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9-2 Variability of the p3 and p4-coding genome region of *European mountain ash ringspot-associated virus* (EMARaV) in *Sorbus aucuparia* of different European regions

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INTRODUCTION

Virus-like symptoms on rowan trees (*Sorbus aucuparia* L.) such as chlorotic ringspots and mottling on leaves, were first described six decades ago (Kegler 1960). In 2007 the symptoms were associated with the *European mountain ash ringspot-associated virus* (EMARaV) (Mielke *et al*. 2007), the type-species of the newly established genus *Emaravirus* (Mühlbach & Mielke-Ehret 2011). The genome of EMARaV consists of four single stranded RNAs (RNA1-RNA4) of negative polarity. Each segment encodes a single protein (P1-P4) translated from the complementary strand. In previous studies, EMARaV variants from different parts of south Finland and Russia were analyzed with respect to the genetic variability of the nucleocapsid-coding region of the viral RNA3 (Kallinen *et al*. 2009, Valkonen & Rännäli 2010). However, these variants showed a low genetic variability within the coding region of the nucleocapsid protein (P3). In this study, genetic variability of P3 of EMARaV variants obtained from infected rowans from Germany, Sweden, Scotland and Finland was compared with the sequences of those samples from southern Finland and Russia. Further, sequence diversity of the non-structural viral protein P4 was investigated.

MATERIAL AND METHODS

Total RNA from symptomatic leaves sampled from infected rowans from Germany, Sweden, Scotland and Finland was isolated according to the protocol by Mielke *et al*. (2007). Viral

RNA3 and RNA4 were amplified by RT-PCR with specific primer pairs. The amplification of three fragments from the RNA3 was performed according to Kallinen *et al*. (2009). The RNA4 encoded P4 protein was amplified with P4-specific primers. The PCR products amplified from RNA3 were directly sequenced. The P4 specific PCR products were cloned prior to sequencing. Neighbour-joining phylogenetic trees were generated using ClustalX 2.0 (Larkin *et al*. 2007).

RESULTS AND DISCUSSION

Amplicons of the expected size (588 bp, 665 bp & 878 bp, RNA3; 699 bp, RNA4) were generated from 18 EMARaV infected rowans of different stands and countries. After sequencing the variability of the non-structural protein P4 and the structural protein P3 were compared with respect to nucleotide and amino acid sequences. Similar results to Kallinen *et al*. (2009) were obtained by comparison of amino acid sequences of P3 from Germany, Finland and Sweden (99-100 %). The Scottish EMARaV variants showed lower identities between 97-99 %. and formed a distinct cluster in phylogenetic analyses. Comparison of the P4 coding-region also showed high sequence conservation (98-100 %). However, phylogenetic analysis did not result in an independent cluster of the Scottish variants.

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