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**ADDENDUM TO PARTIAL REVISION OF THE TEST GUIDELINES FOR TOMATO***Document prepared by the Office of the Union**Disclaimer: this document does not represent UPOV policies or guidance*

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]


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



## The use of DNA markers in the DUS- examination of tomato and tomato rootstocks, proposal to revise the UPOV Test Guidelines

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## 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 same number of individual plants, with the same criteria for distinctness, uniformity and stability 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 characteristic-specific molecular markers.


	<h2 data-bbox="536 344 1171 405">Fusarium race 0 and 1</h2>
	<p data-bbox="331 434 975 472">Important elements of the proposal (1):</p> <ul data-bbox="331 477 1377 891" style="list-style-type: none"><li data-bbox="331 477 847 515">• Test with marker for gene I2.</li><li data-bbox="331 519 1377 701">• Marker is <b>in</b> 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.</li><li data-bbox="331 705 1377 815">• 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.</li><li data-bbox="331 819 1377 891">• The marker is co-dominant, meaning that absence of I2 can be observed.</li></ul>


	<h2 data-bbox="536 1209 1171 1270">Fusarium race 0 and 1</h2>
	<p data-bbox="331 1299 975 1337">Important elements of the proposal (2):</p> <ul data-bbox="331 1341 1377 1733" style="list-style-type: none"><li data-bbox="331 1341 986 1379">• 20 plants per variety, as in bio-assay.</li><li data-bbox="331 1384 1377 1525">• The bio-assay is still possible: <i>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).</i></li><li data-bbox="331 1529 1377 1733">• The basis is the claim of susceptibility or of resistance by the breeder in the TQ: <i>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).</i></li></ul>


 <h2 style="text-align: center;">Fusarium race 0 and 1</h2>			
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 I2, 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

 <h2 style="text-align: center;">Fusarium race 0 and 1: experience</h2>	
	<p>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:</p> <ul style="list-style-type: none"> <li>• All plants with I2: uniformity sufficient</li> <li>• Genetically not uniform, as in the bio-assay: uniformity insufficient</li> </ul> <p>In future a standard check on the presence of I2: if genetically not uniform -&gt; contradiction with claim in TQ -&gt; bio-assay needed to conclude.</p>

	<h2>ToMV strain 0, 1 and 2</h2>
	<p>Important elements of the proposal (1):</p> <ul style="list-style-type: none"><li>• Test with marker for gene <b>Tm2/2<sup>2</sup></b>.</li><li>• Marker is <b>in</b> the protein coding sequence of the gene, so the necessary link is present. This has been validated by Naktuinbouw, also in Harmores.</li><li>• Resistance to strain 0, 1 and 2 is normally caused by gene <b>Tm2<sup>2</sup></b>. Some varieties have gene <b>Tm2</b>, which gives resistance tot strain 0 and 1 only.</li><li>• The marker is co-dominant, meaning that the susceptible allele <b>tm2</b> can be observed.</li></ul>

	<h2>ToMV strain 0, 1 and 2</h2>
	<p>Important elements of the proposal (2):</p> <ul style="list-style-type: none"><li>• 20 plants per variety, as in bio-assay.</li><li>• The bio-assay is still possible: <i>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).</i></li><li>• The basis is the claim of susceptibility or of resistance by the breeder in the TQ : <i>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).</i></li></ul>

 <b>ToMV strain 0, 1 and 2</b>			
Test result DNA marker test	tm2/tm2	Tm2/tm2 or Tm2/Tm2	Tm2 <sup>2</sup> /tm2 or Tm2 <sup>2</sup> /Tm2 <sup>2</sup> or Tm2 <sup>2</sup> /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

 <b>TSWV</b>	
	<ul style="list-style-type: none"><li>• Already accepted in CPVO</li><li>• Gene Sw-5b</li><li>• 20 plants</li></ul>