Tissue culture of CLRV-infected birch species from Northern Finland

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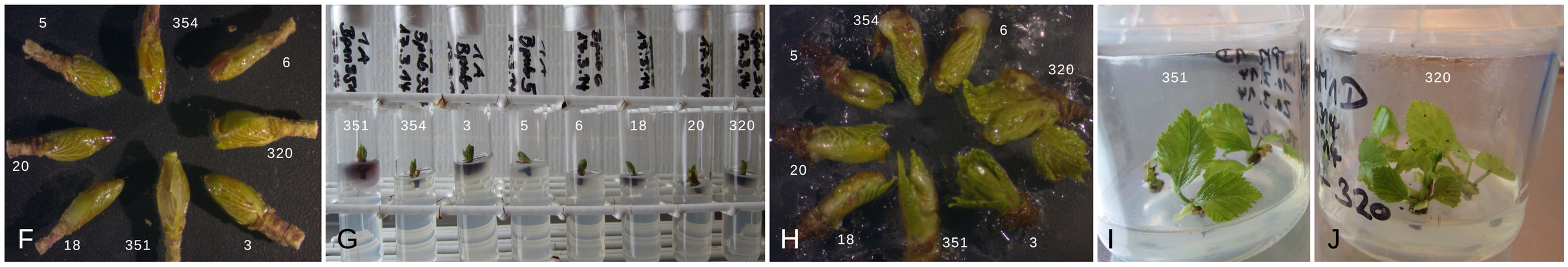
Cherry leaf roll virus (CLRV) has become a serious problem in Finland due to a rapid dissemination and strong symptom expression in birch species in Finnish forest stands, public greens and roadsides.

The establishment of tissue cultures of selected CLRV-infected trees of *Betula pendula* and *B. pubescens* is of great importance for our research studies related to the molecular and epidemiological characterization of CLRV to elude technical difficulties with woody trees as its natural hosts. These are in particular the uneven virus distribution in long-lived trees coherent with low virus titer and high contents of secondary metabolites in sampled plant material from mature woody trees, potentially hampering molecular tools of genome sequencing and characterization as well as CLRV transmission from Finnish provenances to herbaceous test plants as a prerequisite for developing specific antibodies against Finnish CLRV variants for routine diagnosis in a wide area of Fennoscandia. Tissue cultures are also important to preserve the Finnish CLRV variants identified so far as gradually more of our sample trees in Rovaniemi are cut due to their disease.

Since 2006 constant monitoring of the CLRV epidemic in Rovaniemi, Northern Finland, documenting the particularly severe CLRV-associated disease symptoms on leaves, partially adherent with progressive loss of vitality or the death of twigs and branches.
(A, B) CLRV infected *Betula pubescens* no. 3 in June 2013; **(C)** in August 2013. **(D)** Dormant winter buds of CLRV-infected *Betula pubescens* no. 20. **(E)** Dormant winter buds of 8 selected birch trees: CLRV infected *B. pubescens* no. 3, 5, 6, 20, 320, *B. pendula* no. 18; CLRV non-infected controls *B. pubescens* no. 354, *B. pendula* no. 351. Cut twigs with buds were stored in water at 5 °C.



Representative detail of **(F)** peeled buds of each of the 8 genotypes; **(G)** bud culture on initiation medium, one bud per glass tube; after 3 days of regrowth, initiation buds were emerging; individual genotypes were exudating phenolic compounds, visible as dark clouds in the media; **(H)** emerged buds after 3 days of culture; **(I, J)** 10 days old cultures; 3-5 emerged buds were transferred together in one glass jar; viability of bud material gave no indication of CLRV-infected and CLRV-noninfected tree origin.

>> buds were surface sterilized in 70 % ethanol before peeling **>>** 40 buds per each of the 8 tree genotypes; bud culture initiation on woody plant medium (WPM; Lloyd & McCown 1980), 20 buds on **WPM-1A** supplemented with 8.8 µM BAP (6-benzylaminopurine) and 0.2 µM NAA (1-naphthaleneacetic acid), and the other 20 buds per each genotype on **WPM-1D** supplemented with 0.1 µM TDZ (thidiazuron) as growth regulators **>>** growth conditions are a 16 h photoperiod with a light intensity of 95–115 µEm−2 s−1 at 22 °C

Birch genotype CLRV-(+/-)		Rate (%) of phenolic exudation after 5 days of bud growth initiation (WPM-1A/-1D)	Frequency of transfers to new medium due to phenolic exudation after 17 days of bud growth initiation (WPM-1A/-1D)	Rate (%) of microbial contamination after 5/17 days of bud growth initiation
Bpub 3	+	50.0/52.9	0-3 / 0-3	12.5 / 52.5
Bpub 5	+	0/0	0 / 0	20.0 / 27.5
Bpub 6	+	11.1/50.0	0-1 / 0-1	10.0 / 50.0
Bpen 18	+	35.3/89.5	0-3 / 0-4	10.0 / 27.5
Bpub 20	+	26.3/31.6	0-2 / 0-2	5.0 / 50.0
Bpub 320	+	45.0/36.8	0-2 / 0-2	2.5 / 22.5
Bpen 351	-	46.7/89.5	1-3 / 1-2	15.0 / 60.0
Bpub 354	-	21.1/42.1	0-1 / 0-1	5.0 / 42.0

coming next **>>>>** after shoot /adventitious shoot proliferation from growing callus transfer of cultures to woody plant medium WPM-3A supplemented with 2.2 µM BAP and 2.85 µM IAA (indole-3-acetic acid), and alternatively WPM-3B supplemented with 4.4 µM BAP **>>** subsequent rooting of individual shoots in WPM-4 without any growth regulators or alternatively supplemented with 2.25 µM BAP

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