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

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### THE USE OF REFERENCE VARIETIES IN VARIETAL DISTINCTNESS: AN APPROACH UNDER INVESTIGATION IN THE UNITED STATES OF AMERICA FOR POTENTIAL APPLICATION IN PLANT VARIETY PROTECTION

*Document prepared by experts from Monsanto Company*

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#### INTRODUCTION

1. A subcommittee of the United States (U.S.) Plant Variety Protection Office (PVPO) Board, assisted by the American Seed Trade Association (ASTA), has commissioned a study to explore the use of molecular markers in Plant Variety Protection (PVP) for determination of varietal distinctness. This Molecular Marker subcommittee of technical and legal experts, representing both public and private institutions, was formed to advise the U.S. PVPO Board. The objective is to develop robust molecular marker-based descriptors to augment the current morphological descriptors used by the U.S. PVPO. The primary model being explored by the subcommittee utilizes genetic similarity coefficients with a set of reference varieties to determine varietal distinctness. This “reference variety” model is currently being investigated in maize (*Zea mays* L), soybean (*Glycine max*), and lettuce (*Lactuca sativa*).

#### THE REFERENCE VARIETY MODEL

##### Model Overview for Maize

2. The reference variety model utilizes genetic similarity coefficients between PVP candidate subject varieties and a set of pre-determined reference varieties. Reference varieties are selected to provide broad representation of the germplasm in the PVP system. Genotyping is performed with a standard set of SNP markers (ISF, 2014). Genetic similarity coefficients are calculated via pre-determined computational methods.

3. The genetic similarity coefficients between subject and reference varieties would constitute marker-based descriptors for determining distinctness, much in the same way that, for maize, glume color or leaf attitude are current morphological descriptors. Each reference variety is essentially treated as a new ‘trait’ for which the similarity coefficient with the subject variety is calculated (Table 1). It is anticipated that several dozen reference varieties will ultimately be identified within each crop.

##### City Analogy

4. Reference variety identification is an important component in model performance. Consider contiguous U.S. cities in an analogous ‘reference city’ model. If Boston, New York, Philadelphia, Rochester, and Pittsburgh are selected as reference cities, subject cities in the Northeast U.S. are distinguishable by their distances from these reference cities (Table 2). However, if two Western U.S. subject cities are

considered, the model fails to provide adequate distinction between the pair simply because their distances from the reference cities are, in relative magnitude, quite similar. Clearly, additional reference cities in the Western U.S. are needed. In this analogy, an ideal sampling of reference cities would cover the contiguous U.S., with greater representation in areas of higher population density. So it is with reference varieties - they must represent PVP germplasm breadth and must also be dense enough to provide granularity, particularly among more heavily used heterotic pools.

#### Limitations

5. Because the reference variety model is summarizing thousands of base-pair comparisons into several dozen similarity coefficients, there is a loss of information problem – no different from any data reduction method. Therefore the model will, at times, fail to reflect true genetic distinction. However, two fail-safes exist in these cases: 1) morphological characters remain the fundamental means of determining distinctness in U.S. PVP and 2) on a case-by-case basis fingerprints can be used to make similarity comparisons between varieties. The latter is further supported by recent advances in the use of molecular markers for essentially derived variety (EDV) determination (ISF, 2014).

#### Model Features:

- Simplicity in theory and computation.
- Breeders maintain control of fingerprints.
- The U.S. PVPO does not need to store, maintain, or safeguard fingerprints.
- The model fits nicely into the existing PVP framework.
- Genetic similarity coefficients do not replace existing morphological descriptors.

#### Ongoing efforts:

- Reference variety identification.
- Definition of thresholds for distinctness.

#### Example Application

6. Genetic similarity coefficients between six expired PVP maize subject varieties and seven reference varieties are given in Table 3.

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#### REFERENCES

ISF Guidelines for Handling Disputes on Essential Derivation of Maize Lines. ISF 2014.

### Supporting Tables

| Subject Variety | Morph 1 | Morph 2 | ... | Morph <i>i</i> | Similarity Ref Var 1 | Similarity Ref Var 2 | ... | Similarity Ref Var <i>j</i> |
|-----------------|---------|---------|-----|----------------|----------------------|----------------------|-----|-----------------------------|
| x               | 5       | 6       |     | 2              | 0.75                 | 0.80                 |     | 0.84                        |
| y               | 1       | 2       |     | 8              | 0.83                 | 0.78                 |     | 0.89                        |
| z               | 7       | 3       |     | 4              | 0.93                 | 0.84                 |     | 0.75                        |

Table 1 Hypothetical reference variety similarity coefficients in addition to current morphological descriptors.

| Subject City  | Reference Cities |          |              |           |            |            |        |
|---------------|------------------|----------|--------------|-----------|------------|------------|--------|
|               | Boston           | New York | Philadelphia | Rochester | Pittsburgh | Sacramento | Fresno |
| Syracuse      | 423              | 314      | 354          | 120       | 432        | 3806       | 3747   |
| Harrisburg    | 539              | 248      | 151          | 327       | 264        | 3799       | 3719   |
| Albany        | 223              | 217      | 323          | 319       | 575        | 4005       | 3946   |
| San Francisco | 4338             | 4133     | 4055         | 3795      | 3641       | 120        | 261    |
| Los Angeles   | 4174             | 3940     | 3847         | 3638      | 3435       | 582        | 330    |

Table 2 'Reference city' analogy. Distances (km) from subject to reference cities. Without reference cities Sacramento and Fresno, subject cities San Francisco and Los Angeles are poorly distinguished by the model.

| Subject Variety | Reference Varieties |       |       |       |         |       |       |
|-----------------|---------------------|-------|-------|-------|---------|-------|-------|
|                 | B37                 | B73   | C103  | LH38  | LP1NRHt | Mo17  | PH207 |
| DK4676A         | 0.470               | 0.459 | 0.373 | 0.405 | 0.497   | 0.376 | 0.413 |
| LH132           | 0.543               | 0.839 | 0.372 | 0.419 | 0.505   | 0.355 | 0.466 |
| ML606           | 0.484               | 0.427 | 0.439 | 0.733 | 0.429   | 0.464 | 0.484 |
| NK740           | 0.442               | 0.342 | 0.655 | 0.466 | 0.400   | 0.891 | 0.433 |
| NK764           | 0.475               | 0.704 | 0.395 | 0.397 | 0.594   | 0.368 | 0.467 |
| NQ508           | 0.550               | 0.445 | 0.428 | 0.462 | 0.480   | 0.446 | 0.644 |

Table 3 Reference variety model application to expired PVP maize varieties. Similarity coefficients are given between six subject varieties and seven reference varieties of maize. The two most similar varieties here are LH132 and NK764. The two most dissimilar varieties are LH132 and NK740.

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