Characterization of a cDNA full-length clone derived from a beet necrotic yellow vein virus population in Pithiviers (FR)

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Introduction: The A-type of the beet necrotic yellow vein virus (BNYVV) is widely distributed in Europe and one of the major virus types causing rhizomania disease in sugar beet. The closely related P type is mainly limited to a small region in France (Pithiviers). Both virus types possess four RNAs (RNA1-4), but the P-type harbors an additional fifth RNA species (RNA5). The P-type is associated with stronger disease symptoms and resistance-breaking of *Rz1*, one of the two resistance genes which are used to control BNYVV infection. These characteristics are presumably due to the presence of RNA5, but experimental evidence is missing. We generated the first infectious cDNA clone of BNYVV P-type to study its pathogenicity in sugar beet in comparison to a previously developed A-type clone.

Material and Methods: The P-type clone generated in this study was isolated from a BNYVV population collected in Pithiviers (FR). All five RNA components (RNA1-5) were individually cloned into the binary vector pDIVA using Gibson assembly. The infectivity and pathogenicity of the clone was studied in sugar beet using Agrobacterium-mediated infection. A susceptible and an *Rz1* resistant genotype were used for the infection experiments. A previously developed A-type clone was included in the experiments (Laufer et al., 2018). Furthermore, reassortment experiments between both virus types were conducted to study the interchangeability of different RNA components. A double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was applied to measure the viral load of BNYVV in infected lateral roots (DSMZ, Germany, Braunschweig).

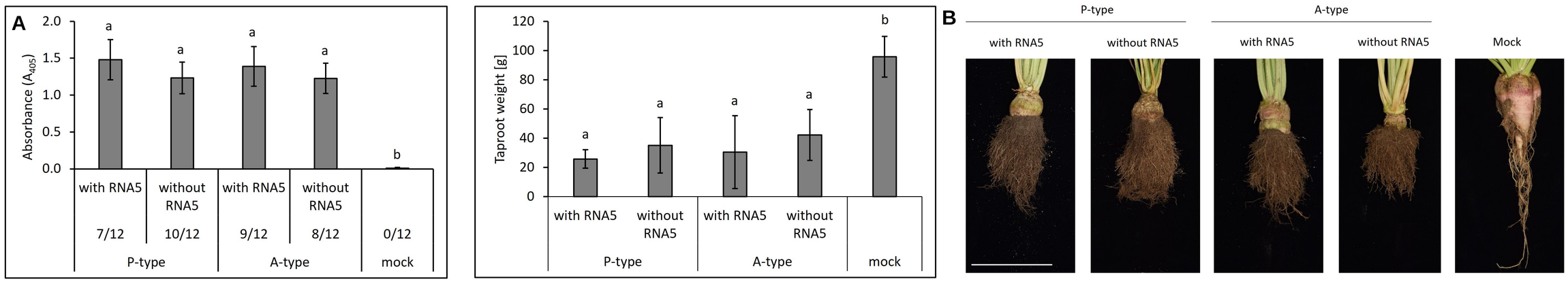

Fig. 1: (A) Mean ELISA absorbance value (A_{405}) and taproot weight of BNYVV inoculated and non-inoculated sugar beet plants. Plants were either inoculated with the A- or P-type in the presence or absence of RNA5 (n=12). Vertical bars indicate standard deviation (SD) and significant differences are indicated as small letters ($p < 0.05$). Only infected samples were used for the mean calculation; the infection rate is indicated in brackets below each bar plot. **(B)** Root phenotype of all variants at 69 dpi. The scale bar represents 10 cm.

Results: After inoculation of the A- and P-type cDNA clones into a susceptible variety, both virus types accumulated a similar virus titer and reduced the taproot weight to the same extend (Fig. 1A). These effects were independent of the presence of RNA5 as there were no statistical differences between all inoculated variants. A strong root beard was observed in all treatments regardless of the virus type and presence of RNA5 (Fig. 1B). To elucidate the effect of RNA5 on resistance breaking, we inoculated a susceptible and *Rz1* resistant genotype with and without the RNA5. The absence of RNA5 had no effect on the infection rate or virus titer in the susceptible variety (Fig. 2). In contrast, both the infection rate and virus titer dropped in the *Rz1* resistant variety when RNA5 was not supplemented to the inoculum. Nevertheless, the P-type was still able to infect the *Rz1* variety to some extent despite the absence of RNA5. Finally, we showed in different reassortment experiments that RNA1, RNA2 and RNA3 can be exchanged between both virus types without losing infectivity (Tab.1). However, the virus titer was lowest when the RNA3 was exchanged in the A- and P-type background.

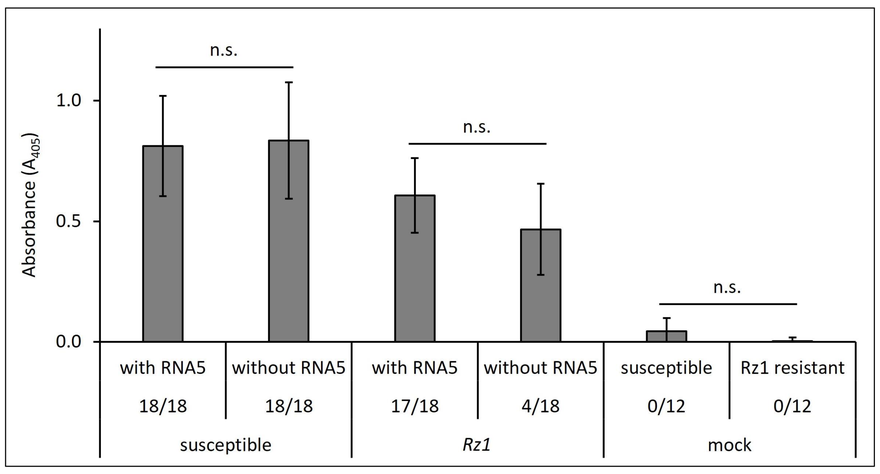

Fig.2: Mean ELISA absorbance value (A_{405}) determined in lateral roots of BNYVV P-type and non-inoculated sugar beets. A susceptible and an *Rz1* resistant variety was inoculated with the P-type with and without RNA5 and lateral roots were harvested after 34 dpi (n=18). Vertical bars indicate standard deviation (SD), horizontal bars indicate which groups were compared ($p < 0.05$). Only infected samples were used for the mean calculation; the infection rate is indicated in brackets below each bar plot.

Conclusion:

Our results showed that both virus types possess a similar pathogenicity after inoculation into a susceptible variety. Furthermore, the P-type clone was able to overcome *Rz1.* We have already shown in a previous study that the A-type clone is not able to infect *Rz1* resistant plants (Liebe et al., 2019). Here, we could show that the RNA5 is crucial for *Rz1* resistance-breaking by the P-type. Finally, the reassortment experiments confirmed the close relationship between both virus types. Therefore, it is very likely that the P-type is originated from an A-type isolate.

Tab.1: Reassortment experiments between the A- and P-type clones. RNA1-3 of both types were exchanged with each RNA from the other type and mechanically inoculated into *B. vulgaris* seedlings by needle inoculation.

Reassortment		Mean A_{405} [a]	SD[b]	Infection rate
P-type background	WT	1.18^{A}	0.17	13/18
	A-type RNA1	1.01^{AB}	0.15	12/18
	A-type RNA2	1.22^{A}	0.25	12/18
	A-type RNA3	0.72^{B}	0.14	13/17
A-type background	WT	0.81^{ABC}	0.20	13/18
	P-type RNA1	0.76^{B}	0.15	16/18
	P-type RNA2	0.52^{CD}	0.07	15/18
	P-type RNA3	0.44^{D}	0.11	15/18
mock		0.01^{E}	0.01	0/12

Literature:

Liebe, S., Wibberg, D., Maiss, E., & Varrelmann, M. (2020). Application of a reverse genetic system for beet necrotic yellow vein virus to study *Rz1* resistance response in sugar beet. Frontiers in plant science, 10, 1703.