Development of a one-step multiplex RT-PCR and Luminex xTAG assay for the simultaneous detection of yellowing viruses infecting sugar beet

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Background

Yellowing viruses are an increasing threat to sugar beet cultivation, due to limitations on insecticide usage and climate change (1). Virus detection, monitoring and resistance breeding are key to secure high sugar beet yields in the future. Thus, validated and specific detection methods are needed. For this, a multiplex RT-PCR method was designed to detect all yellowing viruses prevalent in Europe. In addition, Luminex xTAG array Technology was integrated as a follow-up method to increase assay speed and specificity.

The method detects the well-known beet-infecting viruses: beet mild yellowing virus (BMYV), beet chlorosis virus (BChV) and beet yellows virus (BYV) as well as beet mosaic virus (BtMV), which is sporadically detected throughout Europe (2), and turnip yellows virus (TuYV). TuYV was recently detected multiple times in sugar beet in The Netherlands (3), Sweden (4) and Australia (5). Also, Newbert described a beet-infecting TuYV isolate in his thesis (6). Although TuYV is not a problem in sugar beet yet, it might be good to monitor this virus in the future.

Objectives

- Design a one-step multiplex RT-PCR method for the detection of all yellowing viruses prevalent within Europe.

- Investigate the potential of Luminex xTAG Array Technology as a follow-up method to increase speed and virus specificity.

Method

PCR protocol

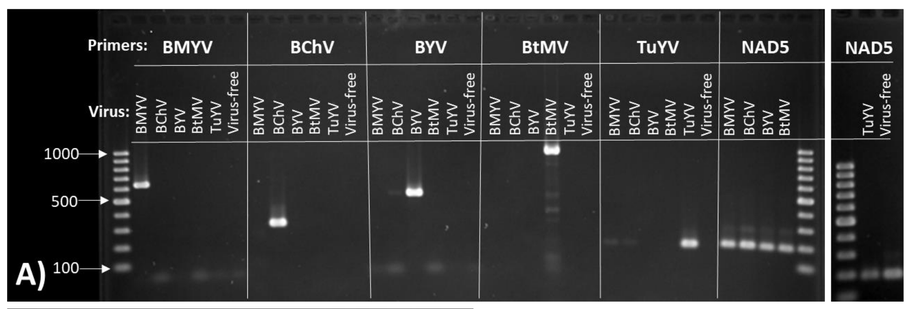
The mRT-PCR protocol was as follows: 30 minutes at 50°C for reverse transcription of the RNA template, followed by 15 min at 95°C for initial denaturation and subsequently, 37 cycles of 30 sec at 94°C, 30 s at 56°C and 1 min at 72°C; and a final step of 10 min at 72°C.

Table 1: Primer sets for the one-step multiplex RT-PCR protocol

Virus species	Target	Product size	Direction	Sequences (5'-3')
BMYV	ORF1	632	Forward Reverse	GTATTCGTTCTCTTCTCGCT CGGGCTTTGTTTGATGGTA
BChV	ORF1	260	Forward Reverse	GACACCAAGTTGAACAGT CATTGATGCTAGTTGTGGC
BYV	ORF1	491	Forward Reverse	CGAGGCGGCAAATTAAGT GTTGTCTTTGTGAATGCTG
BtMV	P3*	834	Forward Reverse	GGAATGGCGAGGTTTAAG CTTGTCCCACCACTTCTC
TuYV	ORF1	142	Forward Reverse	TGGAGCTCGCTAATCTTG GGCAATCCTCCAAAAAGAA

Results

Five virus-specific primer sets (***Table 1***) were tested as single and mixed primer sets on single and mixed virus infections (***Figure 1***). Subsequently, the primers were validated on 22 field samples with single and mixed virus infections and since then used by default for virus detection in beet during this PhD project.



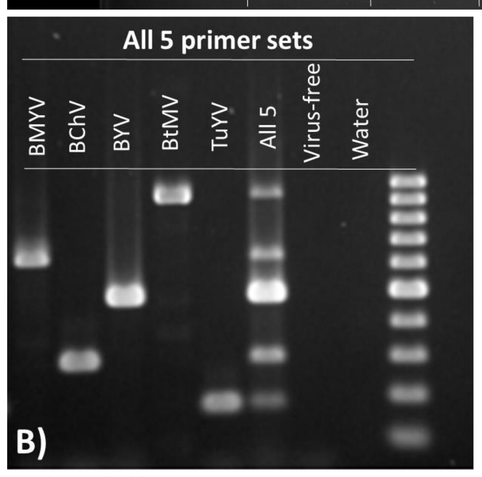


Figure 1: Detection of 5 beet yellowing viruses in a one-step multiplex RT-PCR method. A) Single primers in combination with single infections. B) all five primer sets in combination with single and mixed infections.

The follow-up xTAG assay worked as expected in a simplex (***Table 2***) and multiplex setting, meaning all viruses were detected. Optimization steps would be recommended, regarding BYV detection, as a high background signal was sometimes observed (results not shown here).

Table 2: Median Fluorescent intensity (MFI) values of single infected plant material. The bead address numbers are given in the top row.

Virus	BMYV #51	BChV #34	BYV #72	BtMV #53	TuYV #35
NC	64	103	63	95	93
BYV	51	81	**1526**	85	83
BMYV	**5751**	85	59	77	83
TuYV	49	83	59	82	**1343**
BtMV	67	97	75	**4615**	104
BChV	338	**5758**	62	84	90
Threshold value	701.9	121.4	91.4	119.3	122.3

Conclusions

- The multiplex RT-PCR works as expected and yellowing viruses can be observed via gel-electrophoresis as bands of a different height.
- In the future, the Luminex xTAG assay would be an excellent method for the detection of beet yellowing viruses due to the high specificity and the potential to increase the number of targets in a true multiplex setting, including plant reference genes, generic virus primers or additional primer sets to limit the risks of missing mutants.

References

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Acknowledgements

This project is a research collaboration between Wageningen University, IRS, SESVanderHave, TKI, Cosun Beet Company, and The British Beet Research Organization and received financial support from Topsector Tuinbouw & Uitgangsmaterialen (TKI-T&U 18153)

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