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MOLECULAR MARKER USE IN THE PVP APPLICATION PROCESS
A JOINT PROJECT BETWEEN THE US PVP OFFICE AND THE AMERICAN SEED TRADE ASSOCIATION

Document prepared by experts from Seed Association of America

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1. The United States Plant Variety Protection Office (US PVP Office) and the American Seed Trade Association (ASTA) have commenced a joint study to explore the technical aspects and define a preliminary approach to utilizing molecular markers in the PVP application process to help determine distinctness between varieties. Our specific approach is to evaluate the utility of marker data to help resolve the issue of distinctness in the case of a morphological tie between an existing variety and a candidate variety. The approach is based on previous work done in maize to define Essentially Derived Variety thresholds using molecular markers (ISF. 2014). Therefore the primary goal of this paper is to provide an overview of the approach under consideration such that it may be applied to any crop species.

STAKEHOLDERS

2. A subcommittee of technical and legal experts representing both public and private entities was jointly formed in August 2014 by the United States PVP Board and ASTA. The joint working group consists of 27 individuals collectively representing 18 public and private institutions. The collaborative nature of the project is critical to success and provides complete transparency between the US PVP Office and all public and private stakeholders involved. No entity is excluded from the project, so additional stakeholders are free to engage in the project at any time either directly or as an observer. Currently, the Community Plant Variety Office of the European Union (CPVO) and the Seed Association of the Americas (SAA) are observers of the project.

GROUNDED IN MORPHOLOGY

3. To be clear, the joint project is not a replacement for morphology in the PVP applications process. Morphology continues to serve as the primary method for DUS serving small and large applicants alike. In fact, the choice of inbred lines and varieties for analysis effectively grounds the project in morphology because all genotypes have either been declared as distinct by PVP authorities using morphology or they constitute the reference sets against which candidate varieties have been evaluated for their distinctness based on morphology. To that end, the project is based upon and performs a similar function as the UPOV approved use of markers to manage reference collections (UPOV, 2013).

DEFINING A WORKING PUBLIC DATA SET

4. We used experience gained from developing the maize EDV SNPs and thresholds (ISF, 2014) as a model for this project. A key prerequisite was therefore to define a data set of inbreds or varieties and SNPs that can be publicly accessed and available to all in the working group to analyze and explore. Here are some of the key features for a working dataset:

Inbred Lines and Varieties

5. The approach is to use only publicly available lines to ensure accessibility to all. The other key component is to ensure that the genetic diversity of the crop species is well represented for the appropriate area of adaptation. Key founders and historically important varieties play an important role since they constitute the genetic foundation of most current inbred lines and varieties. It is also important to select inbred lines and varieties used in the study that have themselves either been declared as distinct by comparisons of PVP authorities on their morphological characteristics or they themselves constitute members of reference sets that are used by PVP authorities in the determination of distinctness. This added criterion is meaningful because all marker results can be interpreted with the knowledge that all varieties have been deemed distinct through traditional morphology DUS testing. In other words, marker thresholds represent a marker based metric which is calibrated directly from morphologically based thresholds in use by the PVP authorities. Marker based thresholds are essentially determined by and grounded in morphology.

Molecular Markers

6. As with varieties, the markers used in the study must be publicly known and accessible to all for use. Generally, markers should be high quality, informative, and evenly distributed throughout the genome and collectively be able to achieve high discrimination ability among inbred lines or varieties. Many crop species have high density marker sets available in number from 1000 – 100,000 markers. It is important to ensure use of a sufficient number of markers to have a low standard error i.e. a sufficiently high level of precision in measuring genetic distance between pairs of inbred lines or varieties.

7. It is also important to define the specific public marker set to be used in the process. This avoids the potential issues of using different marker sets for different comparisons in a given species, which also causes problems when applying a specific threshold for distinctness. Defining the specific markers ensures all analysis is uniform across stakeholders, i.e. the same marker set enables harmonization.

Analysis

8. Detailed molecular marker data of inbred lines and varieties can be considered as divulging proprietary information according to the IP environment. In these circumstances, it is important to be able to utilize genetic distance data calculated using marker data without divulging the marker data per se. To address this issue, the working group is focused on simple pair-wise similarities (or genetic distances) for variety comparisons. This provides a clear interpretation that is scientifically derived, while avoiding issues associated with divulging marker profiles of proprietary varieties.

DEFINING PRELIMINARY THRESHOLD

9. With a working data set defined, pairwise similarities among all public varieties can be calculated. A simple step to begin arriving at a similarity threshold is to analyze the frequencies of all pairwise similarity levels. There will likely be a point where the frequency of pairs drops off at the higher similarity levels. At this point it becomes valuable also to look at pedigree information in relation to these higher levels of similar pairs to evaluate the relationships. For example, if only 1% of all pairs in the study are >90% similar by markers, and there is a strong pedigree relationship, then we would anticipate a threshold above 90%. The next factor to evaluate would be morphology. Since all varieties in the test have been proven distinct, evaluation of morphological traits can be helpful since there may be strong trait differences even though broad genome evaluation produces similarities above 90%.

VALIDATION

10. Once a preliminary similarity threshold is agreed upon based on the public data set, the next step would be to validate the threshold on more recently developed inbred lines or varieties. To avoid intellectual property conflicts, we have agreed that each stakeholder within their respective organization will apply the preliminary threshold using their own proprietary varieties to validate. If the threshold is acceptable to all, then the threshold could be considered for implementation. If some stakeholders disagree with the threshold, then the process can revert back to the public data set to arrive at other threshold options. In addition, more varieties may be added to the public data set to address specific stakeholder concerns.

SUMMARY

11. This overview provides a model approach to expanding use of markers in DUS testing that is consistent with molecular maker uses approved by UPOV. The project requires effective collaboration and transparency through the analytical process and avoids potential issues around intellectual property rights. The use of public data sets to arrive at similarity thresholds is important because the inbred lines or varieties under study have already either been declared as distinct on the basis of comparative morphology, or they are used by PVP authorities as reference varieties for those morphological comparisons. The exercise is therefore one of calibrating SNP data using inbred lines and varieties that have already been judged as distinct on the basis of morphology. The use of a simple genetic similarity calculation provides a means to avoid the potential for IP sensitive issues represented by the raw marker data and requires no special databases or software for examination.

REFERENCES

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