nak tuinbouw

Detection of tobamoviruses in seed lots of tomato and pepper using Illumina High Throughput Sequencing (HTS)

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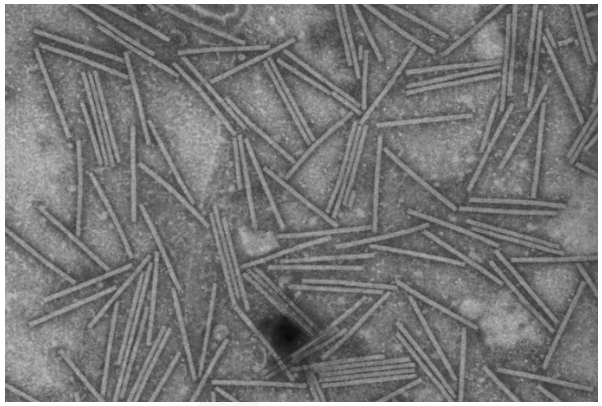
Tobamoviruses

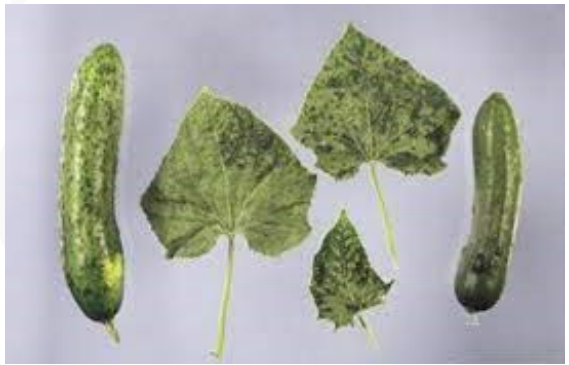


- First description of TMV in 1882
  - 17 species 1999
  - About 70 species today

- Very infectious and present in high concentrations
- Very stable outside the plant
- High concentrations in fruit pulp

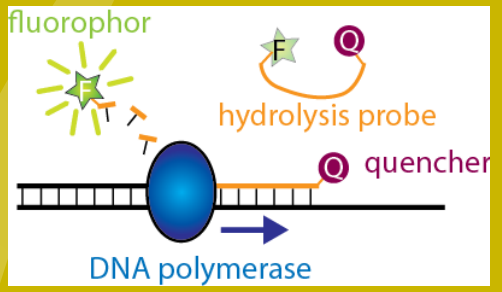
- Present on the seed coat and in many cases penetrates into the seed coat as well

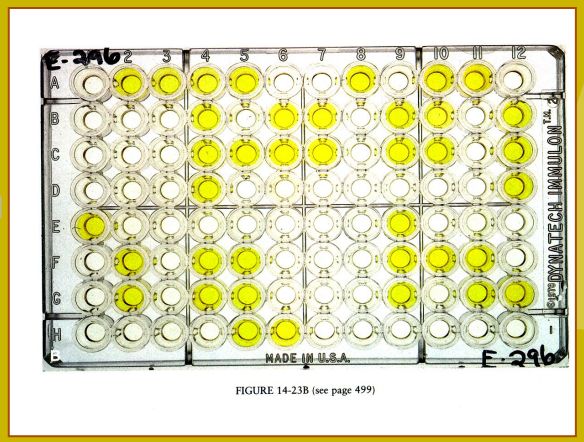
- Seed disinfection and/ or heat treatment do have effect, but not up to 100%

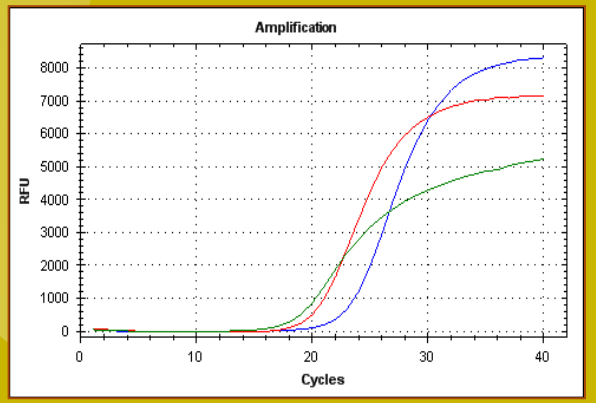


Current validated detection methods

Seed extraction







Bio-Assay

DAS-ELISA

RT-qPCR

Tobamo HTS project

Scope

- Analyzing seed samples from Naktuinbouw's seed collection
- Comparison of HTS, TaqMan PCR and Bio-assay
- Focus on Tobamoviruses, with special attention to the "new" species; ToBRFV and ToMMV
- Seed batches: 72 tomato, 24 pepper

PERFORMANCE FEATURES	SEED HEALT TESTING FORMAT			
	BIOASSAY	**ELISA**	**qPCR**	**HTS**
SENSITIVITY	LOW	MEDIUM	VERY HIGH	?
SPECIFICITY	LOW	LOW	HIGH	?
SPECTRUM	BROAD	BROAD	NARROW	?
INFECTIVITY*	YES	NO	NO	?
SPEED	7 DAYS	2 DAYS	1 DAY	?

*Evaluate seed treatment

Background

- Naktuinbouw has a “seed bank (ZZB) that dates from 1992 and contains characterised seed by ELISA and Bio-assays

- Q status for tomato brown rugose fruit virus (ToBRFV) and the question marks with tomato mottle mosaic virus (ToMMV). Question to be answered is ToMMV a new virus??

- Ever growing use of very sensitive testing methods such as SE-qPCR

  ~ BVS2 generic SE-qPCR

  ~ Rainbow SE-qPCR (RijkZwaan)

  ~ ISHI-Veg protocol ToBRFV (triplex TaqMan qPCR)

Approach (pilot)

- Selection of seed batches by Naktuinbouw in collaboration with vegetable breeding companies

- Pilot experiment with 6 seed samples (3 tomato & 3 pepper)
  - RNA extraction 1000 seed (samples spiked with IIC BaCV) and TaqMan PCR's (generic Tobamo (BVS2) and ToBRFV) ran
  - Bio-assay, 4x 250 seed using standard protocol
  - HTS (Ilumina NovaSeq6000, pe 150bp, incl. DNAse treatment and rRNA depletion)
  - Analysis HTS data (CLC) (stringent and single reads in pairs)
  - Evaluation of data obtained from the three different detection methods

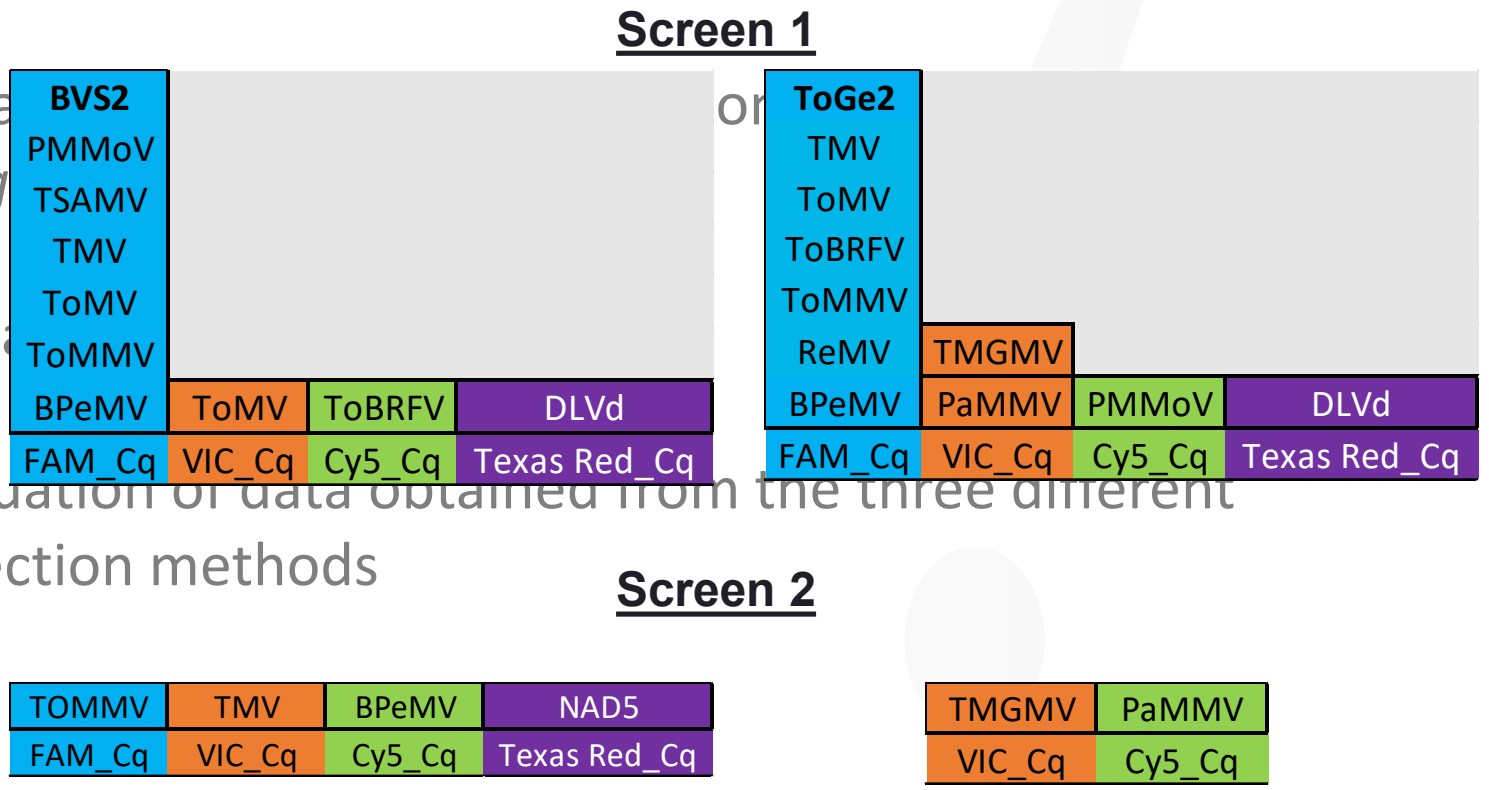
Results HTS pilot study

		number of reads per virus									
seedsample	#trimmed reads (in pairs)	CGMMV	PaMMV	PMMoV	ReMV	TMV	TMGMV	ToBRFV	ToMMV	ToMV	Other Tobamovirusses
tomato 1	112,423,168	-	12	10	28	24	8	-	1,962	73,965,964	-
tomato 2	153,340,530	-	-	32	-	428	-	-	47,765,292	16,226,394	-
tomato 3	94,781,372	-	-	2	-	-	-	-	410	20,580	-
pepper 1	105,769,778	-	-	62,690	-	-	-	-	2	542	-
pepper 1	77,273,370	-	-	144,760	-	988	16	-	-	610	-
pepper 1	73,549,410	-	-	18	-	-	10	-	36	126	-

Seed sample	**result bio-assay**
Tomato 1	Very weakly positive
Tomato 2	Positive
Tomato 3	Negative
Pepper 1	Negative
Pepper 2	Positive
Pepper 3	Negative

Approach

- Pilot experiment was a success
- Rest of the seed batches extracts prepared (90 batches)
- Extension of TaqMan assays with the Rainbow PCR
- Bio-a… or… (*N. g*…
- HTS …
- Evaluation of data obtained from the three different detection methods



Results SE RT-PCR tomato

Discussion on threshold setting - what is still biologically relevant?

Threshold Cq < 30

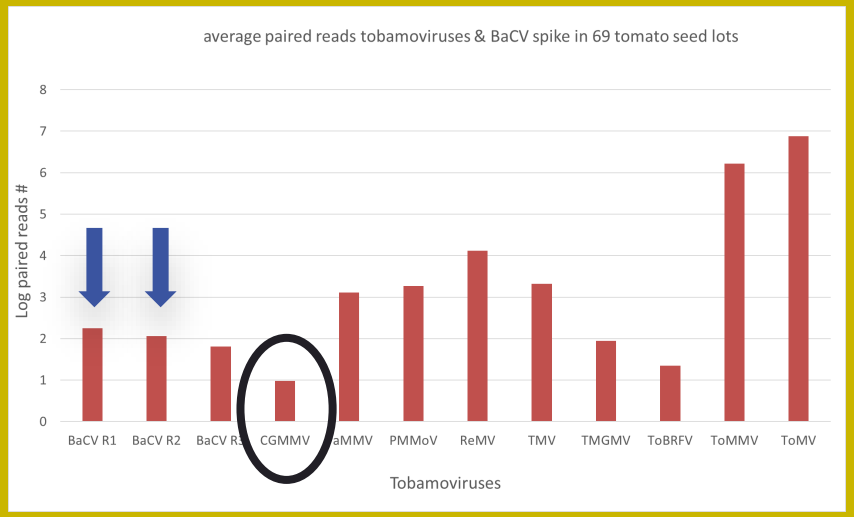
- 21/109 negative seed lots
- 88/109 positive seed lots with 170 tobamoviruses

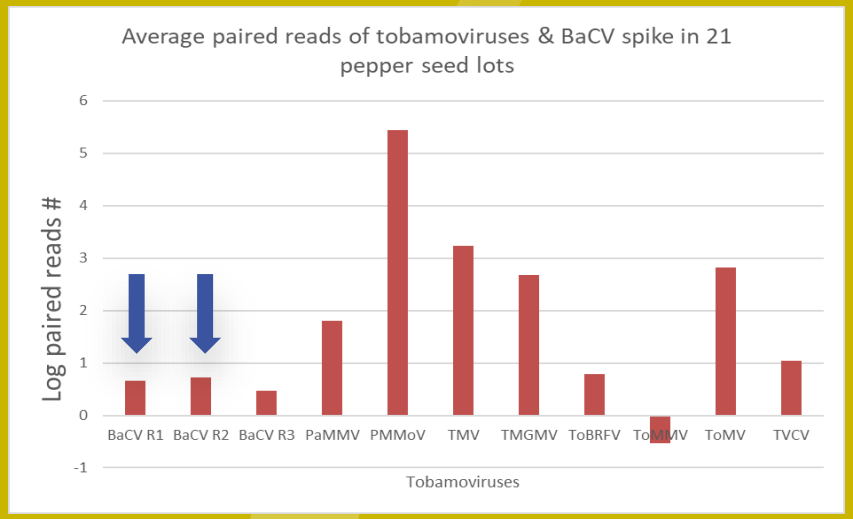
Threshold Cq < 35

- 3/109 negative seed lots
- 106/109 positive seed lots with 304 tobamoviruses

Results HTS

- Tomato seed batches: 68 tobamo positive, 1 negative
- Pepper seed batches: 18 tobamo positive, 3 negative



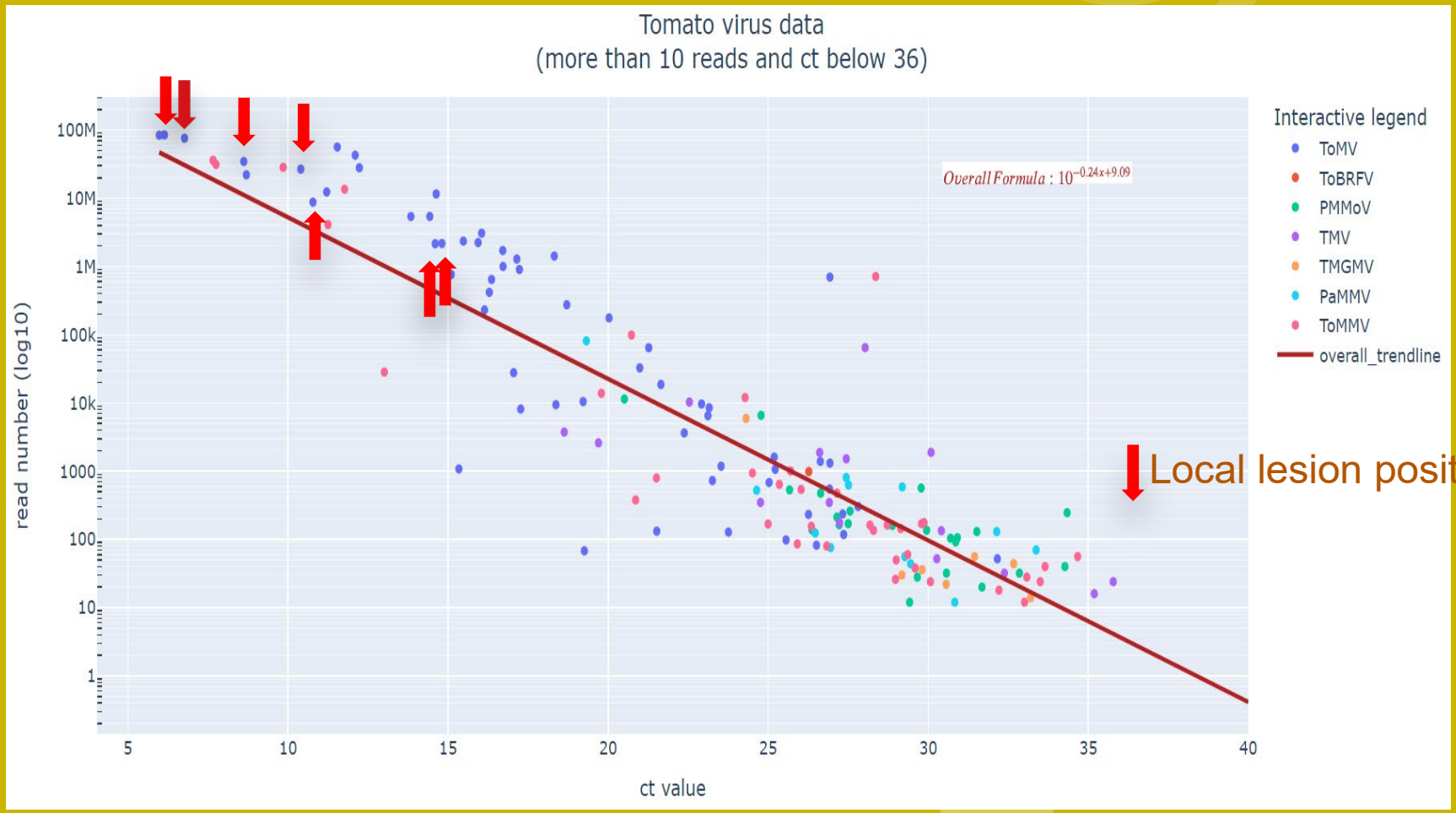


Average number of reads of known tobamoviruses in tomato samples (left) and pepper samples (right). BaCV is internal control (spike).

Inhibition in PCR observed in the pepper matrix.

CGMMV contamination no host for tomato and pepper

Results HTS Illumina & SE TaqMan tomato



Conclusions

- Illumina HTS sequencing detect many tobamo viruses on seeds
- SE PCR even more!
- Majority low virus load but some very high load
- ToMMV widely spread and present on many tomato seed lots
- What are biologically relevant decision thresholds for SE TaqMan and HTS Illumina?

  Bioassay positives > 10^6 read and Cq < 20
- Pepper seed matrix issues with recovery of the BaCV spike in both PCR and HTS
- The high sensitivity of SE-PCR makes it easy to pick up environmental signals (e.g. CGMMV in tomato seed samples), this was also visible when testing with HTS

Acknowledgements

And all other companies who provided seeds used in this study.