


## Diseases Caused by Viruses

### First Report of Odontoglossum Ringspot Virus in *Vanilla* (Orchidaceae) in Madagascar

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Vanilla (*Vanilla planifolia*, Orchidaceae) is Madagascar's leading agricultural export resource, and the country provides 80% of the world's consumption. During a phytosanitary survey from November 2019 to March 2021 in the main vanilla production regions of Madagascar, 250 plots were indexed for Cymbidium mosaic virus (CymMV, *Potexvirus* genus) and Odontoglossum ringspot virus (ORSV, *Tobamovirus* genus), the two most prevalent viruses of cultivated orchids worldwide (Zettler et al. 1990). For each plot, bulk samples (10 leaves taken at random) were assayed using ImmunoStrips (AGDIA, ISK 13301). A quarter of the plots (63/250) tested positive for CymMV. The highest prevalence of CymMV was observed in the SAVA region (57 out of 153 plots = 37.2%) where the virus has been reported since 1997 (Grisoni et al. 2010). Six plots in the district of Mahanoro (Atsinanana) tested positive for ORSV. A few plants in these plots showed chlorotic often annular spots on their leaves. They were individually tested positive for ORSV, and negative for CymMV and potyviruses (ImmunoStrips AGDIA ISK 27200), the other two viruses reported so far in vanilla in Madagascar. To confirm the diagnosis of ORSV, leaf samples from five of the six infected plots were analyzed by Tube Capture-RT-PCR (Grisoni et al. 2017) using two pairs of primers flanking the ORSV coat protein (CP) gene: OrCP1 (5'-GGTCGGTAATGGTGTTAG-3')/OrCP2 (5'-TGCATTATCG TATGCTCC-3'), and CPOR-F (5'-ATGTCTTACACTATTACAGACC-3')/CPOR-R (5'-TTAGGAAGAGGTCCAAGTAAG-3'). The five samples gave amplicons of the expected size (820 and 476 nt, respectively) and

were sequenced with Sanger technology (Macrogen, The Netherlands). The ORSV-CP sequences of the Mahanoro isolates showed very close similarity to 198 ORSV-CP sequences from GenBank (95.8 to 99.6% nucleotide and 94.5 to 100% amino-acid identities), and less than 75.4% nucleotide (80.1% amino-acid) identities with bell pepper mosaic virus (DQ355023), the tobamovirus closest to ORSV. The five ORSV-CP sequences from vanilla were deposited in GenBank under accessions numbers OM847399 to OM847403. These data confirmed that ORSV infects vanilla vines in Madagascar. To our knowledge, this is the first report of this virus in Madagascar and of its ability to symptomatically infect *V. planifolia*. The five ORSV isolates from vanilla had more than 98.7% nucleotide identities of the CP gene and clustered into a monophyletic group in a maximum likelihood phylogenetic tree, suggesting a single origin of these isolates. To further investigate the origin of ORSV in Madagascar, we made use of RNA sequences isolated at different points in time to infer the timing of evolutionary events (Rieux and Balloux 2016). We estimated the CP gene substitution rate at 4.8E-4 subst./site/year [95% HPD 2.1E-4 to 8.7E-4] which is close to the estimate of He et al. (2019) based on a slightly different sequences set (1.25E-3 subst./site/year). We dated the initial contamination of vanilla plots by ORSV between 2004 and 2013. Both ORSV and CymMV have deleterious effects on many ornamental orchids, and the pathogenicity of CymMV is exacerbated when coinfecting with ORSV (Lee et al. 2021). Therefore, ORSV represents a new threat to the Malagasy vanilla crop, especially in regions where CymMV is already rife. Given the economic importance of vanilla cultivation in the country, the implementation of prophylactic measures aimed at preventing the spread of ORSV, in particular through the sanitary control of cuttings, should be a priority for the vanilla industry.

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#### e-Xtra

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