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A possible occurrence of genome reassortment among bipartite rhabdoviruses

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ABSTRACT

Orchid fleck virus (OFV) represents a rhabdovirus with a unique bipartite genome. OFV genetic diversity at the whole genome level has not been described. Using the partial genome sequence of RNA1, we have determined that several OFV isolates derived from orchids in Japan belong to two genetically distant subgroups: subgroup I, the members of which are distributed worldwide but previously not known in Asia, and subgroup II, which is commonly distributed in Japan. However, complete genome sequence analysis of a novel Japanese subgroup I isolate revealed that although its RNA1 sequence differs considerably from those of subgroup II isolates, its RNA2 sequence is almost identical to them. Based on phylogenetic and recombination analyses, the genome reassortment events were predicted to occur between OFV subgroups including other unseen strains. Our data show that genome reassortment contributes to the genetic diversities of the bipartite rhabdoviruses and its occurrence may be geographically constrained.

1. Introduction

Members of the family *Rhabdoviridae* (order *Mononegavirales*) can infect a wide range of organisms and are important pathogens that affect human health (e.g. rabies virus) as well as agriculture and aquaculture industries (Kuzmin et al., 2009). Rhabdoviruses share the typical bullet-shaped or bacilliform virion morphology and their mono- or bipartite negative-strand RNA genome encodes five canonical structural protein genes (N, P, M, G and L) (Dietzgen et al., 2017). Classical rhabdoviruses (nonsegmented RNA genome) have been classified into sixteen genera, consisting of fourteen genera infecting animals and two genera infecting plants (Afonso et al., 2016; Amarasinghe et al., 2017). Recently, two free-floating genera, *Dichorhavirus* and *Varicosavirus*, whose members are plant-infecting negative-strand RNA viruses with unusual bipartite genomes, were also classified into the family *Rhabdoviridae* based on their genome organization and sequence-relatedness to plant rhabdoviruses (Afonso et al., 2016; Dietzgen et al., 2014).

Orchid fleck virus (OFV), the prototype member of genus *Dichorhavirus*, is recognized as an important viral pathogen affecting commercial orchid (Orchidaceae) production (Blanchfield et al., 2001; Kondo et al., 2003; Peng et al., 2013). OFV has non-enveloped bullet-shaped (or bacilliform) particles and is transmitted by the false spider mite (*Brevipalpus californicus* Banks) in a persistent manner (Kondo et al., 2003). OFV RNA1 (6.4 kb) contains five genes in the order 3′-N-P-3-M-G-5′ (gene 3: putative movement protein P3), while RNA2 (6.0 kb) contains a gene coding for L polymerase (Kondo et al., 2006, 2009). Based on nucleotide sequences of N gene fragments, OFV isolates are grouped into two subgroups: subgroup I, which includes most isolates collected from Australia, Germany, South Africa and American Continent countries, but not Asian countries, and subgroup II, which includes only five isolates from Germany, Costa Rica and East Asian countries (Ali et al., 2014; Blanchfield et al., 2001; Bratsch et al., 2015; Freitas-Astúa et al., 2002; Kim et al., 2010; Kubo et al., 2009; Peng et al., 2017). However, molecular information regarding OFV subgroup I isolates is limited, with no genome sequence data available except for the N gene fragment. Recently, a novel OFV strain (namely the citrus strain) infecting citrus trees, whose genome is closely related to OFV subgroup II isolates (~91% nt identities), was reported in Mexico (North America) and Colombia (South America) (Cruz-Jaramillo et al., 2014; Dietzgen et al., 2014; Roy et al., 2013, 2015a, 2015b). In addition, a new dichorhavirus (citrus leprosis virus N, CiLV-N) was also detected in citrus in the southeast region of Brazil (Ramos-Gonzalez et al., 2017). Other dichorhaviruses (e.g., coffee ringspot virus, CoRSV) have also been found in American Continent countries (Dietzgen et al., 2014).

RNA viruses have a high potential for genetic variation through either high error rates during virus replication (genetic drift) or genetic

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exchange by recombination via copy-choice or reassortment of genomic segments (genetic shift) (Duffy et al., 2008; Posada et al., 2002). Genome recombination of RNA viruses occurs at very high rates in positive-strand RNA viruses but is less frequent in negative-strand RNA viruses with RNA genomes that are consistently associated with N protein; this complex probably prevents template-switching by the viral polymerase (Chare and Holmes, 2004). In fact, only a few examples of recombination have been reported for rhabdoviruses (e.g., Liu et al., 2011; Pappi et al., 2016; Xiao et al., 2014). Despite this, their genomes usually contain accessory genes or gene sets, suggesting that ancestral recombination (insertion) events have occurred on their genomes (Walker et al., 2011, 2015). Genome reassortment (the exchange of genome segments), another type of genetic exchange, is well known to occur in segmented RNA viruses during the mixed infection of different viral strains in a host cell. This contributes to the generation of genetic diversity and plays an important role in the emergence and spread of novel strains, e.g. influenza virus and rotaviruses (Simon-Loriere and Holmes, 2011). Although the reassortment does not occur in the nonsegmented RNA genome of classical rhabdoviruses and other mononegaviruses (e.g. filoviruses and paramyxoviruses), this event might occur in dichorhaviruses and varicosaviruses because of their unique bisegment genome structure. However, no genome reassortment events have been reported in bipartite rhabdoviruses.

In this study, we determined partial and complete sequences of several Japanese isolates of OFV. Phylogenetic analysis showed that sequence relationships among Japanese isolates of OFV, including other Asian isolates, differ according to the RNA segments (RNAs 1 and 2), suggesting the occurrence of genome reassortment between OFV isolates. Accordingly, recombination detection programs predicted that the genome reassortment event(s) occurred between OFV and/or related viral strains.

2. Results and discussion

2.1. Sequence analysis of the N gene fragment of the OFV Japanese isolates

RT-PCR using a novel degenerate primer set for the N gene of both orchid subgroups (OFN2F and OFN3R, the N1 region in Fig. 2A) was performed on frozen leaf samples from symptomatic *Cymbidium* and other orchid leaf samples collected from several regions in Japan (Inouye et al., 1996 and unpublished results) (see Table 1 and Fig. S1A). OFV was detected in all leaf samples (Fig. S1B). Nucleotide sequence analysis using the Sanger method confirmed that the N fragments (a 592-nt region) of three samples (Cym07, Cym08 and Cym09 isolates) from different regions of Tokushima Prefecture were mostly identical (~98%) to subgroup I members, while the remaining six samples from Tokushima and other prefectures in Japan were highly similar (~98%) to subgroup II members (Table 1). Nucleotide sequence identities within subgroups I and II were more than 97% and 98%, respectively, whereas nucleotide sequences between two subgroups were relatively distant (82–84%) (Fig. S2A). Two citrus isolates (citrus strain) were more closely related to members of the orchid strain subgroup II (89–91% nt identities) than of subgroup I (83–84% nt identities) (Fig. S2A). The phylogenetic analysis using the 326-nt N fragments (a part of the N1 region) revealed that three Tokushima isolates, Cym07, Cym08 and Cym09, were clustered within subgroup I, while the other six Japanese isolates from different geographical regions (except for Onc10.2 isolate) clustered within subgroup II, which is close to a distinct subclade of the known citrus strain (the Mexican isolates, M2345 and Jal1), except for a novel distinct isolate USA-N0 (Fig. 1A and B).

OFV (orchid strain) was first found in Japan in *Cymbidium* plants showing chlorotic and/or necrotic fleck symptoms (Chang et al., 1976). This virus is likely to occur in many kinds of orchids (Blanchfield et al., 2001; Kondo et al., 2003; Peng et al., 2013). As mentioned above, the OFV subgroup I is distributed worldwide and at least is predominant in Australia and Brazil (33 and 44 orchid samples were tested, respectively), whereas subgroup II so far contains only five isolates obtained from Japan, Korea, China, Costa Rica and Germany (Blanchfield et al., 2001; Kim et al., 2010; Kubo et al., 2009; Peng et al., 2017). Our data suggest that OFV subgroup II already existed in around 1988 in Japan and might be predominant and widely distributed in Japan (Fig. 1 and Table 1). In contrast, the OFV subgroup I appears to have occurred more recently in the limited region of Shikoku Island (Tokushima prefecture) of Japan (Fig. 1 and Table 1). In addition, the Japanese subgroup I isolate Cym07 was transmissible by *Cymbidium* plant-infesting *Brevipalpus* mites (Fig. S1C–E), whose mitochondrial DNA (cytochrome c oxidase subunit I gene) fragment (Accession No. LC222647) was closely related to that of *B. californicus* (data not shown). Therefore, the mite vector might have contributed with the spread of subgroup I in the Tokushima prefecture.

Table 1
List of OFV isolates characterized in this study.

Isolate	Place of origin[a]	Host	Year	Accession no.	Identity[b] (Accession no.)	Sub-group
Cym07	Tokushima (Tokushima city)	*Cymbidium* sp.	2007	R1: LC222629 R2: LC222630	83% (AB244417) 99% (AB244418)	I
Cym08	Tokushima (Awa city)	*Cymbidium* sp.	2008	N: LC222631 G: LC222639 L: LC222643	99% (KR822590) 80% (AB516442) 99% (AB516441)	I
Cym09	Tokushima (Katsuura town)	*Cymbidium* sp.	2009	N: LC222632 G: LC222640 L: LC222644	99% (KR822590) 80% (AB516442) 99% (AB516441)	I
Onc10.2	Tokushima (Kaiyo town)	*Oncidium* sp.	2010	N: LC222633	99% (AB244417)	II
Cym88.9	Okayama	*Cymbidium* sp.	1988	N: LC222634 G: LC222642 L: LC222646	99% (AB244417) 99% (AB244417) 99% (AB244418)	II
Cy50[c]	Chiba	*Cymbidium* sp.	unknown	N: LC222635 G: LC222641 L: LC222645	99% (AB244417) 99% (AB244417) 98% (AB516441)	II
Cal92.7[c]	Miyazaki	*Calanthe triplicata*	1992	N: LC222636	99% (AF321775)	II
Cal94.16[c]	Yamaguchi	*Calanthe hizen*	1994	N: LC222637	99% (AF321775)	II
Cal94.17[c]	Yamaguchi	*Calanthe hizen*	1994	N: LC222638	99% (AF321775)	II

[a] Prefecture.
[b] BLASTn search results for the genome sequence (R1:RNA1; R2:RNA2) or each fragment sequence (N: 592; G: 698; L: 427 nts).
[c] Inouye et al. (1996).