prolonged vase-life, are constantly being generated to meet the market demand.

During cultivation, orchid plants are highly threatened by many phytopathogens, especially the viruses. Due to their high susceptibilities, the cultivation of resistant orchids has become a big challenge to the industry. To date, at least 30 different viruses have been reported to infect orchids, including *Cymbidium mosaic virus* (CymMV) [2], *Odontoglossum ringspot virus* (ORSV) [3], *Orchid fleck virus* (OFV) [4], *Cucumber mosaic virus* (CMV) [5], *Dendrobium vein necrosis closterovirus* (DVNV) [6], and *Tomato spotted wilt virus* (TSWV) [7]. Although these viruses may infect orchids of a specific species, some of these viruses (e.g. CymMV) may produce different diseases in different species.

When orchids are virally infected, they may display severe disease symptoms that affect the quality of the flowers, significant ones being floral and foliar necrosis, color-breaking of flowers causing variation in petal color, size reduction, unpleasant leaf appearances (leaf curl), reduced vigor and stunted growth [8]. These symptoms may vary in orchids and are dependent on factors, such as plant genotype (genus, species, variety), environmental conditions (temperature, humidity), plant management (level of nutrition, abiotic and biotic stresses) and types of virus species and isolates. Plants co-infected with bacteria or fungi may also be factors that contribute to these symptoms. Disease significantly reduces the economic value of orchids. Contradictorily, these 'visible' detrimental disease symptoms are good indicators of viral infection, and are easily distinguished from the healthy plants. In some cases, infected orchids are asymptomatic and appear visibly indistinguishable from healthy ones. This may hinder disease containment, as these plants are equally infectious to their neighbors. Since there is no effective measure to prevent CymMV and ORSV infections, the eradication of infected plants will help prevent further widespread of viruses. Therefore, routine virus screenings in conjunction with quick and reliable routine diagnostic protocols are required to resolve this problem. Alternatively, the establishment of an efficient anti-viral approach would provide a better solution.

## Modes of transmission

Understanding how different viruses are transmitted will help in controlling viral infections in the orchid industry. Some orchid viruses (e.g. potyviruses) are transmitted by the seeds of an infected plant. OFV, CMV and several other potyviruses and tospoviruses are transmitted by arthropods, such as mites, and insects, such as aphids and thrips. Elimination of weeds that grow near orchids may help eliminate viral transmission. Viruses that infect orchids systemically (CymMV and ORSV) are spread through

mechanical transmission by tools and pots contaminated with the viral-containing plant saps. Good horticultural practices such as weed and insect control, and the sterilization of cutting tools and pots prior to use, together with the identification and eradication of virus-infected plants are essential to prevent further spreading of viruses.

### Viruses prevalent in orchids

CymMV and ORSV are two of the most prevalent viruses of orchids, both predominantly present worldwide. They are highly stable viruses that possess similar biological and epidemiological characteristics, and are therefore, commonly co-infected. During single-infection, these viruses may produce symptomatic or asymptomatic diseases in some orchid genera, however plants infected with these two viruses produce severe disease symptoms that are more pronounced than a single-infection [9,10]. Orchids that are affected include Cymbidium, Odontoglossum, Phalaenopsis and Oncidium, and symptoms include mottling, ridging, curling and distortion of flowers and abnormal growth and stunting of plants. Symptom severity during co-infection is attributed to the synergism between ORSV and CymMV. Their co-existence results in enhanced RNA replication, hence causing the viral loads to be highly accumulated in infected plants [11]. This synergism greatly affects the flower quality and quantity, making CymMV and ORSV two economically important viruses in orchid cultivation worldwide that are responsible for significant financial losses in the orchid industry.

Both CymMV and ORSV are relatively heat-stable, highly virulent, and cause systemic infections that affect all parts of the orchid [12,13]. They are therefore, abundant in plant saps, and capable of retaining their infectivity for a long time [14]. Thus, these viruses are transmitted mechanically by plant sap-contaminated tools and potting media used for plant manipulation and nursery management practice. Gardeners working on orchids have become the main transmission agents responsible for the continuous spread of viruses from one plant to another by the dissemination of viruses to plants present in other nurseries. Besides mechanical transmission, CymMV and ORSV are transmitted by contaminated pollen [15–17]. Pre-sterilization of seeds before sowing will ensure that the young seedling plants are free from both viruses.

### Cymbidium mosaic virus

CymMV was first reported by Jensen [18]. It infects cultivated orchids, and is therefore, also known as *Orchid mosaic virus*. It is a species of the genus of *Potexvirus* of the family of *Flexivirida*. It is a flexuous rod of approximately 500 nm in length and 15 nm in width [19]. CymMV has a monopartite, positive-sense single-stranded RNA genome of 6.3 kb nucleotides, containing five open reading frames (ORFs) flanked by a capped 5' end and a polyadeny-lated 3' end [20]. ORF1 encodes a 160-kDa replicase that contains three recognized conserved domains, namely a methyl transferase domain in the N-terminal region, an RNA helicase domain and an RNA-dependent RNA polymerase (RdRp) in the C-terminal region. ORFs 2–4 are three overlapping ORFs, that are referred to as the triple gene block (TGB), and the 26-kDa/13-kDa/10-kDa proteins encoded by TGB, also called the movement protein (MP), are required for cell-to-cell movement [21–23]. TGBp1 also functions

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