Development and validation of an analysis method for the detection of altered resistance in transgenic plants to herbivore-pathogen-complexes

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Project objectives

- Examination of potential variation in virus content resulting from genetic manipulation in both transgenic potatoes and insect vectors colonizing them,
- Development of methods for the quantitative detection of two major potato viruses (PVY and PLRV) in potato plants and in vectors transmitting these viruses.

Quantitative detection of two major potato viruses - Potato virus Y (PVY) and Potato leafroll virus (PLRV)

Partners

BTL Bio-Test Labor GmbH Sagerheide was founded in 1993 and is located in Sagerheide (Northern Germany near Rostock). The company has set its priorities on the development of methods and execution of examinations for the registration of pesticides and transgenic plants. BTL develops suitable methods for breeding target and non-target organisms for the determination of impact of pesticides and for studies on resistance or tolerance as well as for behavioral studies.

Pooled expertise focused on the plant – this interdisciplinary approach defines the Julius Kühn Institute (JKI). The JKI is both a research institution and a higher federal authority affiliated with the Federal Ministry of Food, Agriculture and Consumer Protection. The head office is located in Quedlinburg. The JKI is structured into 15 specialized institutes and several service units at 11 locations in Germany. Among the staff of 1200 people there are 300 scientists. Fields of research encompass plant genetics, agronomy, plant nutrition, soil science, plant protection and plant health.

Quantitative detection of PVY

TaqMan® assays were developed for the quantitative detection of PVY in general and by applying different fluorophores for the discrimination of PVY® and PVYNTH on the one hand, and PVYN and PVYNW on the other hand. RNA-standards were produced as in vitro RNA transcripts. The limit of quantification amounts to 100 virus particles for the general assay (see Fig. 3), and to 1.000 and 10 virus particles for the PVYNTH and PVYNW assays, respectively.

Pre-test for examination of the virus distribution in the plant as well as the influence of cultivar and temperature on the number of virus particles

For the evaluation of a possible change in virus resistance in genetically modified plants (GMPs) in comparison to non-GMPs, it is extremely important to standardize the sampling. As shown in Fig. 4A and 4B, the number of virus particles varies considerably, depending on temperature, cultivar and part of the plant sampled. The virus content changes also by the number of days past inoculation: The virus concentration rises with time in cultivar Hermes, which is highly susceptible to PVY, and decreases in cultivar Princess, which is hardly susceptible to PVY (results not shown).

Extraction method

PLRV and PVY represent the two most important potato viruses, containing RNA as genetic material. For their detection a suitable extraction method is needed. RNA extraction is time-consuming and costly. Therefore, a low-cost, high-throughput (see Fig. 1) and fast sample preparation method was developed without the need for any toxic compounds - the immuno-capture (IC) technique. Furthermore, it allows only the detection of complete virus particles, so that for PLRV, producing subgenomic RNAs, the virus titer will not be overestimated.

Fig.1 ELISA microtiter plate with pierced wells for a flush support of the antibody-coated PCR plate fixed in whole on a Dynex® Washet.

Fig.2 RT-qPCR of RNA-standards based on a PLRV coat protein sequence in a 10⁻¹⁰ dilution series amplified with primers and probes for the detection of PLRV.

Fig.3 RT-qPCR of RNA-standards based on a PVY® strain sequence in a 10⁻¹⁰ dilution series amplified with primers and probes for the detection of all PVY strains.

Fig.4 Influence of cultivar, temperature and sampling position on virus titer in potato plants. Plants were mechanically inoculated with PVY, maintained at the indicated temperature and 16 hours illumination with 10,000 lx. Sampling was accomplished 7 dpi. A cv Princess; B cv Hermes. P < 0.05 (U-test; Wilcoxon-test)