Development and validation of a method for the detection of altered resistance in transgenic plants against herbivore-pathogen-complexes

N. Drechsler^{1*}, A. Habekuß², T. Thieme¹, J. Schubert³

¹ BTL Bio-Test Labor Sagerheide GmbH, * supported by a grant of BMBF (03WKBN05A)

² JKI Institute for Resistance Research and Stress Tolerance

³ JKI Institute for Biosafety of Genetically Modified Plants

Project objectives

- Development of methods for the quantitative detection of two cereal viruses in plants and their vectors
- Examination of potential variation in virus content of genetically manipulated cereals and the insect vectors colonizing them

Detection and quantification of two cereal viruses: Wheat dwarf virus (WDV) and Barley yellow dwarf virus (BYDV)

Partners

BTL Bio-Test Labor GmbH Sagerheide was founded in 1993 and is located at Sagerheide (Northern Germany near Rostock). The company develops methods and carries out the assessments necessary for the registration of pesticides and transgenic plants. BTL develops suitable methods for breeding target and non-target organisms for assessing the effectiveness of pesticides and for behavioural 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.

Test species

Wheat dwarf virus (Geminiviridae) is a circular single-stranded DNA virus located in the phloem of the plant (Fig. 1). It is transmitted by the leafhopper *Psammotettix alienus* Dahlb. in a persistent manner.

The *Barley yellow dwarf viruses (Luteoviridae)* are a complex of different viral species distinguished by their vector species and sequences. BYDVs are linear single-stranded RNA-viruses located in the phloem. In Germany the strain PAV prevails. It is transmitted by the aphids *Rhopalosiphum padi* L. and *Sitobion avenae* F. in a persistent manner.

Fig. 1: WDV infected plant
Both viruses cause similar symptoms: dwarfing, stunting and leaf discoloration leading to yield losses or death of plant.

Extraction methods

Extracting DNA or RNA for the detection of viruses by quantitative PCR (qPCR) with conventional methods is time-consuming, expensive and involves the use of toxic reagents. An approach to reduce working time and cost is the use of immunocapture(IC) for the specific detection of the viruses. To perform this procedure in a 96 well format the method was adapted to the use of PCR plates coated with antibodies. All washing steps were performed in an ELISA washer. This speeds up the procedure and allows the sampling of large numbers of plants necessary to obtain reliable results.

qPCR

qPCR assays with specific primers were designed for the detection and quantification of the viruses. Both Sybr® Green as well as TaqMan® probe assays were developed. They are well suited to quantify virus content in plants and vectors (for examples see Fig. 2 and 3).

Fig. 2: Detection of WDV in *P. alienus* by qPCR with SybrGreen.

Red: plasmid standard ranging from 10^8 to 10^3 copies, blue: test samples, green: negative control, yellow: no template control (NTC).

Efficiency: 0.98, slope: -3.372, y-intercept: 36.09, R^2 : 0.997.

Fig. 3: Detection of BYDV-PAV in plants by IC-RT-qPCR with a TaqMan® FAM probe.

Black: standard dilutions ranging from 10^7 to 10^2 copies, blue: PAV-infected plants, pink: NTC

Efficiency: 0.98, slope:-3.379, y-Intercept: 38.72, R^2 : 0.999.

To determine the sensitivity of the qPCR it was compared to DAS-ELISA. Serial dilutions of sap from WDV-infected plants and healthy plants were subjected to ELISA and DNA extraction followed by qPCR. The ELISA reached its detection threshold at dilutions of 10^{-4} whereas with the qPCR it was possible to detect the virus even at dilutions of 10^{-8} (Fig. 4). Similar tests were done with BYDV, where the ELISA reached its detection threshold earlier due to the lower virus content of the plants.

WDV	ELISA					qPCR				
	A	B	C	D	E	A	B	C	D	E
dilution										
pure sap										
1:10										
1:100										
1:1000										
1:10000										
1:100000										
1:1000000										
1:10000000										

Fig. 4: Comparison of the sensitivity of DAS-ELISA and qPCR for detection of WDV (left) and BYDV-PAV (right)

A-D/E: different plants, red: positive signal, green: negative signal, yellow: positive signal after 2 hours incubation.

Outlook

The methods will be used to determine the virus content of several transgenic wheat lines and their near-isogenic lines, other conventional cultivars and the herbivores colonizing them.