E

BMT/11/6 Add.
ORIGINAL: English
DATE: September 25, 2008

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Eleventh Session
Madrid, September 16 to 18, 2008

ADDENDUM

DEVELOPMENT AND EVALUATION OF MOLECULAR MARKERS LINKED TO DISEASE RESISTANCE GENES FOR TOMATO DUS TESTING (OPTION 1A)

Document prepared by experts from the Netherlands, France and Spain

Development and evaluation of molecular markers linked to disease resistance genes for tomato DUS testing (option 1a)

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Participating organizations:

INRA

G E V E S

MINISTERIO DE EDUCACIÓN Y CIENCIA
INIA
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

nak tuinbouw

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Option

Molecular Characteristics as Predictors of Traditional Characteristics

a) Gene specific markers for predicting individual phenotypic characteristics. Need for reliable linkage between the marker and the expression of the characteristic.

b) Use of a set of molecular characteristics which can be used reliably to estimate traditional characteristics; e.g. quantitative trait loci

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Why an option 1(a) for resistance in tomato?

- In the past 30 years breeding in tomato has focused on disease resistance
- Large number of disease resistance genes are known
- For many genes linked markers are available or the gene conferring the resistance has been cloned
- Basic requirements for the development of an option 1a approach are available

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

In this project we have developed and evaluated an option 1(a) approach for the asterisked (obligatory) disease resistance characteristics in the applicable CPVO tomato DUS protocol TP/44/2.

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Asterisked resistance genes

- *Meloidogyne incognita*, Mi1-2 gene
- *Verticillium dahliae*, Ve1 and Ve2 gene
- *Fusarium oxysporum* f.sp. lycopersici
  - Race 0, I locus
  - Race 1, I2 locus
- Tomato Mosaic Virus – Strains 0, 1, 2 and 1.2
  - No specific strain for 1.2 available. Gene conferring the resistance is usually $Tm2^2$

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Assay development

Different starting situations

- Genes cloned
  - Ve1 and Ve2 gene, Tm2 and Tm2^2 gene
    I2 gene, Mi1-2 gene*
  - Sometimes only resistant allele is known
  - Sometimes highly similar homologues
- Only linked markers
  - Tm1, I gene
- Sometimes more than one assay available

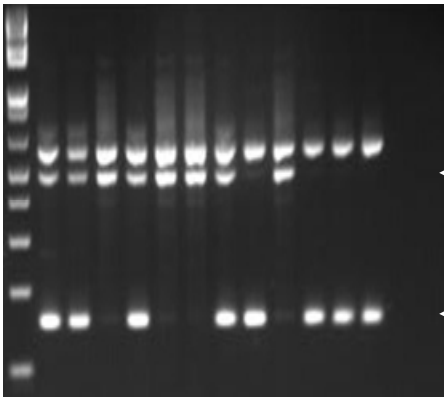
PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Assay development

- Dependent on available information + possibilities
- Different types of assays used
  - Tetra ARMS PCR
  - SCAR
  - CAPS
- In case of +/- PCR reaction additional fragment amplified in same reaction to test for amplification (positive control)
- Tests on a few samples

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Example: Tetra ARMS PCR Ve1 gene

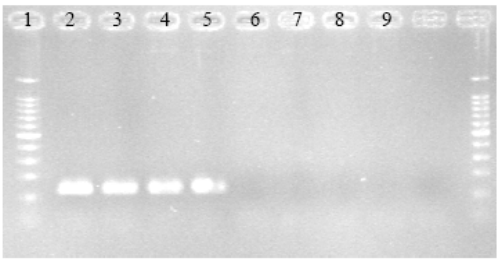


R allele

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Example: SCAR marker I gene (130 bp)

Lanes	Cultivar	R/S	Result At2-F3/R3
2	Marporum	R	130bp
3	Nemo-Netta	R	130bp
4	Campeon	R	130bp
5	Thomas	R	130bp
6	Marporum x Marmande Verte	R	X
7	Montfavet H63.5	S	X
8	Marmande Verte	S	X
9	Marmande	S	X



PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Robustness test

- All 5 labs tested all assays developed
  - On DNA that was used during the development
  - On DNA they had extracted themselves from 4 varieties Marmande and Moneymaker (susceptible varieties) Campeon and Persica (resistant varieties)

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Test	Type marker	INIA	Nakt	GEVES	INRA	PRI
Ve1	gene	OK	OK[1]	OK[1]	OK[1]	OK
Ve2	gene	OK	OK	OK[1]	OK[1]	OK
Tm1	linked marker	OK[1]	OK	OK[1]	OK	OK
Tm2 – CAPS	gene	OK	na	OK	na	OK
Tm2 - ARMS	gene	OK	OK	OK	OK[1]	OK
Mi1-2	gene/linked	OK	OK	OK	OK	OK
I	linked marker	OK	OK	OK	X	OK
I2	gene	OK[1]	OK[1]	OK	OK	OK

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Robustness tests: conclusions

- In general the assays worked well and could be reproduced
  - Sometimes optimization of PCR conditions needed
  - For Tm2/ Tm2^2, tetra ARMS PCR assay preferred
- During the test already a problem with plant material discovered (Marmande was expected to be susceptible, but segregated for Fusarium I resistance)

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Validation of markers

- Usefulness in predicting disease resistance
- Marker assays was carried out on 20 (or 30 Naktuinbouw) varieties for each of the DUS stations
  - GEVES: 1 sample from 2 pooled plants
  - INIA: 2 plants from each variety (separate). In those varieties with heterogeneous or not clear result in the biological assay 5 plants were used
  - Naktuinbouw: 2 plants from each variety (separate)
- Not all the pathogenesis assays were made on the same plant material

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Identical scores markers/pathogenesistest

Testing Station	Verticillium	ToMV ($Tm2^2$)	Meloidogyne incognita	Fusarium I	Fusarium I2
INIA	17/19[a]	20/20	21/21[a]	19/20[a]	20/20[a]
GEVES	18/20	20/20	20/20	19/20	20/20
Naktuinbouw	29/30	30/30	30/30	28/30	29/30
Overall	64/69	70/70	71/71	66/70	69/70

[a] some cultivars for which the pathogenesis assay was inconclusive are included

In 97% of the cases the molecular marker assays confirmed the pathogenesis assays

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

Validation results (1)

- ToMV: exact fit, Tm1 and Tm2 were not found
- Ve: 5 out 39 varieties susceptible in pathotest, whereas markers indicated resistance. In one case it was the other way around. Inoculums differences? Conditions?
- Mi: 4 varieties heterogeneous results in pathotest, markers showed Mi1-2/mi1-2 heterozygous (resistant) phenotype

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

Validation results (2)

- Fusarium I: 2 varieties found susceptible, were markers suggested resistance. In addition, 7 varieties showed heterogeneous results in pathotest, markers showed either resistance or susceptibility. Environmental factors involved? Sampling effect?
- Fusarium I2: In pathotest 5 varieties were inconclusive. Markers showed susceptibility

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Validation: conclusions (1)

- Pathogenesis and marker assays identical for Nematode (Mi) and TMV resistance gene
- 8 % differences for fungus resistance genes (Verticillium and Fusarium)
  - most likely due to the pathogenesis assay
  - more difficult to standardize
  - more subjectively interpreted
  - Possibly the sole presence of either the Ve1 or the Ve2 allele in heterozygous state is insufficient for clear resistant phenotype. Genetic background?

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Validation: conclusions (2)

- Marker assay seem to perform better
  - results are more clear
  - homozygote/heterozygote presence of a resistance gene can be detected in some cases
  - Marker are good at spotting heterogeneity

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Implementation of marker assays: advantages

- Improved reliability
- Gain in cost, time and feasibility (e.g. remove the necessity of the maintenance of a good quality inoculum, and avoid manipulating quarantine pathogens)

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Implementation of marker assays: limitations

- Represent only a specific resistance gene(s). New genes might be introduced.
- No evaluation of the global level of resistance. Effects of genetic background not considered

All issues also apply to isolates of pathogen

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Questions that remain (1)

- Should breeders have the obligation to indicate which resistance genes are present in a particular variety?
- When the resistance data provided by the breeder of the candidate variety exactly match with marker data obtained by a DUS testing station, could it be considered as sufficient evidence for the presence or absence of resistance and a reason not to carry out the pathogenesis test again?

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Questions that remain (2)

- Could an approved molecular test be recognized in the Test Guidelines as a predictor of the resistance? Who would approve it? Based on which criteria?
- If more than one gene can confer the same resistance, could different predictors be considered separately in the Test Guidelines?
- Should DUS testing stations carry out the molecular tests or could they also be subcontracted out?

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

People involved:

Geves
- Laetitia Cavellini
- René Mathis
- Cécile Collonnier

INRA
- Carole Caranta
- Sophie Rolland
- André Moretti

INIA
- Carmen Mansilla
- David Calvache

NAKtuinbouw
- Daniël Deinum
- Diederik Smilde

Plant Research International
- Paul Arens
- Hanneke van der Schoot

PLANT RESEARCH INTERNATIONAL WAGENINGEN UR

Acknowledgements

Sergio Semon for stimulating discussions

This project is co-funded by:

- Community Plant Variety Office
- Ministry of agriculture, Nature and Food quality of the Netherlands
- GEVES
- INRA
- French ministry of Agriculture
- Spanish ministry of agriculture

PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

[End of document]