



First detection of *Raspberry ringspot virus* in mosaic diseased hybrid roses in Germany

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Roses (*Rosa hybrida*) cultivated for several years at the Isle of Mainau, Lake Constance, Germany developed mosaic, leaf chlorosis, vein netting and stunting (Fig. 1a). Various diseased rose cultivars such as Escimo, Diamant, Leonardo da Vinci, Chippendale, Trier 2000, Innocencia, Kurfürstin Sophie, and Alea were sampled in the summer of 2014. Symptoms suggested rose mosaic disease (RMD) that is caused by infection with any of a number of different viruses. The most prevalent virus species associated with RMD occurring worldwide are members of the ilar- and nepoviruses such as *Prunus necrotic ringspot virus* (PNRSV), *Apple mosaic virus* (ApMV), and *Arabidopsis mosaic virus* (ArMV) (Horst & Cloyd, 2007). None of these viruses could be detected by DAS-ELISA in bud and leaf samples of eight diseased roses.

Mechanical inoculation of *Chenopodium quinoa*, *Nicotiana benthamiana*, and *N. tabacum* cv. Samsun with homogenates of buds and leaves from four out of eight rose samples with stunting and leaf chlorosis produced systemic mottle, curling and deformation of leaves in *N. benthamiana*. In *C. quinoa* chlorotic ringspots on primarily infected leaves were induced followed by systemic leaf chlorosis and necrosis of the shoot tip (Fig. 1b). In *N. tabacum* cv. samsun no symptoms could be observed until three weeks post inoculation. Additionally, no symptoms were induced in test plants inoculated with material from symptomless roses serving as negative controls. Isometric particles with a diameter of approximately 28 nm were observed by electron microscopy in dip preparations of leaf homogenates from symptom-bearing test plants (Fig. 2). Induced symptoms in test plants and particle morphology suggested an infection with a nepovirus. Therefore, RT-PCR was carried out according to Wei & Clover (2008) applying two different primer pairs specific for the amplification of a RNA1 fragment (RNA-dependent-RNA-polymerase (RdRp) coding region) of nepoviruses of subgroup A and B, respectively. Specific PCR-products were obtained from symptom-bearing rose samples as well as from respective experimental hosts displaying virus-specific symptoms.

Purified RdRp-fragments amplified from three rose samples were sequenced from both directions (EMBL-EBI Accession Nos. LN877215-LN877217) and subjected to BLASTN analyses revealing

highest identities (88-89%) to a grapevine isolate of *Raspberry ringspot virus* (RpRSV) from Germany (AY310444). We performed a RpRSV-specific RT-PCR using an RNA2-targeted primer pair amplifying a part of the movement protein (MP) coding region (Ochoa-Corona *et al.*, 2005) to validate the infection of roses with RpRSV. The presence of the virus could be confirmed by RT-PCR amplification of the RpRSV-specific 385 bp MP-fragment in samples derived from five different roses (cvs. Alea, Escimo, Kurfürstin Sophie, Leonardo da Vinci, Trier 2000), all exhibiting stunting, mosaic and/or chlorotic vein netting (Fig. 3). Sequencing and BLASTN analyses of selected RpRSV-MP fragments (LN877218-LN877222) revealed maximal 90% nucleotide identity to the corresponding region of the grapevine strain of RpRSV (AY310445). This is the first report of RpRSV in tea hybrid roses exhibiting mosaic, chlorotic vein netting and stunting. As no other isometric viruses previously known to be associated with RMD were detected in the symptomatic roses, it is assumed that RpRSV can induce a similar disease in investigated cultivars.

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Figure 1

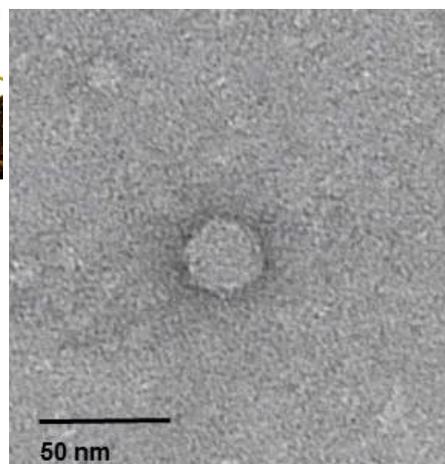


Figure 2

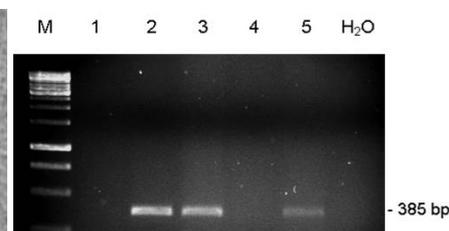


Figure 3

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