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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

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

Fourteenth Session
Seoul, Republic of Korea, November 10 to 13, 2014

MOLECULAR MARKERS AS PREDICTORS FOR 'TRADITIONAL' CHARACTERISTICS

Document prepared by an expert from the Netherlands

Disclaimer: this document does not represent UPOV policies or guidance

The Annex to this document contains a copy of a presentation "Molecular markers as predictors for 'traditional' characteristics" made at the fourteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

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The Netherlands

[Annex follows]

Molecular markers as predictors for 'traditional' characteristics

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UPOV/INF/18/1

Model 1: Molecular Characteristics as Predictors of 'Traditional' Characteristics

a) Gene-specific markers for predicting individual phenotypic characteristics. (Reliable linkage between the marker and the expression of the characteristic required)
- *e.g. disease resistance*

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

Current situation



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DUS testing tomato (only morph)

- Resistance / Susceptibility for the obligatory diseases is used to select relevant reference varieties (grouping characteristics)
- With the information of the candidate on the Technical Questionnaire (TQ) references in the same group are selected.
- Information on TQ for a candidate variety must be confirmed
- Confirmation is done by bioassay
- PCR test is only performed when problems in bioassay as extra confirmation
- **What should be done if a bioassay is not available, not possible or when a bioassay is difficult?**

Proposed strategy



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DUS testing tomato (both morph and DNA)

- First PCR test performed on seedlings:
  - for resistant candidate varieties - min. 20 individual plants (also check on uniformity).
  - for susceptible candidate varieties – 2 individual.
- When resistance gene present and TQ resistant; enough proof – no bioassay needed.
- When resistance gene absent; bioassay will be performed (min. 20 plants, also check on uniformity).
- When PCR result and TQ do not match; bioassay (if possible).
- When there is any (other) doubt; bioassay (if possible).

Benefits of PCR tests



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1. PCR results are complementary to bioassay results. Increased reliability
2. PCR tests are faster and cheaper than bioassays.
3. Good alternative when bioassay is:
    1. Not available
    2. Not possible (because of e.g. Quarantain status)
    3. Difficult to perform and/or to reproduce (false positives and false negatives)

Overview of possible tests I



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Disease resistance in tomato:

- ***Meloidogyne incognita***
    - MI1.2 (traditional PCR)
- ***Tomato Mosaic Virus (ToMV)***
    - Tm1 (traditional PCR)
    - Tm2 and Tm2² (tetra ARMS)
- ***Verticillium dahliae***
    - Ve1 and Ve2 (same locus) (tetra ARMS)
- ***Fusarium oxysporum f. sp. lycopersici***
    - I-2 gene (traditional PCR)

Why these tests I

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- *Meloidogyne incognita*
  - MI1.2 (traditional PCR)
- *Tomato Mosaic Virus (ToMV)*
  - Tm1 (traditional PCR)
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- *Verticillium dahliae*
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- *Fusarium oxysporum f. sp. lycopersici*
  - I-2 gene (traditional PCR)

No significant problem with bioassays

1. Complementary results – more reliability
2. Faster and cheaper – cost efficienty
3. Management of reference collections:
   - Gain new/additional data for old varieties
   - To screen (old) reference varieties

ARMS: example

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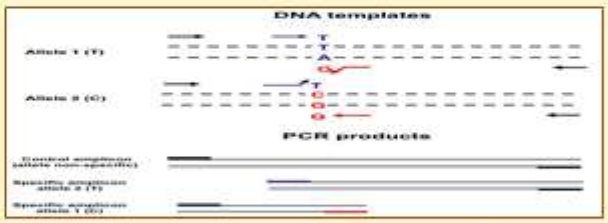
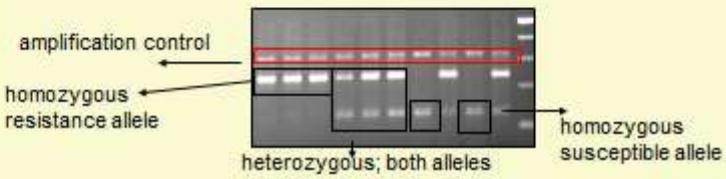
Example: Amplification Refractory Mutation System (ARMS) for the detection of Ve-1/Ve-2 and Tm2/Tm2²





Correlation



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Verticillium dahliae Ve1 and Ve2 genes

Total # varieties	Correlation PCR vs TQ and Bioassay
94	98%
2	Ve1-ve2 new haplotype = intermediate resistance

Discovery of combination of new alleles (=haplotype) that might explain newly observed intermediate resistance levels for Verticillium.

Meloidogyne incognita MI1.2 gene

Total # varieties	Correlation PCR vs Bioassay
130	99%
1 resistant fragment	Susceptible in bioassay

This candidate variety also had intermediate resistance levels for Ve.
This application was not registrated. Not DUS.

Correlation



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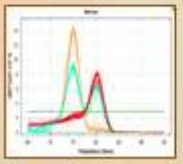
Tomato Mosaic Virus (ToMV) Tm2 and $Tm2^2$

Total # varieties	Correlation PCR vs TQ and Bioassay
100	100%

Fusarium oxysporum f. sp. lycopersici I-2 gene

Total # varieties	Correlation PCR vs TQ and Bioassay
100	100%

Overview of possible tests II



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Disease resistance in tomato:

- ***Tomato Spotted Wilt Virus (TSWV)***
  - Sw-5 (TaqMan PCR)
- ***Tomato Yellow Leaf Curl Virus (TyLCV)***
  - Ty-1 / Ty-3 (Melt Curve analysis)

TSWV reference:
Dianese E.C., Fonseca M.E.N., Goldbach R., Kormelink R., Inoue-Nagata A.K., Resende R.O., Boiteux L.S.(2009) Development of a locus-specific, co-dominant SCAR marker for assisted-selection of the *Sw-5* (*Tospovirus* resistance) gene cluster in a wide range of tomato accessions. Mol Breeding (2010) 25:133-142.

TyLCV reference:
Verlaan M.G.: The Tomato Yellow Leaf Curl Virus Resistance Gene Ty-1 and TY-3 are allelic and Code for DFGD-Class RNA Dependent RNA Polymerases. PLOS Genetics March 2013 Volume 9 Issue 3.

Patent: http://www.google.com/patents/WO2012123025A1?cl=en

Why these tests II

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Disease resistance in tomato:

- ***Tomato Spotted Wilt Virus (TSWV)***
- ***Tomato Yellow Leaf Curl Virus (TyLCV)***

Problems:

TSWV:
- Quarantain pathogens in EU
- difficult bioassay in a tent
- Trips
- Very instable virus
- Many false negatives sometimes false positives

TYLCV:
- Quarantain pathogens in EU
- No bioassay
- White Fly
- Bio Assay based on Agrobacterium inoculation with transgen

TyLCV: melt curve assay

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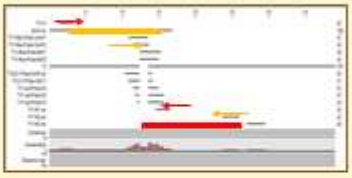
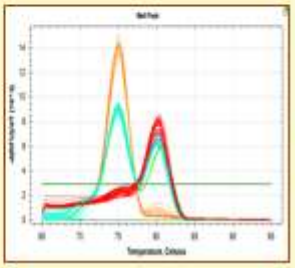
Example: melt curve analysis for TyLCV

Susceptible allele = deletion of 12 base pairs



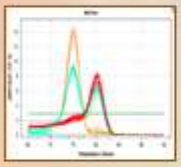


PCR product for resistant allel melts at 75°C (yellow peak)

PCR product for susceptible allel melts at 80°C (red peak)

In a heterozygous variety both peaks are visuable (blue)

Correlation



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TSWV Sw-5

Total # varieties	Correlation PCR vs TQ
118	100%

Total # varieties	Correlation PCR vs TQ and Bioassays
37	100%

TyLCV Ty-1/Ty-3

Total # varieties	Correlation PCR vs TQ
15	100%

Bioassay is not (yet) possible.

Overview of tests III

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Cytoplasmatic Male Sterility (CMS) in *Brassicaceae*

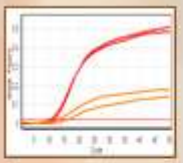
- Broccoli
- Cauliflower
- Red Cabbage
- White Cabbage
- Savoy Cabbage
- Brussels Sprout
- Curly Kale
- Kohlrabi

Ogura-type cytoplasmatic male-sterility from Japanese radish *(covered by confidentiality clause)*

CMS marker is located in *orf138*

Fertile varieties do not have *orf138*

Procedure CMS-PCR testing



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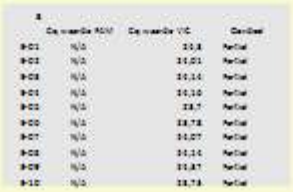
Cytoplasmatic Male Sterility (CMS) in *Brassicaceae*

Seeds are sown on wet filter paper. After one week seedlings are harvested.

For fertile varieties: 5 pools of 5 individuals are sampled and analysed by TaqMan PCR.

For sterile varieties: min. 20 individual plants were sampled and analysed by TaqMan PCR.

Only when problem: uniformity problem or when contradictory to TQ a morphology test will be performed



Internal DNA control NAD5

Correlation CMS

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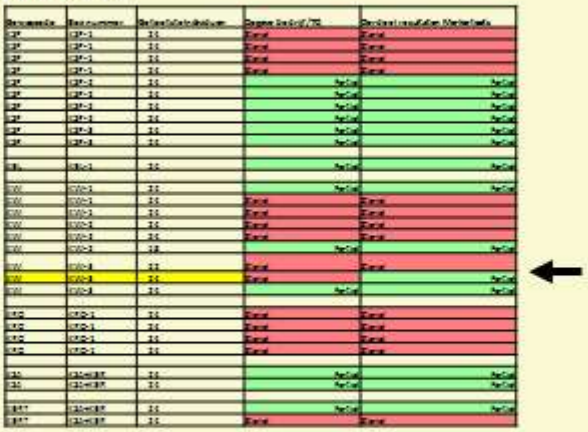
Results 2013:



From 165 candidate varieties (2011-2014) in all *Brassicaceae* tested only one PCR result not in accordance with TQ/morph test (99,4%).

Different source of sterility (not OGURA-type)

Future perspective tests I

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- ***Meloidogyne incognita***
  - Mi1.2 (traditional PCR)
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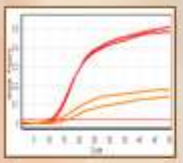
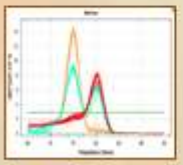
- ***Fusarium oxysporum f. sp. lycopersici***
  - I-2 gene (traditional PCR)

- ❑ PCR is complementary to the bioassay.
- ❑ Faster and cheaper
- ❑ Good help to screen and manage the reference collection

Proposal:
include PCR tests in the UPOV guidelines on crop-by-crop basis

Future perspective tests II





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- *Tomato Spotted Wilt Virus (TSWV)*
  - Sw-5 (TaqMan PCR)
- *Tomato Yellow Leaf Curl Virus (TyLCV)*
  - Ty-1 / Ty-3 (Melt Curve analysis)

Proposal: include PCR tests in the UPOV guidelines

From **CPVO** protocol tomato (TP/044/4 Rev):

11. Observations
11.1 Method ..... visual
11.2 Observation scale ..... Symptoms: top mosaic, bronzing, various malformations, necrosis
11.3 Validation of test ..... evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12. Interpretation of data in terms of UPOV characteristic states
absent [1] symptoms
present [9] no symptoms
13. Critical control points:
TSWV has a quarantine status in some countries. TSWV is transmitted by *Thrips tabaci* and Western flower thrips (*Frankliniella occidentalis*). Pathotype 0 is defined by its inability to break resistance in tomato varieties carrying the resistance gene Sw-5. TSW resistance based on Sw-5 may be jdetected without using the pathogen.

Note: Option for testing without using the pathogen
Resistance to TSWV:0 is often based on the resistance gene Sw-5. The presence of the resistance gene Sw-5 can be detected by molecular marker Sw-5b-LRR (Garland et al. 2005) or by a co-dominant SCAR marker (Dianese et al. 2010). This molecular test is validated to be used instead of the pathotest, as foreseen in UPOV document TC/38/17 Add. – CAJ/45/5 Add. Under Option 1(a). Each molecular marker should be applied to a minimum of twenty plants and validated with proper controls.
If the biolomolecular test is inconclusive, then the pathotest needs to be carried out

Future perspective

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Cytoplasmatic Male Sterility (CMS) in *Brassicaceae*

Ogura-type cytoplasmatic male-sterility from Japanese radish
covered by confidentiality clause

Company is willing to make the marker available for EOs for DUS research only

Legal discussion within UPOV about how to deal with confidentiality
to make sure that the marker is used for DUS purposes only

[End of Annex and of document]