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Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular

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ASSESSMENT OF REPRODUCIBILITY OF 6K SNP GENOTYPING IN SOYBEAN ACROSS LABORATORIES

Document prepared by experts from Seed Association of the Americas (SAA)

INTRODUCTION

1. The United States of America Plant Variety Protection Board and the American Seed Trade Association (ASTA) joint Molecular Marker Working Group (MMWG)[1] are aiming at exploring the use of molecular markers in the PVP application process to help determine distinctness between varieties. This group have done preliminary analysis on exploring using molecular marker genetic similarity threshold to determine distinctness of soybean varieties.

2. The genotyping error or heterozygosity rate of SNP markers may affect the threshold to detect distinctness of soybean varieties. This experiment is to test the repeatability of genotyping across internal and external labs, which will help us to underhand the genotyping rates for further development of sampling guidelines for use of thresholds to establish distinctness in soybean PVPs.

MATERIALS AND METHODS

3. Soybean material— The MMWG selected a panel of 33 varieties with an expired PVP certificate from diverse applicants (universities, public research centers and seed companies), across all maturities, with a PVP certificate issued between 1985 and 1994 (see tables 1 and 2). In addition, the following 5 well known public accessions were included in the panel: Corsoy, Essex, Evans, Lee, Williams 79. The seeds were ordered from GRIN[2], and 20 seeds of each variety were planted in the greenhouse at Monsanto. Leaf samples were collected when plants were 4 weeks old. Leaf tissue from 10 plants for each variety was bulk ground into a powder and stored in a tube.

Applicants	Count
Dairyland Seed Company, Inc.	5
Pioneer Hi-Bred International, Inc.	5
Monsanto Technology, LLC	5
Minnesota Agricultural Experiment Station	3
Novartis Seeds, Inc.	3
Purdue University, Indiana Agricultural Experiment Station	2
Iowa Agriculture and Home Economics Experiment Station	2
FFR Cooperative	1
University of Georgia Research Foundation, Inc.	1
Ziller Seed Company, Inc.	1
Virginia Polytechnic Institute and State University	1
Curators of the University of Missouri	1
Kansas Agricultural Experiment Station	1
Ohio Agricultural Research and Development Center, Ohio State University	1
North Carolina Agricultural Research Service and USDA-ARS	1
Grand Total	**33**

Table 1: PVP applicants of the selected off-PVP varieties

Maturity	Count
0	2
I	5
II	7
III	7
IV	3
V	5
VI	2
VII	2
Grand Total	**33**

Issue date	Count
1985	3
1986	3
1987	3
1988	3
1989	2
1990	5
1991	5
1992	2
1993	3
1994	4
Grand Total	**33**

Table 2: maturity and certificate issue date of the selected off-PVP varieties

4. 96 wells plate design—each of the 38 varieties selected minus 4 slots reserved for controls will be run as a bulk tissue sample of 10 plants, in duplicate. 16 synthetic bulks were also created by mixing tissue powder from different varieties to achieve a high percentage of heterozygote calls, see figure 1. The goal is to assess the error rate of heterozygote calls, which are expected to be different from error rate in homozygous calls. All the samples are run in duplicates to assess the repeatability of genotype calls within a lab, and across all labs.

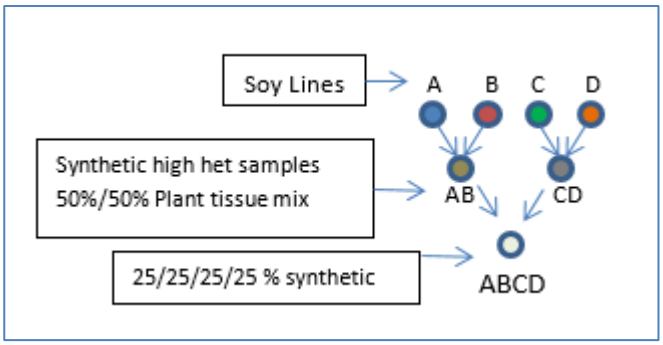

Figure 1: synthetic bulks

5. DNA extraction— DNA was extracted from leaf samples in the lab of Monsanto using protocol of 96-well filter plate method, lysis from lyophilized ground plant tissue, precipitated with potassium acetate, captured on filter plate, wash twice with etoh, eluted in water, then nanodropped to quantify and normalized by adding water to dilute to 30ng/uL.

6. Genotyping—DNA plates were assembled following the design described above and were shipped to the five labs for them to conduct the genotyping (see table 3) on the BARCSoy6K marker platform.

Company	Identity	Contact Name
Neogen GeneSeek	3rd party lab	Jeremy Walker
Eurofins	3rd party lab	Chondra Carlson
Dow AgroSciences	Internal lab	Jon Myrvold
Pioneer	Internal lab	Jamie Rust
Monsanto	Internal lab	Katrina Dickmann

Table 3: list of labs for soybean genotyping experiments

RESULTS AND DISCUSSION

7. The MMWG will have preliminary results to share at the BMT session on the following topics

   a. Compute similarities of like samples within and between labs to establish error rate of homozygous and heterozygous samples.
   b. Calculate percent data return and heterozygosity rate.
   c. Evaluate how genotyping error rates affect threshold of EDV.

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1. Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167, USA
2. Pioneer Hi-Bred International Inc., 7300 NW 62nd Ave., Johnston, IA 50131
3. Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268
4. Seed Science Center, Iowa State University, Ames, IA. USA

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ENDNOTES

[1] The U.S. PVP law (Act) provides for a Plant Variety Protection Board ("Board") to be appointed by the United States Secretary of Agriculture. The Board is composed of 14 individuals who are experts in various areas of development and represent the seed industry sector, academia and government. The duties of the Board are to: (1) advise the Secretary concerning the adoption of rules and regulations to facilitate the proper administration of the Act; (2) provide advisory counsel to the Secretary on appeals concerning decisions on applications by the US PVP Office and on requests for emergency public-interest compulsory licenses; and (3) advise the Secretary on any other matters under the Regulations and Rules of Practice and on all questions under Section 44 of the law. The Joint PVP Board – ASTA Molecular Marker Working Group is a subcommittee which advises the Board and effectively advises the Secretary of Agriculture.

[2] https://www.ars-grin.gov/

[End of Endnotes]