UPOV

International Union for the Protection of New Varieties of Plants

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Technical Working Party for Agricultural Crops

TWA/48/5

Forty-Eighth Session
Montevideo, Uruguay, September 16 to 20, 2019

Original: English
Date: August 30, 2019

THE USE OF BIOMOLECULAR TECHNIQUES IN DUS TESTING FOR PVP IN THE EUROPEAN UNION

Document prepared by an expert from the European Union

Disclaimer: this document does not represent UPOV policies or guidance

The annex to this document contains a copy of a presentation on “The use of biomolecular techniques in DUS testing for PVP”, to be made at the forty-eighth session of the TWA.

[Annex follows]

CPVO
Community Plant Variety Office

The use of biomolecular techniques in DUS Testing for PVP

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Montevidéo, September 2019

Biomolecular techniques (BMT) in the EU

→Improve the quality and efficiency of DUS testing

→Support the enforcement of plant breeders' rights

Overview

1. CPVO-IMODDUS working group
2. Legal Framework to apply BMT
3. Models and Concepts
4. DNA sampling for enforcement purposes

1. IMODDUS working group at CPVO

= "Integration of molecular data into DUS testing"

- Implement CPVO R&D strategy
- Work as a think-tank
- Assess and discuss new molecular developments
- Identify species with model functions
- Harmonize methods
- Propose and promote R&D projects (co-)funded by the CPVO

Participants are experts from :

- Examination offices from the EU network
- Breeders associations (ESA, Plantum, CIOPORA)
- Universities and research institutions (INRA, Hohenheim, Wageningen, …)
- European Commission (DG SANTE, …)
- (non-EU experts : USDA, ASTA…)

2. Legal Framework for the use of BMT

2.1. In the EU (Council Regulation 2100/94)

Art. 56(1): "*Unless a different manner of technical examination relating to the compliance with the conditions laid down in Articles 7 to 9 [DUS] has been arranged, the Examination Offices shall , …, grow the variety or undertake any other investigations required*."

Art. 56(2): "*The conduct of the technical examination shall be in accordance with test guidelines …*"

→ **field trials are the regular tool**
→ **BMT as complementary tools**

2. Legal Framework for the use of BMT

2.2. At UPOV

Guidance on the use of BMT :

Document UPVO/INF/17 : "Guidelines for DNA-profiling: molecular marker selection and database construction ("BMT guidelines")"

Document UPOV/INF/18/1: "Possible use of molecular markers in the examination of distinctness, uniformity and stability (DUS)"

- Characteristic-specific molecular markers (previously called Model 1)
- Calibrated molecular distances in the management of variety collections / Combining phenotypic and molecular distances in the management of variety collections DUS (previously called Model 2)
- Use of molecular marker characteristics (previously called Model 3)
  → negative assessment by UPOV

TGP 15 (associated document to General introduction to DUS (TG/1/3))

BMT in DUS testing → **Examples** for the models

3. Models and Concepts

3. 1 Characteristic-specific molecular markers (UPOV Model 1):

- Use of molecular characteristics directly linked to traditional characteristics (gene-specific markers) = 100% correlation
- Aim: replace field assessment/bioassay by molecular assay
- Conditions:
  - lab test = field test: plant number, growing cycles, DUS standards
  - reliable linkage
  - different markers for the same gene
    = different methods of assessing the same characteristic
  - different genes involved in the expression of a given characteristic
    = different methods of assessing the same characteristic

3. Models and Concepts

3. 1 Characteristic-specific molecular markers (UPOV Model 1):

- Accepted model which has proven to work
- Application to characteristics difficult or cumbersome to assess

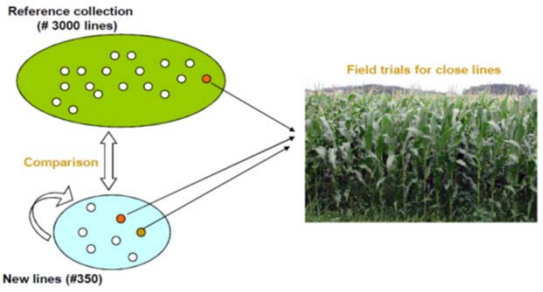
☞ Examples

- Tomato mosaic virus, Tomato spotted wilt virus
- Cabbages, observation of CMS characteristics
- Sugarbeet, resistance to nematode Heterodera schachtii
- Detection of adventitious presence of GM seeds

3. Models and Concepts

3.2 Combining phenotypic and molecular distances (UPOV Model 2) – Principle

Goal = reduce the cost of DUS testing for large and expensive variety collections by safely excluding reference varieties from being grown in the field.



3. Models and Concepts

3.2 Calibration molecular distances in the management of variety collections (UPOV Model 2)

A. **Calibration molecular distances in the management of variety collections** (Calibration of threshold levels for molecular markers against the minimum distance in traditional characteristics).
   - Assessment of genetic distances to predict phenotypic distances.
   - Need for further work to improve the relationship between morphological and molecular distances

☞ Approach which today is not applied

3. Models and Concepts

3.2. Combining phenotypic and molecular distances (UPOV Model 2)

B. Combining phenotypic and molecular distances

- Two independent thresholds to select reference varieties for a side-by-side comparison.
- Varieties exceeding both thresholds do not need to be included in the growing trial.
- Aim: Safely exclude from field comparisons varieties that are beyond "Distinctness plus" thresholds.

☞ Concept approved and applied for maize, lettuce, barley…

3. Models and Concepts

3.2. UPOV Model 2: approaches

C. "Genetic first" selection of similar varieties for the growing trial

- For seed-propagated varieties with two growing cycles

1. DNA profile of the candidate variety
2. Comparison to DNA profiles of the reference collection
   → genetic similarity > 80% → inclusion in field trial (1st growing cycle)
3. Comparing candidate variety description from the field to the DB
4. Only if some are phenotypically close → side-by-side comparison in 2nd growing cycle, otherwise variety declared DUS after 1 cycle.

- Time constrains: reception seed–DNA profiling–selecting ref. varieties

☞ Proposed for adoption by the UPOV Council for French bean (extension planned to further species)

3. Models and Concepts

3.3. Use of molecular marker characteristics only

→ negative assessment

- No consensus whether they would undermine the effectiveness of protection:
  identification of molecular differences not reflected in the phenotype
- Risk of using limitless number of markers to find differences

3. Models and Concepts

3. 4 Prospects

Test on new molecular techniques:

- Whole Genome Sequencing, Genotyping by Sequencing…
  → EU H2020 project INVITE on 10 crops
- Epigenetics

→ CPVO R&D project on Apple for molecular markers allowing the distinction of apple mutants (sports):

Evaluation of differences between Gala and 10-15 mutants

1. Standardized phenotyping of fruit color: intensity, surface, stripes
2. Whole genome sequencing
3. Epigenetic differences assessed genome-wide at the DNA methylation level
   → identification of genes and epigenetic marks potentially involved in the phenotypic changes of mutants

4. DNA sampling for enforcement purpose

Service to breeders

A. Sampling by the EO

- Example roses:
  - ❖ Established in September 2016
  - ❖ DNA sample from the official sample submitted for DUS
    Sample taken at the end of growing trial (to ensure no off-types are collected)
  - ❖ DNA stored without analysis by a lab working for the EO
    → comparative analysis in suspected infringement cases.
  - ❖ Initial storing for 5 years: cost 50 EUR/sample
  - ❖ ≈ 30% of the applicants use the service
- Extension to other crops under discussion, e.g. grape vine

4. DNA sampling for enforcement purpose

B. Sampling by the breeder

- Specific procedure for authorized sample taking
- Material: official sample
  taken during the DUS test or from living reference collection
- Breeder/title holder only → third parties only with written consent
- DNA extraction/analysis by laboratory chosen by the applicant/title holder
- Free of charge
- Applied especially for fruit crops

[End of Annex and of document]