



## GUIDELINES FOR THE HANDLING OF A DISPUTE ON ESSENTIAL DERIVATION IN LETTUCE

(Adopted by the ISF Vegetable and Ornamental Section in Berlin, May 2004)

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 Vegetable and Ornamental section has conducted, in 2001 – 2002, a study to evaluate the inter-variety genetic diversity based on the use of AFLP. Three groups of varieties were used: 35 Butter Head Greenhouse heated (short-day butterhead varieties for heated glasshouse production), 21 Butter Head Field Summer (long day butterhead varieties for outdoor production) and 27 Iceberg varieties of the Salinas-type.
3. The results of the study are available in the ISF internal report posted on the ISF website and in the following publication (Statistical Aspects of Essential Derivation, with illustrations based on Lettuce and Barley, F.A. van Eeuwijk and J.R. Law, Euphytica, 2004), to which it is recommended to look at for further details.
4. Based on the results of the study the ISF working group has decided to propose a threshold of 0.96 Jaccard similarity for the three cultigroups, taking into account the present diversity available on the market but also having in mind the need to protect effectively future breeding work<sup>1</sup>.  
**The technical protocol is annexed to the present guidelines. It has to be followed precisely for assessing the genetic distance.**
5. The above-mentioned threshold is a trigger point to initiate a discussion between the breeders of the putative initial and essentially derived varieties. If the Jaccard coefficient is higher than 0.96, the breeders will try to reach an amicable settlement. If this settlement cannot be reached, the breeder of the i.v. may ask for the arbitration, according to the ISF rules for disputes settlement and the mediators/conciliators or arbitrators may ask for the reversal of the burden of proof. The breeders of the putative EDV will have to provide the information that is relevant to determining the status of his variety. They may be asked to open their breeding records to an independent neutral expert. (See the ISF arbitration rules for further details)
6. The guidelines shall apply on all varieties subject to national laws.
7. After a period of 5 years the protocol and the threshold shall be reviewed in the light of the experience gained and the technical and scientific evolution.

<sup>1</sup> Thresholds for other lettuce cultigroups (and possibly other species) could probably be determined using the same molecular and statistical approach as for the three lettuce cultigroups of the study.

## TECHNICAL PROTOCOL FOR IMPLEMENTATION OF THE ISF GUIDELINES FOR THE HANDLING OF A DISPUTE ON EDV IN LETTUCE

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### ***Plant material***

Three types of lettuce were included in the study, in order to establish this PROTOCOL:

- Butter head for long day outdoor production (acronym FS; 21 varieties)
- Butter head for short day greenhouse production (acronym GH; 35 varieties)
- Crisp head lettuce, USA Salinas type (acronym IC; 27 varieties)

### ***Seed lots***

When a seed sample has been sent in an official office for protection purpose, that sample, or a sample of the same seed lot, has to be the reference for marker studies in arbitration process.

### ***DNA samples***

A leaf disc will be taken of 10 different plants of each variety and the discs will be pooled. The DNA preparation protocol that is used is described in CJ Steward Jr and LE Via, 1993, Biotechniques 14: 748-750.

### ***Primer combinations***

The following 10 AFLP primer combinations will be used: E33/M59, E35/M48, E35/M49, E35/M59, E35/M60, E38/M54, E44/M48, E44/M49, E45/M48, E45/M49.

### ***Genetic similarity***

Markers will be scored as present or absent, where presence is represented by a score of 1, while absence is scored as 0. All bands, whether monomorphic or polymorphic, will be used to calculate genetic conformity. Conformity between varieties will be calculated by the Jaccard coefficient. This means that for a pair of varieties the similarity will be given by the ratio of the number of markers with bands in both varieties (1,1) on the number of markers for which at least one band appears in either of both varieties (1,1; 1,0; 0,1).

### ***Precision of estimates for genetic similarity***

Standard error for similarity estimates will be obtained by the following procedure. Calculate Jaccard similarity estimates between varieties for each of the 10 sets of markers corresponding to the different primer combinations. In this way, for each pair of varieties, 10 similarity estimates will be obtained. Next, calculate standard deviations across the 10 similarity estimates per variety pair. Finally, calculate standard errors by dividing the standard deviations by  $\sqrt{10}$ .

The use of 10 primer combinations leads to standard errors of about 0.007 and confidence intervals of  $\pm 0.014$  for the similarities in the upper tail of the distribution of similarity estimates. This is accepted by the study group as representing a sufficiently high level of precision.

### ***Threshold***

A threshold for potential essential derivation cases is set at a value of 0.96 Jaccard similarity by means of the tail principle. (1)

### ***New marker systems***

Whenever a new marker system is going to be introduced for essential derivation purposes in butter head or crisp head lettuce, the new system should be calibrated on the AFLP system described in this annex. This means that the rank order of the similarity coefficients between current and new system should be high for the collection (or a representative

subset thereof) of FS and GH varieties used in the present lettuce study. In addition, standard errors for individual similarities should be within the same order of magnitude as found in the present study, i.e., be around 1% of the similarity estimate.

(1) Specific remarks :

*(This principle works as follows. First define the reference set of varieties for the particular problem. As for the lettuce study, there existed a clear separation between the three types (FS, GH, IC) on the basis of cluster-analyses and multidimensional scaling on Jaccard similarities, it was decided to treat initially each lettuce type separately. Next, within a reference set of varieties, order the similarities from low to high. Finally, choose a cut-off point, or threshold, in the ordered series of similarities above which variety pairs will classify as being suspect. Such a threshold will typically be placed somewhere in the highest 5% of similarities. A threshold is well chosen when for variety pairs above the threshold arguments can be given why these varieties are so similar. Simultaneously, no variety pairs known to be closely related should end up clearly below the threshold.*

*The lettuce study group has placed the threshold at 0.96 similarity for all three lettuce types included in the study. This threshold was chosen on the basis of the position of 95 percentiles in butter head groups, FS and GH. The 95 percentile is the similarity value for which it holds that only 5% of the variety pairs has higher similarity. For a number of the variety pairs in the upper 5% tail of the distribution of Jaccard similarities for FS and GH convincing reasons could be given for their inclusion in the upper tail.*

*The crisp head group, IC, had less genetic variation than the butter head groups and its 95 percentile corresponded to a similarity value of 0.98. Still, the lettuce study group argued that the amount of genetic variation found in the butter head groups should serve as a standard to be complied with and for that reason also for crisp head lettuce, a threshold similarity value of 0.96 was installed. Further support for this threshold in the crisp head group was given by the breeding history of many variety pairs with similarities larger than 0.96. Many of such variety pairs might be considered to present examples of derivation.)*