INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

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

Twelfth Session
Ottawa, Canada, May 11 to 13, 2010

EDV – THE ISF APPROACH

Document prepared by experts from the International Seed Federation (ISF)
EDV – The ISF approach

Marcel Bruins
Ottawa, May 2010
Outline

1. Essentially Derived Varieties in the UPOV Convention
2. ISF consideration on essential derivation
3. Assessment of essential derivation
4. Burden of Proof
5. Use of molecular markers, a crop-by-crop approach:
   i. Lettuce
   ii. Oilseed Rape
   iii. Ryegrass
   iv. Cotton
   v. Tomato
   vi. Maize
1. Essentially Derived Varieties in the UPOV 1991 Convention

**Predominantly derived**
from the initial variety (or from a variety that is itself predominantly derived from the initial variety), while retaining the expression of the essential characteristics that result from the genotype (or combination of genotypes) of the initial variety.

**Clearly distinguishable from the initial variety**

**Conformity to the initial variety**
in the expression of the essential characteristics that result from the genotype (or combination of genotypes) of the initial variety, except for the differences which result from the act of derivation.

**EDV**
2. ISF consideration on essential derivation

- ISF strongly supports the concept of essential derivation
- Only few internationally agreed-upon professional rules
- Essential derivation is not a new right, but is in the scope of the right of a protected initial variety
3. Assessment of essential derivation

After establishing that the variety is distinct (DUS)

=> consider the following requirements:

- **Conformity** to the initial variety in the expression of the essential characteristics that result from the genotype or the combination of genotypes of the initial variety
- **Predominant derivation** from the initial variety
Proof of predominant derivation

Various criteria or combination thereof:

- Phenotypic characteristics
- **Molecular characteristics**
- Breeding records
- Combining ability
4. Burden of proof

For « prima facie » proof, the following elements should be sufficient:

• Strong phenotypic similarity
• Only small differences in some simply inherited characteristics
• Strong genetic similarity

If the owner of the i.v. has fulfilled one of the above requirements, then the second breeder would have to prove that there is no predominant derivation, or that he had not used the i.v., or a variety essentially derived from that i.v.
Morphological vs. Molecular

- Distance Coefficients to define a threshold (trigger point for the reversal of the burden of proof)

- Geneticists and statisticians: technically equally possible to measure distance coefficients using morphological markers; but that these distances are not always reflective of genetic distances or of pedigree relationships.

- Use of morphological characteristics could be more difficult due to environmental factors, and much more expensive.

- ISF has mainly worked on thresholds (distances measured by molecular markers)
Threshold: divide the scale of conformity into two parts

Threshold

Zone of non-derivation | Zone of putative essential derivation

Genetic Similarity

below the threshold: no presumption of essential derivation

above the threshold: presumption of essential derivation and the burden of proof of non predominant derivation would fall on the breeder of the putative EDV

Threshold will vary from species to species, depending on the existing genetic variability within the species and the established breeding procedures.
ISF recommends to its members

In case of dispute:

- First enter into a conciliation or mediation procedure
- If that does not provide satisfactory results, enter into (binding) arbitration
- According to ISF Procedure Rules for Dispute settlement.
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Choice of markers

- The markers must comply with several requirements:
  - Be “freely” available
  - Meet several technical criteria
    - => addressed in an ISF document “Issues to be addressed by technical experts to define molecular marker sets for establishing thresholds for ISF EDV arbitration” (www.worldseed.org)
How to fix the threshold

- Use of pairs with known genealogy
- Similarity exceeding a percentile point in the distribution of similarities (upper-tail approach)
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Lettuce EDV study

- Three lettuce types: 35 GH, 21 FS & 27 IC
- Long-list made to include most important varieties and maximum variation in each type.
- Varieties collected and shortlist made by ISF secretariat.
- In all ISF studies both varieties and companies are coded.
PCO AFLP data with 95% confidence regions

Explained variation 50%

Colour: popev
Conf. Area: 95%

1
2
3
GH, Jaccard

GH21 and GH22 were selected in the same F4

GH8 and GH11 come from same F3

GH30 and GH35 come from same F3

GH27 is from a cross involving GH25
Lettuce EDV study

- 2004: 0.96 Jaccard similarity for all 3 cultigroups
- Trigger to initiate discussions => amicable settlement => arbitration => court
- Review in 5 years
Oilseed Rape Study

- 4 Studies carried out between 2001-2006
- Bulks of 40 plants have a very high repeatability
- Bulks of 40 plants lead to a clear separation of all the varieties

2007: Dice dist. of 0.85 is trigger to start discussions (assessment according to protocol)

Review in 5 yrs
Ryegrass Study - 1

- 2002 Code of Conduct adopted
- 60 plants/varieté, 5 primer comb.
- Squared Euclidean distance lower than 7=> ask for arbitration
- Apply only to varieties released after adoption
- 2004 concerns by members
- New study initiated
Ryegrass Study - 2

- New study SSR’s (instead of AFLP’s)
- Guidelines (instead of CoC)
- Apply to all varieties
- Court possible, not only arbitration
- 1st Phase: Bulks provide same result as ind. Plants
- 2nd Phase: analyse variability in current varieties => come to threshold.
- Jaccard 0.6 reversal of burden of proof
Guidelines for Handling a Dispute on Essential Derivation in Ryegrass

(Adopted by the ISF Forage and Turf Section, November 2009)

1. The 1991 Act of the UPOV Convention introduced the concepts of essential dependency from an initial variety (i.e.).
2. The Forage Plants Section of ASSINSEL conducted a study in 1997 and tools to determine a possible threshold for assessing putative essential dependency.
3. Based on the results of that study “Principles of a Code of Conduct in Essential Varieties of Perennial Ryegrass” were adopted by the Section in 2002. These replace that Code of Conduct.
4. In 2005 the ISF Forage and Turf Crops Section decided to conduct a new testing protocol using SSR markers on bulked plants instead of using individual plants.
5. As the result of this new research, adequate for:
   a. slow the pace of development of new varieties;
   b. ensure the biodiversity of the bulks;
   c. In the initiation of new varieties
   d. If any

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Table 1. List of 31 SSR markers with fragment size range, number of identified alleles and allele sizes (based on the sizes determined by ILVO, fragment size might vary based on the used platform and laboratory, the use of reference samples is strongly advised, as the specific sizing of the fragments is system- and laboratory-dependent). The data on number of alleles and range correspond to the results obtained in the study described in Roldán-Ruiz et al. (in preparation)

<table>
<thead>
<tr>
<th>Original primer- primer pair</th>
<th>New code name</th>
<th>New primer primer pair</th>
<th>allele range (bp)</th>
</tr>
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<tbody>
<tr>
<td>B1A2a</td>
<td>R01a</td>
<td>F: TTTCAATTGGTTCGCTCGT</td>
<td>17a 116-238a</td>
</tr>
<tr>
<td>B1A8b</td>
<td>R02a</td>
<td>F: TGGAGAGGACCAAGAAGT</td>
<td>11a 180-222a</td>
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<tr>
<td>B1B9a</td>
<td>R03a</td>
<td>F: TACAACTTGGTCGCTCGT</td>
<td>8a 291-403a</td>
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<tr>
<td>B1C9a</td>
<td>R04a</td>
<td>F: GAGGGTTTGAGCTGAGTG</td>
<td>4a 177-184a</td>
</tr>
<tr>
<td>B1C9a</td>
<td>R05a</td>
<td>F: TACGTTGAGTGGTTTCTTT</td>
<td>8a 132-168a</td>
</tr>
<tr>
<td>B26G6a</td>
<td>R07a</td>
<td>F: AGATTTCCAGATACACCACT</td>
<td>7a 99-129a</td>
</tr>
<tr>
<td>B3A1a</td>
<td>R08a</td>
<td>F: GGCAGTTTGGATGGCGAG</td>
<td>3a 309-318a</td>
</tr>
<tr>
<td>B3A3a</td>
<td>R09a</td>
<td>F: GTGACAGGTAAGTGGCG</td>
<td>4a 352-363a</td>
</tr>
<tr>
<td>B3C10a</td>
<td>R12a</td>
<td>F: GGCAGTGGAGTGGGACT</td>
<td>4a 320-323a</td>
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<tr>
<td>B3C50a</td>
<td>R14a</td>
<td>F: TGGACAGGACACACTCGA</td>
<td>13a 190-213a</td>
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<tr>
<td>B3D2a</td>
<td>R16a</td>
<td>F: GAGCAAAATGAGGCTGAC</td>
<td>9a 392-425a</td>
</tr>
<tr>
<td>B3E6a</td>
<td>R18a</td>
<td>F: CAACGCAACAGCTCAAGT</td>
<td>4a 350-354a</td>
</tr>
</tbody>
</table>

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Cotton Study

- Literature review on mol. mrkrs in cotton
- Gen. Div. within allotetraploid cotton varieties => no EDV threshold assigned
- Different approach: parentage
- 2007: If phen. or gen. char's suggest that 2 or more BC's were used or coefficient of parentage value is >87.5% => put. EDV
- Threshold is trigger point for discussions
- No settlement => arbitration
Tomato Study

- Started 2006, Daniella type
- 21 hybrids & 35 parent lines
- 93 SSR markers used for data analysis
- Dice coefficient of 0.78 between F1 and parent line: trigger point for suspected use of proprietary line in production of a hybrid.
- Continuation with cherry type => 0.80 Dice
Maize Study

- 150 SSR markers, highly polymorphic
- Uniformly distributed, 80% coverage
- Avg 2 mrkrs/bin, Distance > 5cM
- Min. 3 alleles/mrkr, PIC min 0.3 [0.6-0.7]

- 2008: At 82% conformity: burden of proof shifts to breeder of put. EDV
- At 90% conformity: strong indication of predominant derivation
Essential Derivation

ISF has adopted arbitration procedure rules tailored to the technical and legal aspects of essential derivation in plant breeding.

Regulation for the Arbitration of Disputes concerning Essential Derivations (RED)

Explanatory notes clarify and provide guidance on numerous provisions of these rules. RED is based on a threshold for essential derivation, measured as the genetic distance between varieties, above which the breeder of the putative essentially derived variety must demonstrate that his variety has not been predominantly derived from the initial variety. The ISF Crop Sections have adopted guidelines for dealing with disputes on essentially derived varieties of perennial ryegrass, maize, oilseed rape, cotton and lettuce. The technical rules for establishing a threshold for essential derivation are also available.

RED does not anticipate any special requirements for the arbitrators and the choice of arbitrators is at the discretion of the disputing parties. To assist parties ISF provides a list of international arbitrators who have the necessary knowledge to handle a dispute on essential derivation.
Intellectual Property

2009
- ISF View on Intellectual Property
- Use of Proprietary Parental Lines of Hybrids

2007
- Hybrids
  - Implementation of Articles 14(2) and 14(3) of UPOV 1991 in Relation to the Phrase: Reasonable Opportunity

2006
- Provisional Protection
  - Use of DNA Markers for DUS Testing, Essential Derivation and Identification

2005
- Essential Derivation from a Not-yet Protected Variety and Dependency
Thank you for your attention