Detection of *Cherry leaf roll virus* in birch pollen by an improved IC-RT-PCR

Ulrike Bütow, Maria Landgraf, Martina Bandte, Karl-Christian Bergmann, Heidrun Behrend, Peter Beyerlein, Janina Kneipp und Carmen Büttner

Humboldt-Universität zu Berlin, Faculty of Agriculture and Horticulture, Department of Crop- and Animal Sciences; Division Phytomedicine; Lentzeallee 55/57, D-14195 Berlin

Introduction

Cherry leaf roll virus (CLRV) is a globally spread *Nepovirus* of the family *Secoviridae* and one of the most important viruses infecting *Betula* spp. (Bandte et al., 2009; Büttner et al., 2011). Pollen has not been used for the detection of CLRV. This work focussed on the establishment of a more sensitive detection method of CLRV in birch pollen.

Fig. 1: Sampling of *Betula* spp. with virus-like symptoms. (a) CLRV-infected birch (b) chlorotic veinbanding(c) mottling (d) Use of a tree climber, to collect branches from different tree areas (e) mature catkins (f) preparation of the pollen samples in the greenhouse under full protective clothing of the participants (g) seperation of the pollen from the catkins

Materials & Methods

Pollen from 48 birch trees (*Betula pendula*, *Betula pubescens* and hybrids of both) were collected in spring of 2011 and 2012 (Fig. 1). Birch trees with typical virus-symptoms and those without symptoms were sampled. From these, a total of 69 pollen samples were comparatively investigated with two CLRV-specific IC-RT-PCR methods (Tab. 1). Leaves from CLRV-infected *Chenopodium quinoa* (10^{-1}-10^{-4}) were used in the IC-RT-PCR to test the sensitivty of the two primer combinations.

Tab. 1: Overview of the IC-RT-PCR methods under specification of the attached homogenate, the used primers, the expected fragment size and the PCR-conditions (method A = Gentkow et al. (2007); method B = this work)

		method A	method B
Dilution homogenate		10^{-1}, 10^{-2}	10^{-1}, 10^{-2}
cDNA-syntheses		GTC GGA AAG ATT ACG TAA AAG G; Werner et al. (1997)	
Primer PCR	*FW:*	GTC GGA AAG ATT ACG TAA AAG G; Werner et al. (1997)	
	RW:	TGGCGACCGTGTAACGGCA Werner et al. (1997)	CATGCGACCGGTCCTAGTAGTA (this work)
Fragment size		420 bp	353 bp
PCR-protocol		2 min 94 °C; 30 x 1 min 94 °C, 45 s 55 °C, 1 min 72 °C; 5 min 72 °C	

Results

- Using the new primer combination (method B) leads to an at least 10fold higher sensitivty of IC-RT-PCR (Fig. 2)
- Substances present in birch pollen extracts inhibited IC-RT-PCR when using pollen dilutions of 10^{-1} (Fig. 3); applying at least a dilution of 10^{-2} overcomes this problem
- The modified IC-RT-PCR led to higher amplification rates (Fig. 3)

- Using the same pollen samples the modifications (method B) led to 31 samples with CLRV-positive test result (Tab. 2)
- Of 15 positive tested trees pollen could be analysed in both years. 8 of them were CLRV-positive in both years, while the birch pollen from the other 7 trees were only in one year CLRV-positive
- The detection of CLRV in pollen doesn´t correlate with the expression of leaf symptoms.

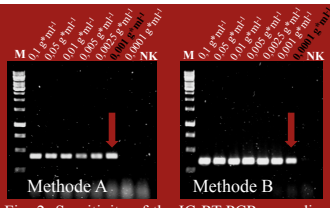

Fig. 2: Sensitivity of the IC-RT-PCR according to the used primers; method A: Werner et al. 1997; method B: this work

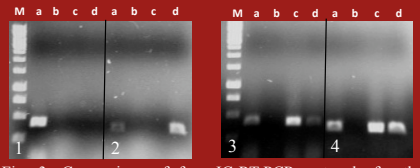

Fig. 3: Comparison of four IC-RT-PCR-protocols for the detection of CLRV in birch pollen. **Primer**: (1;3) Werner et al. 1997, (2;4) this work; **dilution pollen extract**: (1;2) 10^{-1}, (3;4) 10^{-2}; a-d: pollen of four individual trees

Tab. 2: Number of CLRV-positive tested birch pollen according to the location and the choosen IC-RT-PCR method (method A = Gentkow et al. (2007); method B = this work)

	2011			2012		
location	trees	CLRV pos.		trees	CLRV pos.	
		method A	method B		method A	method B
Im Schwarzen Grund	5	1	4	10	2	4
Vogelsang	9	3	6	10	2	4
Grunewald	9	0	4	10	0	4
Vogelsang	3	0	1	13	1	4
In total	26	4	15	43	5	16

Summary

- A sensitive and reliable CLRV-detection method could be established
- With the new primer combination the CLRV-specific IC-RT-PCR is more sensitive
- Substances present in pollen extracts have an inhibitory effect on IC-RT-PCR
- CLRV was detectable in pollen from symptomatic and asymptomatic birches

➢ Nearly half of the tested trees (23/48) are CLRV-positive, indicating a wide distribution of the virus in birch population

Literature

Bandte et al. (2009): *Jahrbuch der Baumpflege,* 215-221.

Büttner et al. (2011): *Virus and Virus-like Diseases of Pome and Stone Fruits*, 119-125.

Gentkow et al. (2007): *Jahrbuch der Baumpflege,* 279-302

Werner et al. (1997): *Journal of Forest Pathology* 27, 309-318.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (BU890/14-1, BU890/23-1).