

Resistance-specific molecular markers in DUS testing

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TGP/15/1



DOCUMENT TGP/15

Guidance on the use of BMT techniques in DUS examination.



MODEL 1:

Characteristic –specific molecular markers

- Herbicide Tolerance
- Disease Resistance



Benefits of PCR tests



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1. PCR results

- complementary to bio-assay results.
- More reliable, especially in cases of difficult bioassays.

2. PCR tests

- can replace a bioassay when a bioassay is not possible

3. PCR tests

- faster
- often cheaper than bioassays.

Current situation resistances



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As example: DUS testing in tomato

- 25 Disease Resistance Characteristics (out of 61)
- 5 Disease Resistance Characteristics in TQ
 - Mi / Va and VD / Fol race 0.1 and ToMV race 0
 - used to select relevant reference varieties (grouping characteristics)
 - to be confirmed by Examination Office
- Confirmation - bioassay
- TG/44/11 Rev. includes - test protocol
 - number of plants
 - test circumstances
 - description of symptoms
 - standards

But...

Current situation resistances



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Bioassay not always easy

- difficult to perform
- difficult to reproduce (false positives and false negatives or mild symptoms as in Fol).

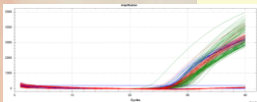
PCR test - additional confirmation.

Bioassay not always / not for everybody possible

- not available (specific circumstances needed)
- not possible (quarantine status as for Rs, TYLCV, TSWV).

PCR test - to confirm breeders' information on TQ.

Requirements for PCR tests



- Robust - repeatable results
- Validated - link marker - resistance
- Sufficient number of plants
- Knowledge of gene(s) in use
- Preferably co-dominant marker



Lower band: 24 homozygous resistant plants



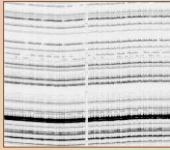
2 bands: 24 heterozygous resistant bands



12 plants (heterozygous) resistant and 12 plants (homozygous) susceptible

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Proposed strategy



Classification	Group	Value	Ref
A.1.1. Resistance to Powdery mildew (q-PCR) (M.1.1.1)	Group	0.00	0.00
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Preconditions

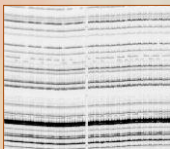
- Bioassay - not possible or difficult
- Validated PCR test - available

Procedure

- 1: check breeders' claim on the TQ by a PCR test
- 2: if needed (depending on the type of marker and the conclusion of the PCR test) perform a bioassay
- 3: in case of contradiction between TQ and PCR test: perform a bioassay

- In case a bioassay is needed, but not possible, the characteristic can not be used in the DUS test

Proposed strategy



Classification	Group	Value	Ref
A.1.1. Resistance to Powdery mildew (q-PCR) (M.1.1.1)	Group	0.00	0.00
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PCR result	Resistance marker present (dominant marker)	Resistance marker absent (dominant marker)	Homozygous resistant or heterozygous (co-dominant marker)	Homozygous susceptible (co-dominant marker)
Conclusion DNA	Resistant	- Susceptible, - mistake in the test, - Resistant (based on a different gene)	Resistant	- Susceptible, - Resistant (based on a different gene)
TQ – RES	Okay: conclusion resistant	Not okay: bioassay	Okay: conclusion resistant	Not okay: bioassay
TQ - SUSC	Not okay: bioassay	Confirmation by bioassay (less # plants)	Not okay: bioassay	Okay: conclusion susceptible

Quality in Horticulture