THIEL, HEIKE and MARK VARRELMANN*, Institute of Sugar Beet Research, Department of Phytopathology, Holtenser Landstrasse 77, 37079 Göttingen, Germany. **The P25 pathogenicity factor of *Beet necrotic yellow vein virus* physically interacts with several sugar beet proteins possibly involved in virus pathogenicity or plant resistance.**

ABSTRACT

Beet necrotic yellow vein virus (BNYVV), the causal agent of rhizomania in sugar beet, consists of four (to five) RNA-segments and is transmitted by the soil-borne plasmodiophorid *Polymyxa betae* and induces severe lateral root proliferation, necrosis and strong root yield reduction. The growth of partially (*Rz*1, *Rz*2) resistant hybrids stabilizes yield but does not prevent virus infection and replication entirely. Control of the disease is achieved by growth of BNYVV partial resistant sugar beet cultivars. The viral RNA3 encodes the pathogenicity factor P25 which is responsible for the induction of rhizomania symptoms and virus translocation in the root system as well as for symptom development and yield reduction, is suggested to function as an avirulence (Avr) gene product in resistant and pathogenicity factor in susceptible sugar beet genotypes. In addition previous studies have shown that recently occurring resistance breaking isolates possess increased P25 variability.
To better understand P25 functions and the molecular basis of the virus-host interactions, the BNYVV encoded P25 was used for investigations of physical protein-protein interactions with sugar beet proteins in order to elucidate the sugar beet resistance mechanism against BNYVV and to better understand P25 pathogenic mode of action. Therefore P25 was applied in a yeast two-hybrid screen (YTH) of an *Rz*2 resistant sugar beet cDNA library. The YTH screening was performed using a quantitative liquid mating and resulted in approximately $3x10^6$ independent transformants. The mating resulted in 430 putative positive clones. 37 cDNA candidates remained after elimination of false positives (i.e. transcriptional autoactivators). This screen identified several candidate proteins, for which orthologues from other plant species are well-known to be expressed following pathogen infection and involved in plant defense response.

The interaction of candidate genes with P25 was confirmed by applying the bimolecular fluorescence complementation (BiFC) technique. This *in planta* assay is based on the splitting of the red-fluorescent protein (mRFP) into N- and C-terminal fragments. Fusing each of the two halves to the putative interacting candidates, followed by transient expression in *Nicotiana benthamiana* leaves led to reconstitution of the functional fluorophore. The red fluorecence suggesting protein interaction could be monitored by epifluorescence microscopy after 3-5 days. Finally ten out of 37 cDNA clones interacting displayed BiFC *in planta* interaction with P25.

Sequencing and database analysis revealed putative functions of many candidates. Several P25 interacting sugar beet derived proteins share nucleotide and amino acid sequence homologies to proteins involved in plant-pathogen-interactions. Some interactions may be necessary for the virus life cycle or might serve to suppress the sugar beet defense. Among the candidates are members of the plant ubiquitin/proteasome system and proteins involved in phytohormone signalling, cell cycle and structure as well as stress and pathogen response.