

Technical Working Party for Vegetables

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ADDENDUM TO PARTIAL REVISION OF THE TEST GUIDELINES FOR TOMATO

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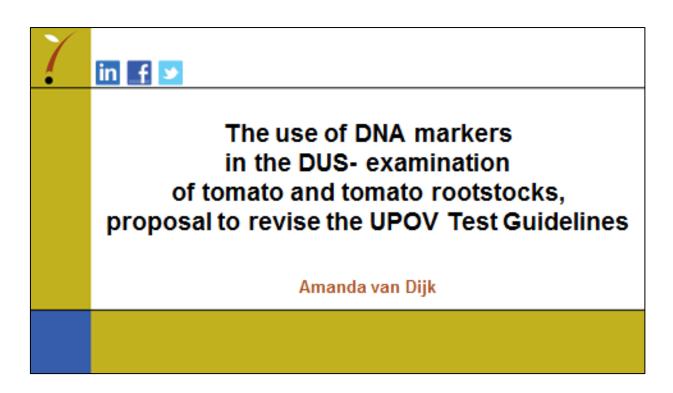
The annex to this document contains a copy of the presentation made at the fifty-first session of the Technical Working Party for Vegetables, by Ms. Amanda van Dijk (Netherlands) on "The use of DNA markers in the DUS of tomato and tomato rootstocks, proposal to revise the UPOV Test Guidelines".

[Annex follows]

ANNEX

THE USE OF DNA MARKERS IN THE DUS- EXAMINATION OF TOMATO EN TOMATO ROOTSTOCKS, PROPOSAL TO REVISE THE UPOV TEST GUIDELINES

BY AN EXPERT FROM THE NETHERLANDS





UPOV and DNA markers

TGP/15/1

- 2.1 Characteristic-Specific Molecular Markers (see Annex 1)
- 2.1.1 Molecular markers can be used as a method of examining DUS characteristics that satisfy the criteria for characteristics set out in the General Introduction, Chapter 4, section 4.2, on the following basis:
 - (a) the test for the marker is conducted on the <u>same number of individual plants</u>, with the <u>same criteria for distinctness</u>, <u>uniformity and stability</u> as for the examination of the characteristic by a bioassay;
 - (b) there is verification of the reliability of the link between the marker and the characteristic;
 - (c) different markers for the same characteristic are different methods for examining the same characteristic:
 - (d) markers linked to different genes conferring expression of the same characteristic are different methods for examining the same characteristic; and
 - (e) markers linked to different regulatory elements for the same gene conferring expression of the same characteristic are different methods for examining the same characteristic: 2.1.2 Annex I to this document "Gene Specific Marker for Herbicide Tolerance" provides an example of the use of characteristicspecific molecular markers.



Fusarium race 0 and 1

Important elements of the proposal (1):

- Test with marker for gene I2.
- Marker is in the protein coding sequence of the gene, so the necessary link is present. Naktuinbouw validated this in more than 120 varieties: haven't seen any example of a broken link between the proposed marker and the phenotype.
- Resistance to both race 0 and 1 is most often caused by this gene I2. Some varieties with this resistance do not have I2, but I3. Also gene I7 gives this result.
- The marker is co-dominant, meaning that absence of I2 can be observed.



Fusarium race 0 and 1

Important elements of the proposal (2):

- 20 plants per variety, as in bio-assay.
- The bio-assay is still possible: Resistance to race 0 (ex 1) and race 1 (ex 2) to be tested in a bio-assay (method i) and/or in a DNA marker test (method ii).
- The basis is the claim of susceptibility or of resistance by the breeder in the TQ: in case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the resistance is absent or present for the variety (on another mechanism).



Fusarium race 0 and 1

Claim TQ	Resistance to 0, susceptible to 1	Resistant to 0 and 1	Resistant to 0, 1 and 2
	Several varieties (older/determinate/special fruit types)	Large majority of varieties	A few varieties known
DNA marker I2: Homozygous susceptible allele	Probably not gene 12, but I: additional bio- assay for 0	contradiction: bio- assay	Probably gene I3 or I7: additional bio-assays
DNA marker I2: Heterozygous or homozygous resistant allele	Contradiction for race 1: bio-assay needed for 1	Agree	Race 0 en 1 agreed, for race 2 a bio-assay needed



Fusarium race 0 and 1: experience

In 2016/7 for prox. 20 candidates used in cases where resistance has been claimed, but some plants show symptoms. The genetic background of the plants with symptoms was checked:

- All plants with I2: uniformity sufficient
- Genetically not uniform, as in the bio-assay: uniformity insufficient

In future a standard check on the presence of I2: if genetically not uniform -> contradiction with claim in TQ -> bio-assay needed to conclude.



ToMV strain 0, 1 and 2

Important elements of the proposal (1):

- Test with marker for gene Tm2/2².
- Marker is in the protein coding sequence of the gene, so the necessary link is present. This has been validated by Naktuinbouw, also in Harmores.
- Resistance to strain 0, 1 and 2 is normally caused by gene Tm2². Some varieties have gene Tm2, which gives resistance tot strain 0 and 1 only.
- The marker is co-dominant, meaning that the susceptible allele tm2 can be observed.



ToMV strain 0, 1 and 2

Important elements of the proposal (2):

- 20 plants per variety, as in bio-assay.
- The bio-assay is still possible: Resistance to strain 0, 1 and 2 to be tested in a bio-assay (method i) and/or in a DNA marker test (method ii).
- The basis is the claim of susceptibility or of resistance by the breeder in the TQ: in case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the resistance is absent or present for the variety (on another mechanism, e.g. gene Tm1).

ToMV strain 0, 1 and 2				
Test result DNA marker test	tm2/tm2	Tm2/tm2 or Tm2/Tm2	Tm2 ² /tm2 or Tm2 ² /Tm2 ² or Tm2 ² /Tm2	
		(occurs incidentally)		
51.1 Strain 0	[1] absent	[9] resistant	[9] resistant	
51.2 Strain 1	[1] absent	[9] resistant	[9] resistant	
51.3 Strain 2	[1] absent	[1] absent	[9] resistant	

