



High genetic variability found among *Cherry leaf roll virus* variants from symptomatic birch trees in Rovaniemi (Finland)

Rumbou A.¹, von Bargaen S.¹, Rott M.¹, Jalkanen R.² and Büttner C.¹

¹ Humboldt University, Division Phytomedicine, Berlin

²The Finnish Forest Research Institute METLA, Rovaniemi, Finland



Cherry leaf roll virus (CLRV) (*Nepovirus* subgroup C, Fam. *Secoviridae*) is a worldwide distributed plant pathogen that infects many deciduous trees and shrubs. During the last decade it has expanded widely in the birch forests in northern Finland and is considered to correlate strongly with the epidemic (von Bargaen *et al.*, 2009). Symptoms include leaf malformation, interveinal chloroses and rolling (Fig.1,2). The appearance of declining affected trees is common even in the wild forest (Fig. 3).



Fig. 3 Declining birch tree with strong leaf chloroses and scattered canopy in the forest near Rovaniemi.

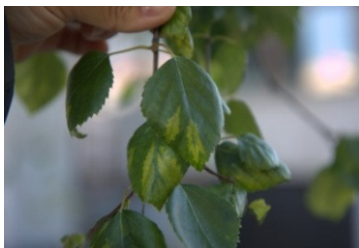


Fig.1,2 *Betula pubescens* leaves showing typical CLRV symptoms.

Materials and Methods

14 birch trees (*B. pubescens* and *B. pendula*) exhibiting strong symptoms were selected. After total RNA-isolation from leaf samples, RT-PCR-RFLP-analysis and partial sequencing of the genome were performed. A 416 bp-fragment from the 3'-untranslated region (3'-UTR) was cut with three restriction enzymes. For the genetic analysis, fragments from three genome regions were cloned and sequenced; 416 bp from the 3'-UTR, 627 bp from the coat protein region (CP) and 318 bp from the RNA-dependent-RNA polymerase region (RdRp) (Fig. 3).

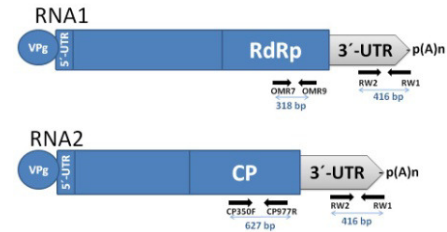


Fig.3 The three genome regions (3'-UTR, CP and RdRp) selected for sequencing.

Results

The RFLP analysis of the 3'-UTR region showed 4 different patterns, some of them in the same tree (Fig.4, Table 1). The sequencing analysis showed 4 genotypes in the 3'-UTR region, 3 genotypes in the CP region and 6 genotypes in the RdRp region. Most polymorphic was the 318 bp-fragment of the RdRp region. In this region, two different sequence variants were found in five out of 13 trees. In the other regions two trees were found to be infected with different virus variants.

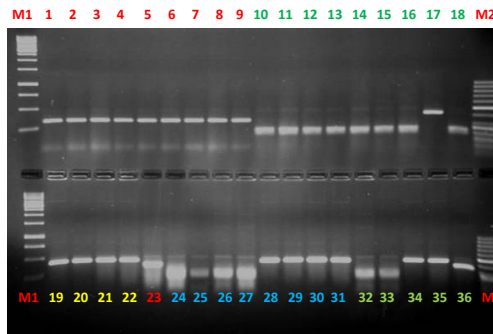


Fig. 4. RFLP analysis of cloned fragments of the partial 3'-UTR (416 bp, RW2-RW1) with the restriction enzyme *RsaI*. M1: 1Kb ladder; M2: 50 bp ladder. Samples with the same color originate from the same tree.

No of tree	No of sample	RFLP 1	RFLP 2	RFLP 3	RFLP 4
B4	1-9			332/84	
B5	10-18	221/195			416
B6	19-22				416
B7	23			332/84	
B12	24-31		280/136		416
B18	32-36		280/136	332/84	416

Table 1. Four different RFLP patterns revealed by restriction analysis with *RsaI* of the 416bp 3'-UTR-fragment from 6 birch trees (see Fig. 4).

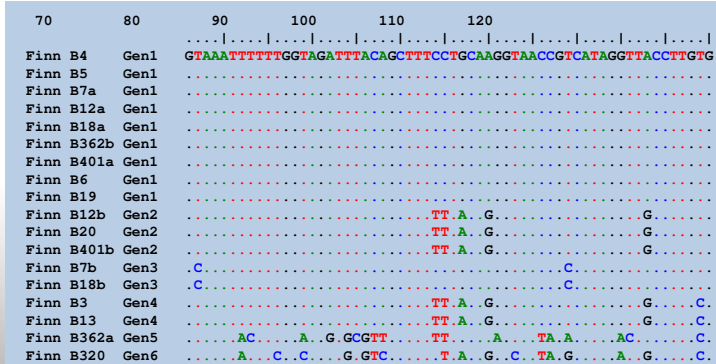


Fig. 5 Sequence alignment of 318 bp-fragments from the RdRp region from 13 birch trees in Rovaniemi revealing six different genotypes. The first 60 nucleotides are shown here.

Discussion

These data constitute preliminary results concerning the genetic variability of the Finnish CLRV population. The finding of two different variants in many trees and in all genetic regions studied, suggests the presence of at least two different virus variants within those trees. Considering the fact that the 14 investigated birches were growing in the city of Rovaniemi, thus spatially distributed within a limited area, we assume that the Finnish population of the virus among trees is more diverse than the populations studied earlier in Germany and Great Britain; this may be an explanation for the high disease severity in Finland.

References

von Bargaen, Grubits, Jalkanen & Büttner (2009): *Silva fennica*, 43 (5), p. 727-738
 Büttner, von Bargaen, Bandte & Myrta (2011): *Virus and virus-like diseases in pome and stone fruits*, p. 119-127