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Applying P25 pathogenicity factor of beet necrotic yellow vein virus in yeast two-hybrid interaction hunt on the *Rz2* sugar beet proteome identifies several candidate proteins possibly involved in virus pathogenicity or plant resistance

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INTRODUCTION

Beet necrotic yellow vein virus (BNYVV) is responsible for one of the most devastating diseases of sugar beet, the rhizomania. The virus is transmitted by the soil-borne plasmodiophorid *Polymyxa betae* and induces severe lateral root proliferation, necrosis and strong root yield reduction (Fig. 1.). Only growth of partially resistant hybrids carrying monogenic dominant resistance genes (i.e. *Rz1, Rz2*) stabilizes yield but does not prevent virus infection and replication entirely. RNA3 encoded pathogenicity protein P25 is responsible for symptom development and yield reduction and its composition is associated with the resistance response and formation of necrotic lesions in mechanical inoculated leaves of *B. maritima* and therefore suggested to function as an avirulence (Avr) gene product in resistant and pathogenicity factor in susceptible genotypes. In addition previous studies have shown that recently occurring resistance breaking isolates possess increased P25 variability.

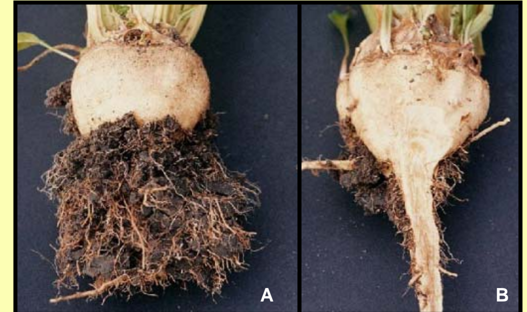

Figure 1: BNYVV symptoms (a) root beard, (b) vein necrosis

MATERIAL & METHODS

In order to identify interacting plant proteins from resistant genotypes which are able to recognize and target P25, a yeast two hybrid (YTH) screen with an *Rz2*-derived cDNA library was carried out. After several de-selection steps of false-positive P25 interactors (i.e. transcriptional autoactivator proteins), database comparison and selection of genes with known functions were identified, resulting in 36 candidates. To confirm the interaction of the putative positive candidates with P25 an *in planta* assay Bimolecular fluorescence complementation (BiFC) was carried out.

RESULTS

This screen identified several candidate proteins, which orthologues from other plant species are well-known to be expressed following pathogen infection and involved in plant defense response (Fig 2.). 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 (Tab. 1.). The interaction of several of the candidate genes with P25 was confirmed in *Nicotiana benthamiana* leaf cells by transient agrobacterium-mediated expression applying the bimolecular fluorescence complementation (BiFC) technique (Fig 3.).

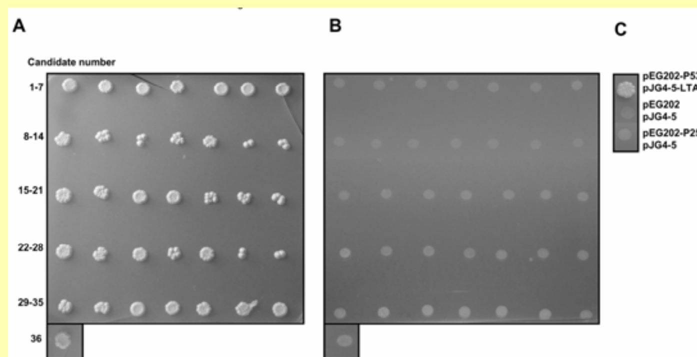

Figure 2: YTH analysis. Interaction of (A) cDNA candidates with P25, (B) cDNA candidates with empty vector, (C) System controls

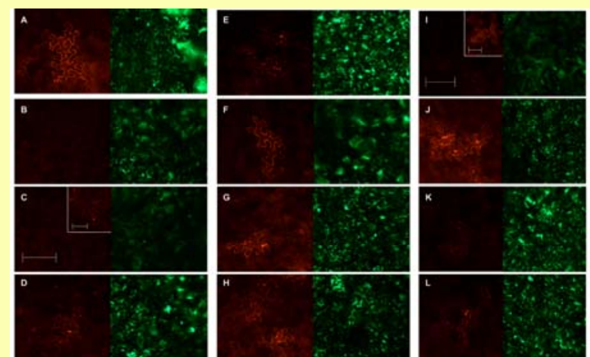

Figure 3: BiFC analysis. (A) positive control, (B) negative control, (C) - (L) cDNA candidates interating with P25

The BiFC *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 36 cDNA clones interacting displayed BiFC *in planta* interaction with P25 (Fig 3.).

Table 1: cDNA candidates identified in the YTH analysis with possible functions and responsibility in plant defense or viral pathogenicity

No.	cDNA length (bp)	Database analysis NCBI Blastn		
		Accession No.	Function (Organism)	e-value
1	1149	NM_126342.3	**F-box** Kelch repeat-containing F-box family protein (*Arabidopsis thaliana*)	$1e^{-85}$
2	508	NM_102054.3	**F-box** Kelch repeat-containing F-box family protein (*A. thaliana*)	$5e^{-36}$
3a	751	EF091878.1	ACRE 276-like protein (*Solanum tuberosum*)	$1e^{-120}$
3b		AY220483.1	Avr9/Cf-9 rapidly elicited protein 276 (ACRE276) (*Nicotiana tabacum*)	$3e^{-109}$
3c		NM_102674.3	**U-box** PUB17 (PLANT U-BOX17); ubiquitin-protein ligase (PUB17) (*A. thaliana*)	$5e^{-74}$
4	311	NM_103837.1	**LRR-Extensin** Leucine rich repeat family protein/extensin family protein (*A. thaliana*)	$5e^{-19}$
5	751	AF123508.1	**Nt-iaa28** deduced protein (*N. tabacum*)	$3e^{-17}$
6a	442	AJ507317.1	**HIP2** Potyviral helper component protease-interacting protein 2 (*hip2* gene) (*S. tuberosum*)	$3e^{-22}$
6b		NM_118840.4	**TOR1** (TORTIFOLIA 1) (*A. thaliana*)	$1e^{-13}$

DISCUSSION

To confirm the P25 interactions detected and investigate its biological significance, full-length cDNA clones of the interesting candidates are required from BNYVV resistant and susceptible sugar beet genotypes. Therefore full-length sequences still need to be identified by genome walking. Subsequent repetition of interaction assays including *in vitro* interaction assay will demonstrate if genotype dependent functional polymorphisms exist. Further the confirmation of the database candidate functions is planned using different functional assays and study of RNA expression levels.