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BMT-TWA/Maize/2/7-a
ORIGINAL: English
DATE: November 26, 2007

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR MAIZE

Second Session
Chicago, United States of America, December 3, 2007

EDV IN CORN:

CONCEPTS OF ESSENTIAL DERIVATION AND DEPENDENCE;
POSSIBLE USE OF DNA MARKERS
THE MAIZE CASE

Document prepared by an expert from the International Seed Federation (ISF)

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CONCEPTS OF ESSENTIAL DERIVATION AND DEPENDENCE

Possible use of DNA markers
The maize case

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Ad Hoc Crop Subgroup on Molecular Techniques for Maize

Chicago, 3 December 2007

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Outline

1. Definition of Essentially Derived Varieties in the UPOV Convention
2. ISF consideration on essential derivation
3. ISF interpretation of article 14.5 of the 1991 Act of the UPOV Convention
4. Assessment of essential derivation
5. Burden of Proof
6. Use of molecular markers, the maize example

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"A variety shall be deemed to be essentially derived from another variety (the initial variety) when

i. It is 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;
ii. it is clearly distinguishable from the initial variety and
iii. except for the differences which result from the act of derivation, it conforms to the initial variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety.

Essentially derived varieties may be obtained for example by selection of natural or induced mutants or of a somaclonal variant, the selection of a variant individual from plants of the initial variety, backcrossing or transformation by genetic engineering."

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ISF consideration on essential derivation

- ISF strongly supports the concept of essentially derived variety
- Only few international agreed-upon professional rules
- Essential derivation is not a new right, but is in the scope of the right of a protected initial variety

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The technical aspect

- Clear distinctness in the sense of the UPOV Convention
- 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
- Predominant derivation from an initial variety

If one of these requirements is not fulfilled, there is no essential derivation

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The legal aspect: dependency

- The initial variety must be a protected one
- Dependence can only exist from one protected variety alone
- It is possible to have a “cascade” of essential derivation. However, a cascade of dependence does not exist

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Assessment of essential derivation

Takes place after establishing that the variety is distinct (DUS) and should 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

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Proof of predominant derivation

Various criteria of combination thereof:

- Combining ability
- Phenotypic characteristics
- **Molecular characteristics**
- Breeding records

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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.

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The use of distance coefficients to define a threshold which would be a trigger point for the reversal of the burden of proof is another interesting approach. Up to now, ISF has mainly worked on thresholds based on distances measured by molecular markers. Geneticists and statisticians consider that technically it is equally possible to measure distance coefficients using morphological markers but that these distances are not always reflective of genetic distances or of pedigree relationships. Additionally, use of morphological characteristics would probably be more difficult due to environmental factors, and much more expensive.

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The threshold would divide the scale of conformity into two parts: below the threshold there would be no presumption of essential derivation, above the threshold there would be presumption of essential derivation and the burden of proof of non predominant derivation would fall on the breeder of the putative e.d.v..

The threshold will certainly vary from species to species, depending on the existing genetic variability within the species and the established breeding procedures.

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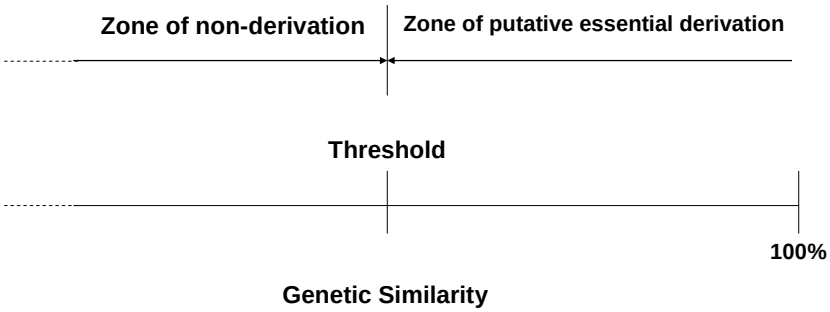
Possible use of a threshold



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ISF recommends to its members, in any case of dispute, to first enter into a conciliation or arbitration procedure according to ISF Conciliation and Arbitration Procedure Rules before resorting to legal action.

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Choice of markers

- The markers must comply with several requirements:
  - Be “freely” available
  - Meet several technical criteria that are addressed in an ISF document “Issues to be addressed by technical experts to define molecular marker sets for establishing thresholds for ISF edv arbitration”

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The maize example: 3 approaches

- The French approach, based on the genetic distance of already protected lines
- The German approach, based on computer simulation and validation by the production of triplets

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The maize example: 3 approaches

- The US approach, in three phases:
  - Phases 1 and 2: Examination of pedigree and DNA markers relationships among material already developed by breeders
  - Phase 3: DNA data from controlled and carefully monitored study reflecting actual breeding methods

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The maize example

- The adopted ISF Guidelines

(see enclosed document)

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Guidelines for the Handling of a Dispute on Essential Derivation of Maize Lines
(Adopted by the Maize and Sorghum Section, May 2007)

1. The 1991 Act of the UPOV Convention introduced the concepts of essential derivation and dependency from an initial variety (i.v.).
2. The ISF View on Intellectual Property (adopted June 2003) supports the UPOV Convention and clarifies various technical and legal aspects of essentially derived varieties (EDV).
3. There have been several studies conducted to determine if genetic markers systems can be used as a tool to determine the possibility that one variety may have been derived from an initial variety. The key papers are:

   a. Identifying Essentially Derived Varieties with Molecular Markers. Heckenberger et al. 2005 TAG 111:598
   b. Study on Essential Derivation in Corn in North America. Charles W. Stuber. North Carolina State University. 2005
   c. Synthesis of Studies conducted by SEPROMA on the estimate of genetic distances between maize inbred lines. B. Andreau, D. Dubrevil, D. Perret, F. Azanza, A, Charcosset. IRNA Station de Genetique Vegetale Ferme du Moulon F-91190 Gif/Yvette, France. SEPROMA 17 rue du Louvre, F75001 Paris, France December 2003

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Guidelines for the Handling of a Dispute on Essential Derivation of Maize Lines
(Adopted by the Maize and Sorghum Section, May 2007)
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An additional study was commissioned using the statistical model used in the Heckenberger et al study and the markers selected for use by the SEPROMA studies. The results are described in a paper ISF EDV Study, May 8, 2006. Martin Bohn, University of Illinois-Crop Science.

The conclusion of these studies is that molecular marker systems can be used to differentiate between inbred lines of maize. It is further concluded that a threshold can be set that could initiate the discussion as to the derivation of one variety from an initial variety.

4. ISF recognizes that marker systems and specific marker sets will change over time as the technologies develop. As of the date of the adoption of this paper, simple sequence repeat (SSR) systems are the most commonly used. The use of this system is most effective when the following criteria are applied:
   a. A minimum of 150 SSR markers are employed
   b. The SSR markers must be highly polymorphic
   c. The SSR markers should be uniformly distributed across the chromosomes – 80% coverage of the genome (minimum of 75 bins)
      i. Average of 2 markers/bin (minimum = 1, maximum =4)
      ii. Distance > 5 Cm
      iii. Minimum of 3 alleles/marker
      iv. PIC minimum 0.3
      v. Average PIC of the set between 0.6 and 0.7

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5. The studies suggest that using a marker set as described in point 4 would indicate, with a low error rate, that EDV might be a question if the homology, based on the Rogers distance, is 82% or higher.
6. The ISF Maize and Sorghum Section does not support a central database on the DUS of maize lines as described by the molecular markers. It is the burden of the inventor of the initial variety to determine if there is a question of EDV. Once the threshold of 82% is determined, other criteria should be evaluated including combining ability, phenotypic characteristics, and breeding records. With a sufficient weight of evidence, the burden of proof shifts to the breeder of the putative essentially derived variety in question.
7. In order to help arbitration in a case of dispute, the ISF Maize and Sorghum Section recommends considering a second threshold of 90% using all the markers as a strong indication of predominant derivation.
8. Because of the rapid pace of the technology development, the threshold and measurement technique described in this paper will be reviewed every five years and adjusted as necessary. In case of change in the measurement technique, the new protocol will be tested against a set of lines used for the establishment of the agreed threshold. This set of lines should be kept in a gene bank.

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Thank you for your attention