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9-7 Identification of protein-protein-Interactions in the host-pathogen-system *Arabidopsis thaliana*/*Cherry leaf roll virus* (CLRV)

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INTRODUCTION

Cherry leaf roll virus (CLRV) is a worldwide distributed *Nepovirus* (family *Secoviridae*) that infects a wide range of herbaceous and woody plants (Büttner *et al*. 2011). In regard to appearance, symptom severity and crop losses an infection by CLRV has a high economic relevance as it is known from fruit trees such as cherry (*Prunus* spp.) or English walnut (*Juglans regia* L). CLRV is naturally transmitted by pollen and seed, but knowledge on mechanisms is still missing. In this study, specific interactions between the CLRV-movement protein (MP, 385 aa, 42 kDa), the coat protein (CP, 512 aa, 56 kDa) and plant proteins were investigated applying the yeast two-hybrid system (YTHS). Systemic infection of the host plant including the reproductive organs by the virus is achieved by cell-to-cell movement via plasmodesmata and long distance transport through the vascular system. Members of the family *Secoviridae* are transported as virions, thus requiring the coat protein. Further, the viral movement protein inducing tubular structures by multimerization within plasmodesmata is necessary for passage of virus particles to adjacent cells.

MATERIAL AND METHODS

Protein-protein interaction experiments were performed in *Saccharomyces cerevisiae* strain Y190 (Harper *et al*. 1993) applying the Matchmaker Two-Hybrid system (Clontech). Total RNA was isolated from *Chenopodium quinoa* leaves systemically infected with a CLRV isolate from rhubarb. CLRV-MP and -CP sequences were amplified by RT-PCR and cloned into the GAL4 DNA binding domain (BD) vector pAS2 and the GAL4 DNA activation domain (AD) vector pACT2. Using the lithium acetate method *S. cerevisiae* Y190 cells were

co-transformed with pAS2- and pACT2-fusion plasmids. Selection of positive transformants was performed by combination of the reporter genes *his3* and *lacZ*.

RESULTS AND DISCUSSION

The yeast two-hybrid assay revealed dimerization of the viral movement protein and the coat protein of CLRV by expression of both reporter genes. Self-interaction of CLRV-CP was expected since it is a prerequisite for encapsidation of the two CLRV RNAs by CP subunits (Jones & Mayo 1972; Walkey *et al*. 1973). Likewise, dimerization of the viral MP was detectable in the YTHS corroborating the essential multimerization of the movement protein into tubular structures. Consequently, specific interaction of the CP with the MP could be confirmed in YTHS supporting the hypothesis that CLRV is also transported as a virion along MP-tubules through plasmodesmata. Strong binding of the CLRV-MP to the plant protein At-4/1 could be detected in YTH assay. This plant protein has been shown to interact with the movement protein of *Tomato spotted wilt virus* (TSWV) and sequence alignment revealed a relation of At-4/1 to myosin- and kinesin-like proteins participating in cellular motility (von Bargen *et al*. 2001). Localization of the protein at the plasmodesmata supports its role in directed cellular movement processes (Paape *et al*. 2006). This suggests that CLRV and TSWV are not only sharing the tubule-guided mechanism but utilizing the same cell-to-cell transport in their host plants.

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