


**Technical Working Party for Vegetables****TWV/53/7 Add.****Fifty-Third Session  
Seoul, Republic of Korea, May 20 to 24, 2019****Original:** English  
**Date:** May 29, 2019

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**ADDENDUM TO  
PARTIAL REVISION OF THE TEST GUIDELINES FOR TOMATO***Document prepared by an expert from the Netherlands**Disclaimer: this document does not represent UPOV policies or guidance*


The annex to this document contains a copy of a presentation on the “I2 marker and resistance to Fol – UPOV status May 21, 2019” by an expert from the Netherlands, made at the fifty-third session of the Technical Working Party for Vegetables (TWV).

[Annex follows]




## I2 marker and resistance to Fol - UPOV status May 21, 2019 -

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TWV 53, Seoul, 2019



## DNA markers are a useful tool


Reliable and efficient DUS test





New genetics, lower levels of resistance, more difficult to assess Uniformity


Useful tool: DNA marker test

- In case of variable phenotype, genotype may be conclusive:  
Example: some plants with strong symptoms of the disease, other plants healthy -> in DNA marker assay all plants resistant -> conclusion Uniform  
Can be more efficient than testing bigger plants.

	<h2 style="text-align: center;">Decision rules</h2>
	<p>Clear decision rule needed for each characteristic/bio-assay/marker assay: Example: “if both homozygous resistant and heterozygous resistant plants occur, this is <u>not</u> a lack of Uniformity, as both genotypes confer to phenotypical resistance.” Example: “if the number of off-types in the DNA marker assay is more than allowed, the variety lacks Uniformity.” or “if the number of off-types in the DNA marker assay is more than allowed, the bio-assay is conclusive for the Uniformity.”</p>

	<h2 style="text-align: center;">Marker validation</h2>
	<p>Good correlation between genotype and phenotype: a reliable link</p> <ol style="list-style-type: none"><li>1. Validation of the SNP</li><li>2. Validation of the method</li></ol>
	<p style="text-align: right;">Fits well in INF/17, is being revised by BMT</p>

	<h2 data-bbox="607 296 1138 342">1. Validation of the SNP</h2>
	<p data-bbox="323 380 565 407">100% correlation</p> <ul data-bbox="323 420 1422 562" style="list-style-type: none"><li data-bbox="323 420 643 447">- All example varieties</li><li data-bbox="323 457 618 485">- All control varieties</li><li data-bbox="323 495 1422 562">- A large number of varieties, covering all types in the crop and a large part of the applicants</li></ul> <p data-bbox="323 611 428 638">In 1 lab</p> <p data-bbox="323 648 857 676">Other labs may ask for validation data</p> <p data-bbox="323 686 1117 714">Strong doubt on one or more varieties: test by more labs</p>

	<h2 data-bbox="566 1205 1175 1251">2. Validation of the method</h2>
	<p data-bbox="323 1289 618 1316">Many relevant steps:</p> <p data-bbox="323 1327 1409 1436">Harvest leafmaterial, DNA extraction, DNA concentration, purity, PCR machine and its specifications, PCR program, visualisation in a graph or on a gel</p> <p data-bbox="323 1484 1336 1551">All steps to be examined in each lab and may need optimization (time, money)</p> <p data-bbox="323 1600 1008 1627">Cooperation by offering assistance in test phase.</p> <p data-bbox="323 1675 1266 1703">Lack of reproducibility in a number of labs: postpone final proposal.</p>


## I2 marker for resistance to Fol: 0 and Fol: 1 - current status (1) -

- Efficient? Yes!
  - Normally faster and cheaper than bio-assay
  - Bio-assay more and more difficult to assess due to interaction between Fusarium and tomato plants (penetrance, modifiers)
  - Even to be improved: I3 marker validated at Naktuinbouw for tomato, I marker to be developed.
- Decision rule? “as both homozygous resistance and heterozygous resistance give a resistant phenotype, both may occur in one variety.”  
To be put more clearly in the protocol?
- SNP validated? Yes!
  - By Naktuinbouw, on examples, control varieties, prox. 200 varieties

## I2 marker for resistance to Fol: 0 and Fol: 1 - current status (2) -

- Method validated?
  - Yes, at Naktuinbouw
  - No, at other labs
  - Conclusion: no

Variety	lab A			lab B			lab C		
	Homo I2	Hete I2	Homo S	Homo I2	Hete I2	Homo S	Homo I2	Hete I2	Homo S
Cherry type control 2 = H	20			1	8	1	4		
H	13	7					4		

	<h2>I2 marker for resistance to Fol: 0 and Fol: 1 - how to proceed -</h2>
	<ul style="list-style-type: none"><li>• Naktuinbouw offers assistance to optimize result in other labs</li><li>• Postpone the addition as alternative method until 2020</li></ul>