Detection and quantification of cereal geminiviruses

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*supported by a grant of BMBF, FKZ 03WKBN05A
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3rd Young Scientists Meeting 2010

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Cereal geminiviruses

*Wheat dwarf virus*, *Barley dwarf virus*, *Oat dwarf virus*, *(WDV, BDV, ODV)*, transmitted by the leafhopper *Psammotettix alienus* Dahlb. in a persistent manner.
Detection with qPCR: absolute quantification

qPCR with SybrGreen, WDV-standard dilution series: $3 \times 10^8$ to $3 \times 10^2$ copies
blue: plasmid standard mixed with negative plant DNA (30 ng), red: plasmid standard without plant DNA, yellow: NTC, black: negative plant DNA.
TaqMan-Probes - differentiation between BDV and WDV

BDV-specific FAM-probe ($\lambda_{\text{max}}$520nm)

WDV-specific Yakima Yellow-probe ($\lambda_{\text{max}}$549nm)
Comparison of sensitivity of DAS-ELISA and qPCR

WDV- positive Greenhouse plants (A-E), 8 weeks p. i., sap diluted with sap of WDV-negative plants
Field plants

winter barley, early June

photo: Nadine Drechsler

Field plants

Pearson correlation: 0.932

Spearman correlation: 0.592

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Virus titer and symptom expression

Rubina, 16 weeks past inoculation with *Psammatettix alienus*

- 6.3 x 10^8 copies
- 1.3 x 10^8 copies
- Negative

photo: Nadine Drechsler
Distribution of WDV in the plant

Winter barley 'Rubina', grown under greenhouse conditions, inoculated with 3 leafhoppers for 1 week, leaf tips were harvested every week, after 4 weeks harvesting of whole plant.
Detection of WDV in the vector *Psammotettix alienus*

persistent transmission, non-propagative

Hougenhout et al. 2008, modified

top: *P. alienus* stored in ethanol
bottom: *P. alienus* stored at -80°
n=64, winter barley 'Rubina' inoculated with one viruliferous leafhopper for 7 days, afterwards analysis of leafhoppers by qPCR, plants were tested for infection after 4 weeks, red – WDV-positive plant, green – WDV-negative plant (testet with qPCR, viral load of plants not plotted).
Summary and outlook

-qPCR assays were developed which allow sensitive and specific detection and quantification of cereal geminiviruses

**Application of the method:**

- Monitoring the spatial and chronological development of viral infection of the plant
- Investigations of transmission behaviour of the vector
- Comparisons of virus titre of transgenic and non-transgenic wheat lines (mildew-resistant wheat developed at the University of Zürich)
Thanks to

- Gudrun Meißner, Bärbel Apel and Martina Nielitz
- Dr. Edgar Schliephake
- Greenhouse staff of JKI Quedlinburg
- BMBF for financial support

and for your attention.