DUS Training for Maize
Case Study 1 (DUS Test and Uniformity Assessment, CPVO)

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Presentation of the CPVO and the EU PVP SYSTEM

Outline

• The CPVO, organisation
• Main features of the EU system for PVP
• Technical aspects and set up of DUS testing
• Uniformity – requirements of the UPOV Convention
• DUS testing of maize organized by the CPVO
• Standards for decisions - Uniformity
The CPVO

The EU Plant Variety Protection system is run by the Community Plant Variety Office (CPVO):

- Official Agency of the EU
- Based in Angers (France)
- Operational since 1995
- Around 50 staff members
- Independent legal status
- Fully self-financed
Institutional Framework

- CJEU
  - General Court
  - BOARD OF APPEAL
  - ADMINISTRATIVE COUNCIL
    - CPVO
      - Examination Offices
Main features of the EU PVP system

- The system for the EU protection of plant varieties was established by a Regulation of the European Community in 1994 (EU 2100/94)
- The intellectual property rights granted under this system are valid throughout the territory of the 27 Member States of the European Union
Main features of the EU PVP system

EU / National

- The EU system co-exists with the national systems of the EU Member States (24 of 27 have a national system)
- It is the applicant’s choice: national or EU plant variety rights
- The EU PVR protection cannot be combined with national PVR

UPOV

- The EU system is in line with the UPOV 1991 Act
- The EU is a full member of UPOV as an inter-governmental organisation (since June 2005)
Examination offices

- When the EU PVR system was created it has been decided to use the testing facilities already existing in the MS, for the assessment of the DUS criteria of candidate varieties.

The office collaborates with offices outside the EU, for example where no examination office within the EU is available for testing a particular species or where specific agreements have been made.
Examination offices

• The DUS examinations are carried out by entrusted Examination Offices

• The Examination Offices must fulfil quality requirements (audits)

• The entrustment is made by the Administrative Council
The red dots represent countries where DUS examinations are carried out in the European Union on behalf of the CPVO.
To be protected, a variety should:

- be **New**
- have a suitable **variety denomination**
- be **Distinct**
- be **Uniform**
- be **Stable**
Technical aspects and set up of DUS testing

DUS Criteria:

• be **Distinct**

• be **Uniform**

• be **Stable**
Uniformity – Requirements of the UPOV Convention

• Article 6(1)(c) of the 1961/1972 and 1978 Acts of the UPOV Convention:
• a variety is deemed uniform if it is “sufficiently homogeneous, having regard to the particular features of its sexual reproduction or vegetative propagation.”

Article 8 of the 1991 Act:
• a variety is uniform if, “subject to the variation that may be expected from the particular features of its propagation, it is sufficiently uniform in its relevant characteristics”. 
What should be taken into account

- Relevant characteristics
- Feature of propagation
- Type of expression of characteristics (QN, QL, PQ)
Relevant characteristics

- Relevant characteristics of a variety include at least all characteristics used for the examination of DUS or included in the variety description established at the date of grant of protection of that variety.

- Therefore, any obvious characteristic may be considered relevant, irrespective of whether it appears in the Test Guidelines or not”. Hence, it is a matter for the authority to decide, which other characteristics it may include in its consideration of distinctness, which must also be considered for uniformity and stability.

- (Exceptions – dry matter content, content of drugs, weight thousand seed ....Usually observed in bulk sample)
Feature of propagation

- Different level of uniformity according to particular feature of propagation:
  - Truly self-pollinated varieties (low level of genetic variation, variation results predominantly from environment)
  - Mainly self-pollinated varieties
  - Inbred lines
  - Vegetatively propagated varieties
  - Cross-pollinated varieties (high genetic variation, variation results from genetic and environment)
  - Mainly cross-pollinated varieties
  - Synthetic varieties
  - Hybrids (single-cross, three-way crosses, double crosses)
  - Some modification within group are acceptable (e.g.- segregation for three-way hybrid, for synthetic varieties, for male sterile varieties maintained by near-isogenic maintainer lines ...)
Segregating characteristics

• for hybrids other than single-cross hybrids, for synthetic varieties and, in some cases, for varieties maintained by near-isogenic maintainer lines, a segregation for certain characteristics, in particular for qualitative characteristics, is acceptable if it is compatible with the expression of the parental lines and the method of propagating the variety.
Segregating characteristics

- If the inheritance of a segregating characteristic is known, the variety is considered to be uniform if the characteristic behaves in the predicted manner. This can be determined by using a statistical method, such as one based on the $\chi^2$ test.
Segregating characteristics

• If the inheritance of a clear-cut segregating characteristic is not known, the observed segregation ratio should be described.
Segregating characteristics

- In quantitative characteristics, segregation in multiple-cross hybrids and synthetic varieties may result in a continuous variation. In such cases, uniformity is assessed as in cross-pollinated varieties, on the basis of standard deviations.
Type of expression

• Type of expression of characteristics
• QN - quantitative
• QL - qualitative
• PQ – pseudo-qualitative
• the variation in the expression of the relevant characteristics within variety - the basis for assessment of uniformity
<table>
<thead>
<tr>
<th>Method of propagation of the variety</th>
<th>Type of expression of characteristic</th>
<th>Method of propagation of the variety</th>
<th>Method of propagation of the variety</th>
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<tbody>
<tr>
<td>Vegeatatively propagated</td>
<td>Off-types</td>
<td>Off-types</td>
<td>Off-types (visual observation) Standard Deviations (measurement)</td>
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<tr>
<td>Self-pollinated</td>
<td>Off-types</td>
<td>Off-types</td>
<td>Off-types (visual observation) Standard Deviations (measurement)</td>
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<td>Cross-pollinated</td>
<td>Off-types</td>
<td>Off-types</td>
<td>Standard Deviations</td>
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<tr>
<td>Single-cross hybrid (in-bred parent lines)</td>
<td>Off-types</td>
<td>Off-types</td>
<td>Off-types (visual observation) Standard Deviations (measurement)</td>
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<tr>
<td>Other hybrids</td>
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Off-types approach

- off-type - it can be clearly distinguished from the variety in the expression of any characteristic of the whole or part of the plant that is used in the testing of distinctness, taking into consideration the particular features of its propagation.

- in the assessment of uniformity, the standard for distinctness between off-types and a candidate variety is the same as for distinctness between a candidate variety and other varieties.
Off-types approach

What we need to know?
- a sample size, e.g. 100 plants;
- a fixed population standard, e.g. 1%;
- an acceptance probability, e.g. at least 95%
Off-types approach - Self-pollinated, vegetatively propagated and single-cross hybrid varieties

• The acceptable number of off-types tolerated in samples of various sizes is often based on a fixed “population standard” and “acceptance probability”. The “population standard” can be expressed as the maximum percentage of off-types to be accepted if all individuals of the variety could be examined. The “acceptance probability” is the minimum probability of accepting as uniform a variety with the population standard of off-types.
Off-types approach - Cross-pollinated varieties

- In some cases, in particular for qualitative and pseudo-qualitative characteristics, the great majority of individuals of a variety may have very similar expression, such that plants with a clearly different expression can be detected as off-types (e.g. root color in fodder beet, root color in fodder radish). In such cases the off-type procedure is appropriate. The number of off-types of a candidate variety should not significantly exceed the number found in comparable varieties already known. Thus, the population standard should reflect the level of uniformity found in comparable varieties.
Standard deviation approach

- Overall level of variation observed across all individual plants is considered and compared with comparable varieties
- The candidate variety should not be significantly less uniform than the comparable varieties
Standard deviation approach

- the choice of visual observation or measurements for quantitative characteristics, may take into account the following factors:
- (a) visual observations are generally quicker and cheaper than measurements but, because they are based on the expert’s judgement, they have a particularly important requirement for training and experience to ensure that observations by a DUS examiner for a characteristic are consistent and that repeatability between observers can be achieved; visual observations are appropriate if the resultant data fulfill the conditions for calculation of the mean and standard deviation;
- (b) measurements may be required in order to provide the appropriate precision for the assessment of variation.
The comparison between a candidate variety and comparable varieties is carried out on the basis of standard deviations, calculated from individual plant observations. Comparable varieties are varieties of the same type within the same or a closely related species that have been previously examined and considered to be sufficiently uniform.
Standard deviation approach

- several statistical methods for dealing with uniformity in measured quantitative characteristics. One method, which takes into account variation between years, is the Combined Over Years Uniformity (COYU) method
- calculates a tolerance limit for each characteristic on the basis of varieties within the same trial with comparable expression for that characteristic
DUS testing of maize organized by CPVO

• Entrusted examination offices for DUS testing of maize: **9 examination offices** all over the Europe
Testing of maize

- According to the CPVO Technical Protocol
- CPVO-TP/002/3 dated on 11/03/2010

- Harmonized with UPOV Technical Guideline

- [https://cpvo.europa.eu/sites/default/files/documents/zea_mays_3_0.pdf](https://cpvo.europa.eu/sites/default/files/documents/zea_mays_3_0.pdf)
Testing of maize

- TP contains:
  SUBJECT OF THE PROTOCOL
  SUBMISSION OF SEED AND OTHER PLANT MATERIAL
  CONDUCT OF TESTS:
  - Variety collection
  - Material to be examined
  - Characteristics to be used
  - Grouping of varieties
  - Trial designs and growing conditions
  - Special tests
  - Standards for decisions – DUS

REPORTING OF RESULTS
Standards for decisions - Uniformity

- Inbred lines and single-cross hybrids a population standard of 3% with an acceptance probability of 95% should be applied.

- In the case of a sample of 40 plants, the maximum number of off-types allowed would be 3.

- The same population standard and acceptance probability should apply to clear cases of out-crossed plants in inbred lines as well as plants obviously resulting from the selfing of a parent line in single-cross hybrids (clear difference in plant height, size of ear or earliness as well as proof through electrophoresis of enzymes in case of lack of uniformity on morphological level).
Standards for decisions - Uniformity

• In three-way cross hybrids and double-cross hybrids, characteristics may segregate with the effect that several states of expression occur side by side in a variety.

• Certain characteristics which from experience are known to give rise to such segregations in three-way cross hybrids and double-cross hybrids

• "S" in the table of characteristics (Ear: type of grain, Ear: color of top of grain)
Standards for decisions - Uniformity

- **ear-rows**, a population standard of 3 % with an acceptance probability of 95 % should be applied.
- The candidate is considered to be sufficiently uniform if the number of off-type ear-rows does not exceed **1 in 6 ear rows** examined.
- For sown plots the above-mentioned population standard is applicable. By analogy this provision also applies to any plant progeny test.
Standards for decisions - Uniformity

- use of enzyme electrophoresis, the Office follows the actual UPOV approach
- out-cross - any plant in an inbred line where two or more loci are heterozygous with one allele of the locus of the inbred line (e.g. AX)
- off-type - one locus is heterozygous or where two foreign alleles are present at least one locus
Testing of maize

• Different systems as regards the hybrid testing
• different approaches amongst Examination Offices:
  • DUS “hybrid first” approach
  • DUS parental lines approach
Thank you for your attention!

Questions?

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