



ISF Guidelines for Handling Disputes on Essential Derivation of Maize Lines

(Adopted by the ISF Field Crop Section in Beijing in May 2014)

1. Introduction / Background

- 1.1. The 1991 Act of the UPOV Convention introduced the concepts of essential derivation and dependency from an initial variety.
- 1.2. The ISF View on Intellectual Property (adopted in June 2012) supports the UPOV Convention and clarifies various technical and legal aspects of essentially derived varieties (EDV).
- 1.3. Several studies have been conducted to decide if genetic marker systems can be used as a tool to help determine the possibility that a variety may have been derived from an initial variety (see references). The conclusion of these studies is that molecular marker systems can be used to differentiate between inbred lines of maize. Additionally, it has been determined that a threshold can be set from where to initiate the discussion as to the derivation of a putative derived variety from an initial variety.
- 1.4. 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, single nucleotide polymorphism (SNP) systems are developing and tend to replace rapidly the simple sequence repeat (SSR) systems.

2. The ISF Study

- 2.1. Between 2010 and 2013 a group of ISF experts carried out a study aimed at understanding the impact on the reliability and comparability of data obtained when switching from SSR to SNP methods. The study has matched the Modified Rogers' distance of results obtained from both sets of markers and found them closely correlated; additionally the experts have identified a set of 3,072 SNPs markers that deliver a high correlation with SSR data.
- 2.2. The study has concluded that the use of this SNP set of markers (details in Annex 1) will indicate with a low error rate a putative EDV when the homology based on Rogers' distance is 91% or higher.
- 2.3. Aiming to help arbitration in a case of dispute, the ISF Field Crop Section recommends adopting a second threshold of 95% as a strong indication of predominant derivation.

3. Concluding remarks

- 3.1. The ISF Field Crop Section does not support the creation of a central database on the DUS of maize lines as described by the molecular markers. It is the responsibility of the breeder of the initial variety to determine if there is EDV. Once the threshold of 91% is exceeded the burden of proof shifts to the breeder of the putative essentially derived variety. For completeness of information other criteria should then be evaluated, including combining-ability, phenotypic characteristics and breeding records.
- 3.2. Because of the rapid pace of technology development, the threshold and measurement techniques described in this paper will be reviewed every five years and adjusted as necessary. Higher throughput SNP assay platforms including genome sequencing are already in use. Nonetheless, these assay platforms can also be used to assay the same SNP loci cited here so that the methods described here will not become redundant as new assays are developed to assay SNPs. However, in case of future changes in marker or sequence-based characterization and measurement techniques, it is advisable to maintain in long-term storage a set of lines that can be used for defining the agreed thresholds.

References

1. Heckenberger M., Bohn M., Frisch M., Maurer H.P. and A.E. Melchinger (2005). Identification of essentially derived varieties with molecular markers: an approach based on statistical test theory and computer simulations. *Theoretical and Applied Genetics*, 111(3): 598-608.
2. Heckenberger M., Muminović J., Rouppe van der Voort, J., Peleman, J., Bohn M. and A. E. Melchinger (2006). Identification of essentially derived varieties obtained from biparental crosses of homozygous lines. III. AFLP data from maize inbreds and comparison with SSR data. *Molecular Breeding*, 17(2): 111-125.
3. C.W. Stuber (2007). EDV in Corn: Study on Essential Derivation in Corn in North America - Abbreviated Report on Phase 3. Document No. BMT-TWA/Maize/2/7-d, Second Session of the Ad Hoc Crop Subgroup on Molecular Techniques for Maize, Chicago (USA). The International Union for the Protection of New Varieties of Plants (UPOV): Geneva (CH). http://www.upov.int/meetings/fr/doc_details.jsp?meeting_id=14203&doc_id=177398
4. Andreau B., Dubrevil D., Perret D., Azanza F. and A. Charcosset (2003). Synthesis of the studies conducted by Seproma on the estimation of genetic distances between maize inbred lines. INRA-UPS-INAPG, Station de Génétique Végétale, Ferme du Moulon, F-91190 Gif-sur-Yvette, France, and UFS (Union Française des Semenciers), 17 rue du Louvre F-75001 Paris, France.
5. Kahler A.L., Kahler J.L., Thompson S.A., Ferriss R.S., Jones E.S., Nelson B.K., Mikel M.A. and J.S.C. Smith (2010). North American study on essential derivation in maize: II. Selection and evaluation of a panel of simple sequence repeat loci. *Crop Science*, 50: 486-503.

Annex 1: About the Study on SNP Markers

In 2010 a joint study by ASTA and UFS was undertaken with the purpose of re-calibrating SSR based similarity thresholds to their equivalent SNP based thresholds in the framework of evaluating EDV in maize. A set of 58 inbred lines, representative of US and the EU germplasm, completed with 34 pairs of closely related proprietary lines was analyzed with a publicly available set of 50,000 SNP from the Infinium array technology. A high correlation of pair-wise genetic distances for SNP data compared to SSR data was found. Different subsets were examined and it was established that 3,072 SNPs gave a very high level of correlation and precision ($R^2 = 0.99$; SD 0.004) compared to the overall set of SNPs. Subsets of SNPs were not chosen based on their ability to discriminate the inbred lines but to provide an even coverage of the genetic and physical maps of maize. Thus, the final 3,072 SNPs should be useful to estimate genetic distances between inbred lines from a very broad base of *Zea mays* germplasm.

It was also found that genetic distances estimated using SNP data were generally lower than those estimated using SSRs. Consequently, equivalency to a predetermined SSR threshold required that the SNP threshold be raised in accordance with the different slopes generated by comparing inbred pairwise distances using SSR vs. SNP profiling methods. As a result of this recalibration the study concluded that SSR based maize EDV threshold of 82% similarity should be recalibrated to 91% similarity when using SNPs. Likewise, an SSR threshold of 90% should be recalibrated to 95% when using SNPs.

The list of the 3,072 SNP markers is available in [Annex 2](#).