UPOV

International Union for the Protection of New Varieties of Plants

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DO RESISTANCE MARKERS FOR TOMATO FULFIL THE REQUIREMENTS OF TGP/15?

Document prepared by an expert from the Netherlands

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The Annex to this document contains a copy of a presentation on “Do resistance markers for tomato fulfil the requirements of TGP/15?”, prepared by an expert from the Netherlands, to be made at the seventeenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (BMT).

[Annex follows]

ANNEX

DO RESISTANCE MARKERS FOR TOMATO FULFIL THE REQUIREMENTS OF TGP/15?

Presentation prepared by an expert from the Netherlands

Do resistance markers for tomato fulfil the requirements of TGP/15?

BMT/17, Montevideo 2018

Amanda van Dijk

Status of the question

- TWV51 (July 2017, Roelofarendsveen) proposal to partially revise
  - Tomato guideline (TG/44/11 Rev.)
  - Tomato rootstocks guideline (TG/294/1 Corr. Rev. 2)
- A presentation was given, titled *The use of DNA markers in the DUS of tomato and tomato rootstocks, proposal to revise the UPOV Test Guidelines*
- Proposal accepted by TWV

Status of the question

- TC-EDC (March 2018) raises a question:

DNA marker test to be presented to the BMT to check whether method corresponds to TGP/15

- Conclusion of BMT/17 to be shared with TWV/52, September 17 to 21 2018, Beijing.
- Only if TWV/52 accepts, TC/54, October 29 and 30 2018, can adopt the partial revisions.

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

Verification of the reliability is succesful if one can not find the exception among varieties tested in the last 3 years

?

1 exception = show stopper (broken link)

Fusarium race 0 and 1 (and 2)

Important elements of the proposal (1):

- Test with **marker for gene I2**.
- The I2 marker is positioned **in** the protein coding sequence with a validated key function in disease resistance.
- Further validation by Naktuinbouw in a set of more than 120 varieties:
  - **no** varieties with the marker but without the resistance phenotype
  - some varieties without the marker but with the resistant phenotype (and with other resistance genes)
- Proposed text in the guideline: “Dominant resistance gene I2 is always associated with resistance to both race 0 (ex 1) and race 1 (ex 2). The presence or absence of the resistance allele can be detected by the co-dominant marker as described in this method.”

Fusarium race 0 en 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) or in a DNA marker test (method ii), if appropriate. Resistance to race 2 (ex3) to be tested in a bio-assay (method i).*
- 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 variety is resistant e.g. on another mechanism like gene I3.*

Fusarium race 0 en 1

UPOV characteristics		Genes	I	I2	I3	I7
tomato	tomato rootstock	Races				
48.1 *	24.1 *	**0 (ex 1)**	R	R	R	R
48.2 *	24.2 *	**1 (ex 2)**	S	R	R	R
48.3	24.3 *	**2 (ex 3)**	S	S	R	R

Claim TQ	Susceptible to 0 and 1	Resistant to 0, susceptible to 1	Resistant to 0 and 1	Resistant to 0, 1 and 2
	Several varieties (older/determinate/ special fruit types)	Several varieties (older/determinate/ special fruit types)	**Large majority of varieties**	A few varieties known in tomato, common in tomato rootstocks
DNA marker i2i2	**Agreed** (susceptible to race 0 and 1)	Probably not gene I2, but I: additional bio-assay for 0; for race 1 **Agreed** (susceptible)	contradiction: bio-assay	Probably gene I3 or I7: additional bio-assays
DNA marker I2i2 or I2I2	contradiction: bio-assay	Contradiction for race 1: bio-assay needed for race 1	**Agreed** (resistant to race 0 and 1)	Race 0 en 1 **Agreed** (resistant), for race 2 a bio-assay needed

Better judgement of uniformity with markers

In 2016/7 approx. 20 candidates with a low number of symptomatic plants

- Plants with symptoms were analyzed with I2 marker
- When in all these plants I2 marker was present, the variety was judged uniform
- This happened in most cases

12. Interpretation of test results in comparison with control varieties

absent	[1]	severe symptoms
present	[9]	mild or no symptoms

Fusarium race 0 en 1: experience

Since 2018 routine DNA marker test for all DUS candidates.

10% also in bio-assay. Good correlation.

Added in national TQ to prevent breeders' to claim resistance only based on an I2 marker test.

Also for resistances the phenotype should be leading for declaration.

ToMV strain 0, 1 and 2

Important elements of the proposal (1):

- Test with marker for gene Tm2/2^2.
- Marker Tm2/2^2 is positioned **in** the protein coding sequence with a validated key function in disease resistance.
- Validated by Naktuinbouw, also in Harmores.
- Resistance to strain 0, 1 and 2 is usually caused by gene **Tm2^2**. Some varieties have gene **Tm2**, which gives resistance to 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):

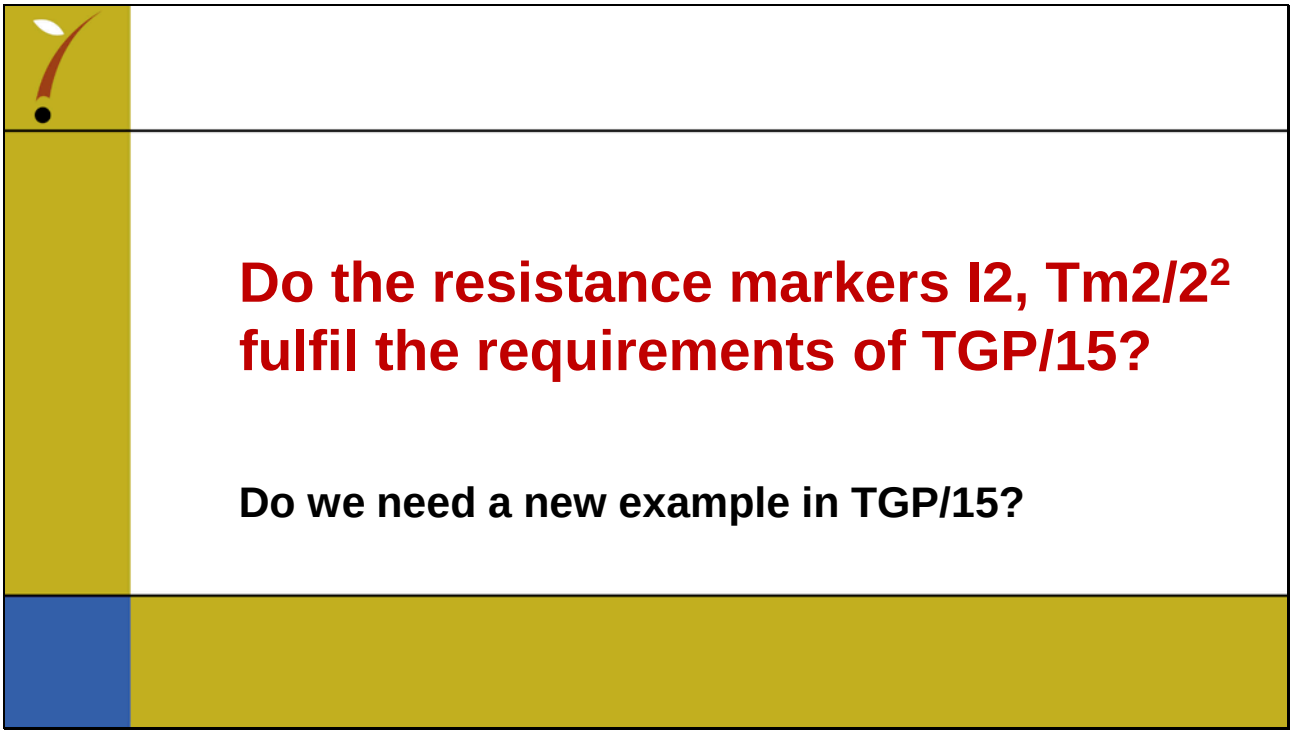
- 20 plants per variety, as in bio-assay.
- The bio-assay is still possible: *Resistance to strain 0, 1 and 2 to be tested in a bio-assay (method i) or in a DNA marker test (method ii), if appropriate.*
- The basis is the claim of susceptibility or of resistance by the breeder in the TQ : *in case the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

ToMV strains 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	Reliable, robust marker not yet developed
				Tm1
		(occurs incidentally)		
51.1* Strain 0	[1] susceptible	[9] resistant	[9] resistant	Resistant
51.2 Strain 1	[1] susceptible	[9] resistant	[9] resistant	Susceptible
51.3 Strain 2	[1] susceptible	[1] susceptible	[9] resistant	Resistant

Resistance markers I2 and $Tm2/2^2$ fulfil the requirements of TGP/15

- More genes play a role. I2 is well linked to resistance to Fusarium race 0 and 1, but other genes can give the same phenotype. $Tm2/2^2$ is well linked to resistance to ToMV strain 0, 1 and 2, but Tm1 can give (for strain 0 and 2) the same phenotype.
- This will always be the case. We decide on knowledge of today. Breeding will always be ahead of us.
- TGP/15/1 (d): markers linked to different genes conferring expression of the same characteristic are different methods for examining the same characteristic
- Without information in the TQ a DNA marker test for I2 is decisive on Fol: 0 and 1 when the resistance allele is present.
- Without information in the TQ a DNA marker test for $Tm2^2$ is decisive on Tm: 0, 1 and 2 when the resistance allele is present.



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