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Characterization of the resistance protein Rz2 and its interaction with the avirulence protein triple gene block I from beet necrotic yellow vein virus.

Beet necrotic yellow vein virus (BNYVV) causes the devastating disease rhizomania in sugar beet. For decades, the disease has been successfully controlled by using the resistance genes *Rz1* and *Rz2*. However, the spread of *Rz1*-resistance breaking populations emphasizes the importance of *Rz2*. The resistance protein encoded by *Rz2* has been identified as a nucleotide binding (NB) and leucine-rich-repeat receptor (Capistrano-Gossmann et al., 2017). In a transient assay in *Nicotiana benthamiana*, Rz2 was shown to recognize BNYVV triple gene block I (TGB1) as the corresponding avirulence protein leading to a hypersensitive response (HR) with cell death (Wetzel et al., 2021). Colocalization experiments and interaction assays were conducted to characterize the interaction. A mutation was introduced into the phosphate binding loop inside the NB domain of Rz2 to abolish the rapid HR for fluorescence visualization. Coexpression of this K201A Rz2 mutant fused to GFP and BNYVV TGB1 fused to mRFP demonstrated that Rz2 and BNYVV TGB1 colocalize in the cytoplasm and nucleus. Manipulation of the subcellular localization by fusion of Rz2 and BNYVV TGB1 with a nuclear localization signal (NLS) resulted in a greatly reduced HR, whereas fusion of both with a nuclear export signal (NES) did not affect the HR, suggesting that the cytoplasmic distribution of Rz2 and BNYVV TGB1 is important for Rz2 mediated resistance. In a yeast-two hybrid (Y2H) assay, no direct physical interaction between Rz2 and BNYVV TGB1 was detected. Similar to Y2H, bimolecular fluorescence complementation (BiFC) with the K201A Rz2 mutant failed to show a direct interaction *in planta*. Missing evidence for a direct interaction suggests an indirect interaction with a conserved host protein present in sugar beet as well as in *N. benthamiana*. Proximity labeling coupled with mass spectrometry will help to identify a potential intermediate host protein as well as other unknown interaction partners.