

**Working Group on Biochemical and Molecular Techniques
and DNA-Profiling in Particular****BMT/16/12 Add.****Sixteenth Session
La Rochelle, France, November 7 to 10, 2017****Original:** English**Date:** November 3, 2017**ADDENDUM TO
UPDATE ON THE AMERICAN SEED TRADE ASSOCIATION & U.S. PVP OFFICE MOLECULAR
MARKER WORKING GROUP***prepared by an expert from the Seed Association of the Americas (SAA)**Disclaimer: this document does not represent UPOV policies or guidance***ABSTRACT**

1. In 2014, the United States Plant Variety Protection (PVP) Board and the American Seed Trade Association (ASTA) formed a joint molecular marker working group (MMWG) to explore methods for using DNA markers in determining distinctness, uniformity, and stability (DUS). This working group is comprised of several dozen scientists and observers from both public and private institutions. The MMWG meets at least quarterly and the agendas are technically focused. MMWG members have presented at the fourteenth and the fifteenth sessions of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (BMT) (Achard and Nelson, 2016, Nelson et al., 2014) and several will present at the sixteenth session of the BMT. The MMWG will make recommendations to the U.S. PVP Board on rationale, methods, and procedures for the implementation of DNA markers in DUS determination.

BACKGROUND

2. DNA markers have revolutionized biological and medical sciences where adoption has been swift and application has been broad. Plant breeding is no exception, particularly in the crops of greatest economic importance where competition between breeders drives innovation into the genomic space. Genomic prediction and selection, marker-trait association, seed purity monitoring, and germplasm characterization are just a few examples of DNA marker applications in plant breeding. DNA marker techniques are also used to protect intellectual property of varieties, where DNA-based essential derivation thresholds have been established (ISF, 2014) and civil litigation and even criminal cases within the seed industry have been resolved (Kamm, 1995; Genoways, 2015). The use of DNA markers in these fields goes back a quarter century including a variety of methods following from the use of isozyme molecular markers during the 1980s. DNA comparisons of pods from different trees of Palo Verde (*Parkinsonia aculeate*) were even used to convict a murder suspect (Mestel, 1993; Yoon, 1993).

3. The use of DNA markers with application to DUS has been a topic of discussion for decades (BMT, 1993). UPOV guidelines allow for the use of markers as proxy for traits so long as there is a reliable marker-trait association so that marker data are a surrogate for specific phenotypic characteristics including disease resistance (UPOV, TGP/15 "Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)"). UPOV can also accept the use of markers to help in the organization of reference collections. For example, GEVES applies genotypic data in the management of reference collections allowing varieties to complete distinctness evaluation in a single year when a candidate variety exceeds a marker threshold of "super-distinctness" compared to other candidate varieties and reference varieties. (Maton et al., 2014). The CPVO permits the use of isozymes "as a complement to other differences in morphological or physiological characteristics" in Barley (CPVO-TP/019/3, 2012).

4. Various PVP authorities and seed trade associations have formed working groups with similar goals and expertise as the US MMWG: the IMODDUS working group of the CPVO and the SAA molecular marker working groups are two such examples.

5. As the MMWG explores methods for applying DNA markers in DUS, the focus is on using SNP markers which broadly sample the genome, are of high quality, are publicly available, and collectively have a high capability to distinguish different varieties. For most of these markers, any associations they may have due to genetic linkage with other agronomic traits or morphological characteristics remain unknown.

MMWG PAST

6. Previous work by the ASTA corn variety identification subcommittee (CVIS) included the selection of a set of 3000 SNP markers for use in EDV (ISF, 2014). The MMWG determined that this same set could be used for DUS applications. For soybean, a set of 6000 SNP markers was identified and validated (Achard and Nelson, 2016). Various South American PVP authorities have chosen this same soy 6K SNP set for use in their DUS evaluations.

7. In the United States of America, breeders regard plant genotypic data as information that can be useful to facilitate further breeding. Consequently, they are reluctant to allow detailed marker profiles to be available in the public domain at least during the period of protection of the variety or parental line. This poses a potential challenge if PVP examiners cannot make direct genotype comparisons between pairs of varieties. However, this potential barrier was removed in 2014 when the MMWG developed the “reference variety model” whereby genetic distances to a set of standard reference varieties can be used in varietal comparisons, rather than the raw genotypic data per se (Nelson et al., 2014). Van Ettekoven (2016) expanded this model to fit within the context of a UPOV characteristic. At the sixteenth session of the BMT, Nelson et al. further explored the selection of reference varieties and highlighted challenges and opportunities with the model. This model is potentially useful for DUS, but requires further research to identify the number and identity of reference varieties, databasing, and analyses. The MMWG decided in 2015 to deprioritize work on this model so other higher priority projects could be accomplished.

MMWG PRESENT

8. In 2015, the MMWG shifted its focus toward a simpler approach of examining the use of direct varietal comparisons as supplemental evidence in cases where it is necessary to request additional morphological data in order to assess distinctness, much in the same way that the Community Plant Variety Office of the European Union (CPVO) uses isozymatic data for distinctness evaluation in barley. Such difficulties usually arise when the varieties are from the same breeder. Therefore, the breeder can make the marker comparisons in their own lab and then report the simple similarity data to the PVP office, thus removing the need to provide full genotypic profiles to the PVP office.

9. As with any data-based comparison, a measure of statistical significance via confidence intervals or a least significant difference is needed to guide the practitioner in determining distinctness. For our application, this will take the form of a similarity threshold. If a direct similarity comparison is above this threshold then the PVP examiner will conclude that DNA marker evidence is not sufficient to determine distinctness. Two components constitute a statistically valid threshold, the threshold itself and then applying a genotyping error component to the threshold. The genotyping error component is primarily derived from genetic heterogeneity in the variety or from the lab genotyping process itself. Currently we are addressing genotyping error and have initiated a study which is being presented at the sixteenth session of the BMT (Achard et al., 2017, Nelson et al., 2017). Efforts are ongoing in soybean to establish a distinctness threshold based on analysis of varieties that have already been through the PVP system and have been determined to be distinct on the basis of morphology. Consequently, this comparison of varietal marker differences is grounded within the context of morphology.

MMWG FUTURE

10. At the conclusion of the threshold analysis, the MMWG will make a technical recommendation to the PVP board. This recommendation will include the threshold, with the addition of other supporting evidence, plus guidelines and protocols for genotyping and analysis. These data will also allow a recommendation for an EDV boundary for soybean. These results will be prepared for peer review and publication in a reputable scientific journal.

11. The error analysis and threshold recommendation will be immediately useful to the PVP office and plant breeders. But, it is an ex-post approach, and there is still great opportunity in developing a model which applies DNA-based descriptors at the onset of the application process which will allow the PVP examiner to assess distinctness relative to all varieties of common knowledge. Our threshold analysis lays a foundation for methods and standards in the application of DNA markers to DUS.

REFERENCES

- Achard, F., and B. Nelson. 2016. Evaluation of Soybean Molecular Marker Public Resources for Potential Application in Plant Variety Protection. Retrieved Oct 24, 2017, from
http://www.upov.int/edocs/mdocs/upov/en/bmt_15/bmt_15_13.pdf
- BMT, 1993. The Advantages and Limits of the Use of DNA Polymorphism with Relation to Plant Variety Protection. Retrieved October 24, 2017, from
http://www.upov.int/edocs/mdocs/upov/en/bmt_1/bmt_1_3.pdf
- CPVO-TP/019/3. 2012. Protocol for Distinctness, Uniformity, and Stability Tests. Barley. Retrieved October 24, 2017, from
http://cpvo.europa.eu/sites/default/files/documents/TP/agr/TP_HORDEUM_VULGARE_019-3.pdf
- Genoways, T., 2015. Corn Wars. Retrieved October 24, 2017, from
<https://newrepublic.com/article/122441/corn-wars>
- ISF Guidelines for Handling Disputes on Essential Derivation of Maize Lines. (2014). Retrieved October 24, 2017, from http://www.worldseed.org/wp-content/uploads/2015/10/ISF_Guidelines_Disputes_EDV_Maize_2014.pdf
- Kamm, T. Patented Plums give French Fruit Sleuth his raison D'etre. The Wall Street Journal. September 18th 1995
- Maton, C., M. Thomasset, A. Bernole, A. Remay, B. Aizac, and R. Mathis. 2014. The Use of Molecular Markers (SNP) for Maize DUS Testing. Retrieved October 24, 2017, from
http://www.upov.int/edocs/mdocs/upov/en/bmt_14/bmt_14_10.pdf
- Mestel, R. 1993. Murder trial features tree's genetic fingerprint. New Scientist 29 May 1993 p 6.
- Nelson, P.T., F. Achard, M. Butruille, and S. Madjarac. 2014. 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. Retrieved October 24, 2017, from
http://www.upov.int/meetings/en/doc_details.jsp?meeting_id=34524&doc_id=287358
- UPOV TGP/15. Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS). Retrieved October 24, 2017, from
http://www.upov.int/edocs/tgpdocs/en/tgp_15.pdf
- Yoon, C. K. 1993. Botanical Witness for the Prosecution. Science, 260: 894-895

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