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Development of a quantitative Pea Necrotic Yellow Dwarf Virus (PNYDV) screening system for the selection of resistant pea (*Pisum sativum* L.) accessions

Introduction

- Pea necrotic yellow dwarf virus (PNYDV) is an obligate aphid transmitted nanovirus, emerged in Central Europe only recently and leads to substantial yield reduction or even complete loss in highly epidemic years.
- Control of this virus is challenging particularly in organic agriculture where insecticidal treatment against the aphid vector is limited or not allowed.
- The selection and breeding of resistant pea varieties is therefore one of the most promising approach.
- This requires a reproducible quantitative screening method of the virus content of inoculated plants.

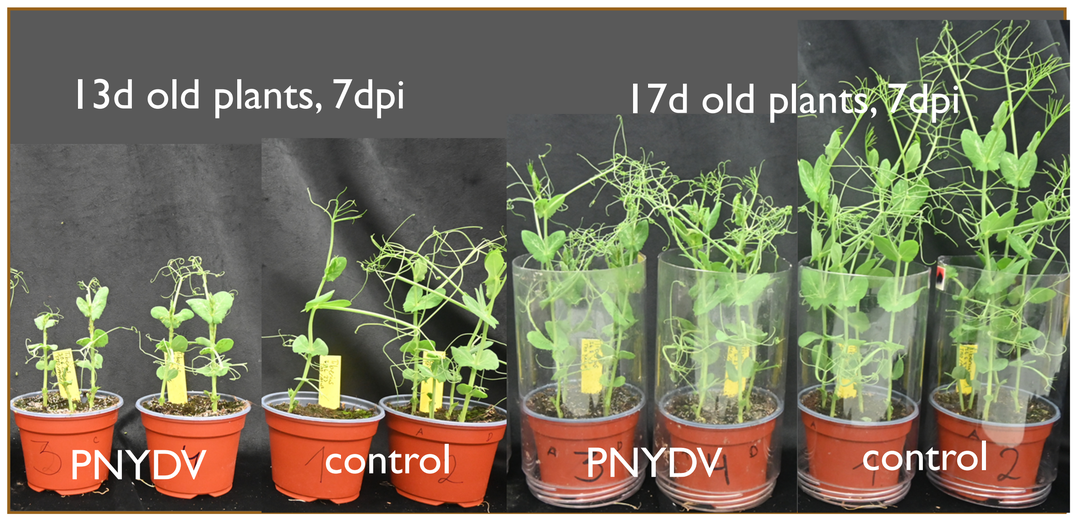

Fig.1: Variety «Tiberius», Left 13d old plants inoculated with PNYDV 6d after sowing. Right 17d old plants, incoculated 10d after sowing.

Methods

- Pea seedlings were PNYDV inoculated by colonizing with 25-30 aphids (*Acyrthosiphum pisum* Harris) per pot carrying PNYDV at 7d and 10d after sowing (4 seeds/pot).
- After 7dpi leaves were sampled in 96-deepwell plates and kept frozen at -20°C.
- DNA was extracted from leaves using the simplified PEX method (Hataya 2021) entirely performed in 96-well deep well plates of 2000µl and 1000µl volume per well, respectively.
- PNYDV was quantified by duplex qPCR in relation to the plant 18S rDNA as „house keeping gene“ and shown as ΔΔCq.

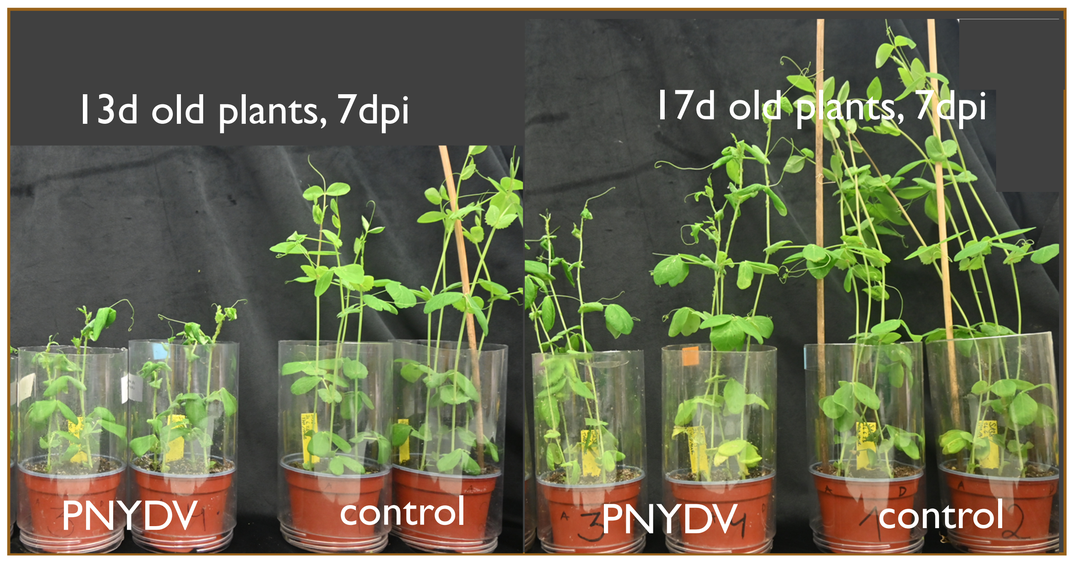

Fig. 2: Variety «S138», Left 13d old plants inoculated with PNYDV 6d after sowing. Right 17d old plants, incoculated 10d after sowing.

Results

- PNYDV symptoms at 7dpi were visually less severe when older plants were inoculated, (Fig. 1+2), but nonetheless after 14-21dpi all plants were entirely necrotic.
- Sampling and DNA extraction with the PEX method was straight forward (4h hands-on time for 4 plates and 384 samples) and gave consistent qPCR results of both PNYDV (coat protein gene) and 18S rDNA house keeping gene.
- No significant differences in the relative PNYDV content could be detected in 20 pea varieties such as shown for the two varieties „Tiberius“ and „S138“ in Fig. 3.

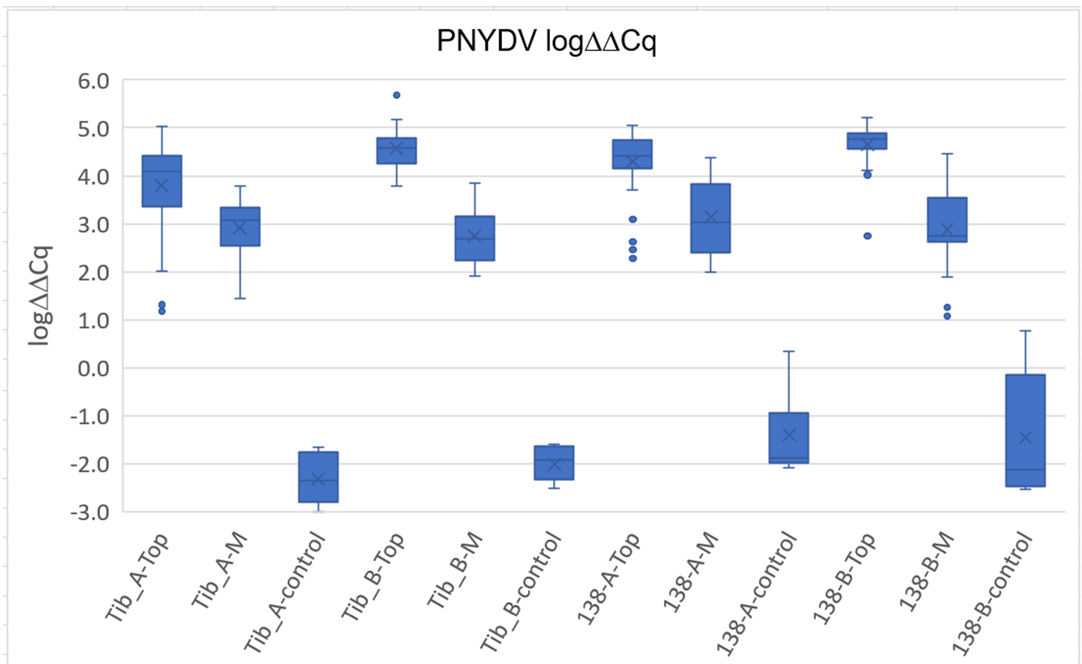

Fig. 3: Relative PYNDV concentration in leaf tissue of the two varieties Tiberius and S138 expressed as logΔΔCq. The relative virus content was 10-100x higher in top leaves (top) than in leaves sampled in the middle of the shoot axis (M) independent of the plant age (A: 13d. B: 17d). Control: non inoculated plants.

Conclusions

- PNYDV is one of the biggest threads for pea production in epidemic years, and all pea varieties tested so far are highly susceptible.
- It is crucial that more pea varieties are tested from different origins to find resistant or at least robust varieties, which can serve as base for future breeding strategies.

References
Hataya, T. 2021. An Improved Method for the Extraction of Nucleic Acids from Plant Tissue without Grinding to Detect Plant Viruses and Viroids. Plants 2021, 10, 2683. https://doi.org/10.3390/plants10122683

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