Interaction studies of the *Cherry leaf roll virus* (CLRV)-encoded proteins involved in intercellular movement in host plants

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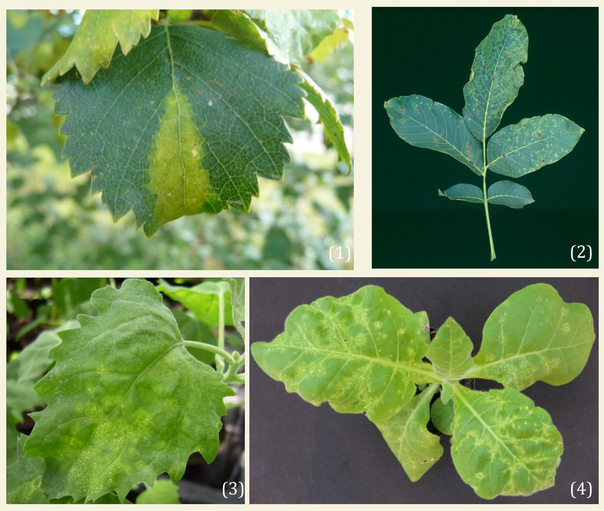

fig. 1: CLRV-infected *Betula pendula* (1), *Juglans regia* (2), *Chenopodium quinoa* (3), *Nicotiana tabacum* cv. *samsun* (4)

INTRODUCTION

Cherry leaf roll virus (CLRV) is a worldwide distributed *Nepovirus* (family *Secoviridae*), that infects many deciduous trees and shrubs including stone fruits, such as cherry (*Prunus avium*) and walnut (*Juglans regia*, fig. 1), and small fruits, for instance raspberry (*Rubus idaeus*). CLRV is reported to spread in nature mainly by seed (Büttner et al., 2011). Members of the family *Secoviridae* are transported as virions, thus requiring the viral coat protein (CP). Further, the movement protein (MP) inducing tubular structures by multimerization within plasmodesmata is necessary for passage of virus particles to adjacent cells. In case of CLRV, virus-like particles (VLPs) have been observed within tubules in anther cells and in pollen grains of virus-infected birch and walnut (Massalski and Cooper, 1984). The yeast two-hybrid system (YTHS, fig. 2) was applied to investigate the underlying interactions of the CLRV-movement protein (385 aa, 42 kDa) and the viral coat protein (512 aa, 56 kDa) involved in cell-to-cell movement and gametophyte infection.

MATERIALS AND METHODS

The movement and coat protein coding regions of a CLRV-isolate from rhubarb (Fig. 2) were cloned into the GAL4-based vectors pAS2 (GAL4-BD) and pACT2 (GAL4-AD).The plasmids were co-transformed and expressed in the yeast *Saccharomyces cerevisiae* Y190. A positive protein-protein interaction was detected by a His-auxotrophy and the colorimetric lacZ-assay. Additionally, the YTHS was used to examine specific binding of the CLRV-encoded proteins to a plant protein (At-4/1).

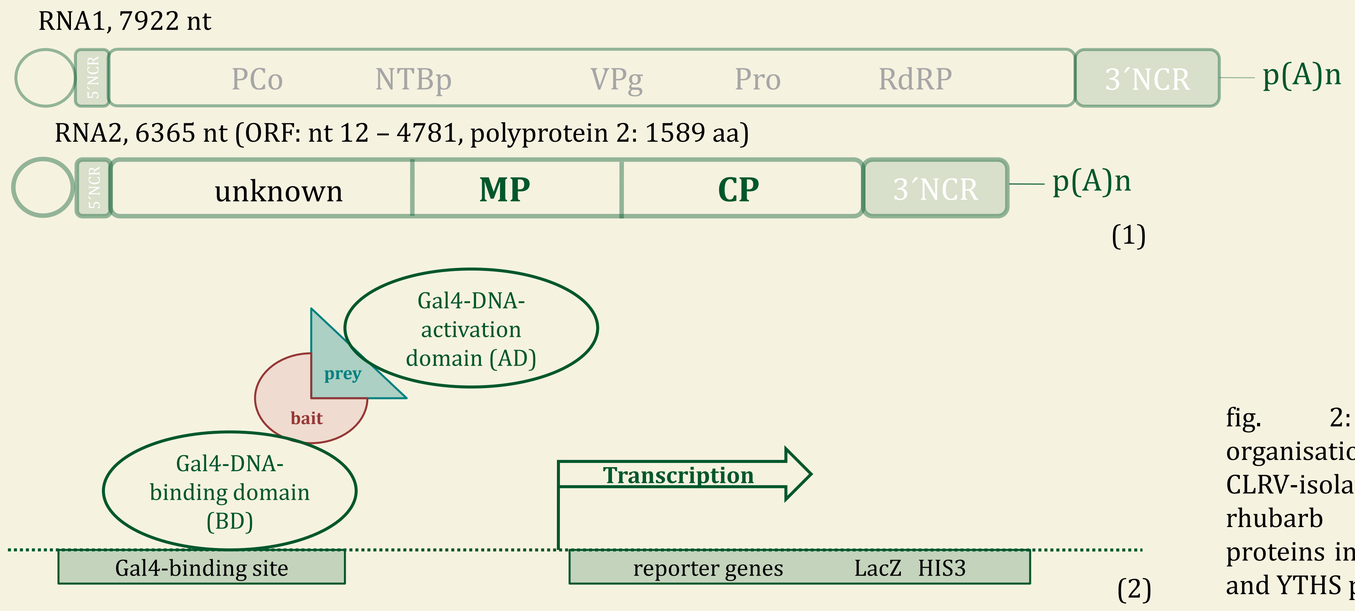

fig. 2: Genome organisation of the CLRV-isolate from rhubarb (1), viral proteins investigated in, and YTHS principle (2)

RESULTS of reporter gene assay and CONCLUSIONS: (fig. 3)

- **Dimerization of CLRV-MP**

suggests a possible multimerization of the MP into tubular structures within the plasmodesmata that are essential for the transport of CLRV.

- **Interaction between CLRV-MP and CP**

suggests that the transport of CLRV as virions along the MP-tubuli is possible.

- **Dimerization of CLRV-CP**

is a prerequisite for encapsidation of the two CLRV RNAs by CP subunits and is essential for the transport as a virion.

- **Interaction between CLRV-MP and the plant protein At-4/1**

suggests that CLRV is possibly transported analogous to *Tomato spotted wilt virus* (TSWV). The plant protein At-4/1 localized at plant cell plasmodesmata facilitates intra- and intercellular trafficking (Paape et al., 2006) and interacts specifically with the tubuli-forming MP (NSm) of TSWV.

- **Specificity of CLRV-CP and -MP interactions**

were confirmed in control experiments against the replicase of *Wheat dwarf virus* (WDV-RepA), the TSWV silencing suppressor protein (NSs) and nucleocapsid protein (N), and the *Arabidopsis thaliana*-PRL1 protein.

(1)

		GAL4-AD fusion (pACT2)							
		GAL4-AD		CLRV-MP		CLRV-CP		At-4/1	
		HIS3	LacZ	HIS3	LacZ	HIS3	LacZ	HIS3	LacZ
GAL4-BD Fusion (pAS2)	GAL4-BD	++		++		++		++	
	CLRV-MP	++		+++		+++		+++	
	CLRV-CP	++		++		+++		++	

(2)

Yeast double transformants		**Reporter gene assays**	
GAL4-BD fusion	GAL4-AD fusion	HIS3	LacZ
TSWV-N	CLRV-MP	+	
TSWV-NSs		+	
WDV-RepA		+	
A. thaliana-PRL1		+	
TSWV-N	CLRV-CP	+	
TSWV-NSs		+	
WDV-RepA		+	
A. thaliana-PRL1		+	
TSWV-NSm-1-240	*A. thaliana*-At-4/1	+++	
S. cerevisiae-SNF1	*S. cerevisiae*-SNF4	+++	

Fig 3: Results of YTHS-reporter gene assays: Protein-protein interactions of CLRV-MP and -CP (1) and control experiments (2)

OUTLOOK

The specific interactions of the MP and CP of CLRV will be confirmed by bimolecular fluorescence complementation (BiFC) as a second independent method.

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