

Technical Working Party on Testing Methods and Techniques**TWM/1/16****First Session****Virtual meeting, September 19 to 23, 2022****Original:** English**Date:** September 14, 2022

THE US PVPO SOYBEAN MOLECULAR MARKER METHOD*Document prepared by an expert from the United States of America**Disclaimer: this document does not represent UPOV policies or guidance***SUMMARY**

The US PVPO has been working closely with the US agricultural industry for more than 10 years to research and develop a reliable molecular method using markers to distinguish two similar soybean varieties on a case-by-case basis. The US PVPO is continuing to assess the method as a confirmatory tool after morphology has been used to initially distinguish the two varieties. The research and method were published by the development team in Crop Science on May 6, 2020. The presentation will provide an overview of the method development and PVPO's plan with regard to the method.

[Annex follows]

US Plant Variety Protection Office

Soybean Molecular Marker Method Update



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Soybean Molecular Marker Method

- Welcome to a short update on the US Plant Variety Protection Office (PVPO) Soybean Molecular Marker Method project
- This has been an ongoing project with PVPO and stakeholders for many years
- As you know, many stakeholders and our office have provided presentations at previous meetings about the progress of the project
- An update was provided to the Plant Variety Protection Board members in December of 2021 on the history and accomplishments of the project
- Here is the short history of how the project was started



History of Soybean Molecular Marker Method Development

- Leading up to 2010, there were discussions at PVP Board meetings regarding the use of molecular marker data to distinguish corn and soybean varieties
- A PVP Board Molecular Marker Subcommittee was created to discuss ideas
- Eventually, the focus shifted from corn to soybean because soybean varieties were perceived as more difficult to distinguish due to the limited number of characteristics
- The ASTA Molecular Marker Working Group and PVP Board Molecular Marker Subcommittee started separately and later merged to develop a method that could be used as a new type of characteristic to help with future distinctness determinations



PVPO Soybean Examination Updates

- While the team was working on the method, PVPO continued to examine soybeans with the following improvements:
 - The US Objective Description of the Variety - Exhibit C form was revised to include additional characteristics
 - Applicants were requested to thoroughly complete the Exhibit C form before submission
 - Other characteristics from the UPOV Test Guidelines were also added
- As a result of these changes, there have been enough characteristics to determine distinctness and complete soybean examinations with little difficulty



PVPO Soybean Method Now

- Moving from 2010 to 2022 we now have a molecular method
- The method was published in the Crop Science Journal in August 2020, and it can be accessed here:
www.betterseed.org/wp-content/uploads/Molecular-Marker-Article.pdf
- The method compares the similarity of two varieties using a Single Nucleotide Polymorphism (SNP) analysis and the percent difference is calculated based on the profiles. Similarity of 96% or less confirms distinctness. The similarity of 97% or higher indicates that the varieties are very similar but does not exclude morphological distinctness
- The reference data for the soybean SNP analysis is available to the public and here is the link:
- <https://soybase.org/>

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PVPO Soybean Method Now

- The method uses the public BARCSoySNP6K data set which includes 5,346 markers
- 10-15 bulk plant samples of the two soybean varieties are analyzed
- If the results show that the two varieties have a similarity of below 96% (inclusive of 96%) then they can be considered distinct from each other
- Two varieties with a similarity above 96% do not preclude distinctness, morphology can show distinctness
- For reference, a 4% difference out of 5,346 SNP markers is 214 individual SNP differences

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PVPO Soybean Method Example

- Here is an example of the results from the soybean method and the morphological characteristic(s) used for distinctness:

Case #1	Applicant Variety	Similar Variety
# Standard morphology insufficient	VarietyXA	VarietyXB
# Additional morphology distinguishable (PVP granted)	Additional Characteristics maturity, protein, oil	
# Molecular markers distinguishable	Similarity 80%-90% range	

Case #1(b)	Applicant Variety	Similar Variety
# Standard morphology insufficient	VarietyWA	VarietyWB
# Additional morphology collected (PVP granted)	Additional Characteristics plant height	
# Molecular markers distinguishable	Similarity 70%-80% range	

Case #3	Applicant Variety	Similar Variety
# Standard morphology insufficient	VarietyZA	VarietyZB
# Additional morphology distinguishable (PVP granted)	Additional Characteristics brown stem rot	
# Molecular markers indistinguishable	Similarity >96%	

These are examples where initial characteristics provided were insufficient to prove distinctness and then additional characteristic data was provided to confirm distinctness. The soybean molecular method was performed on these varieties after protection was granted

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PVPO Soybean Method Example

- Here is another example of the results from the soybean method and the morphological characteristic(s) used for distinctness:

Scenario	Description	6k SNP sim	Candidate Variety		Similar Variety		Ex B Traits
			PVPCERTNUM	Variety Name on Application	Comparison Variety	PVPCERTNUM	
Scenario 1	Indistinguishable using standard morphology, distinguishable using MM, and distinguishable with additional morphology data	<96%	AB1000	AB1000	CD2000	CD2000	Candidate variety AB1000 is most similar to CD2000 however, AB1000 is susceptible to iron chlorosis and is tolerant to Dicamba herbicide, whereas CD2000 is tolerant to iron chlorosis and is intolerant to Dicamba herbicide.
Scenario 3	Neither Distinct by standard morphology and augmented with SNP similarity of over 96%, but other agronomic or physiological trait supports Distinctness (Traditional, but New Approach doesn't preclude Distinctness)	>96%	EF3000	EF3000	GH4000	GH4000	Candidate variety EF3000 is most similar to variety GH4000 however, EF3000 has an elongated seed shape and is tolerant to Dicamba herbicide, whereas GH4000 has a spherical-flattened seed shape and is intolerant to Dicamba herbicide.

These are also examples where initial characteristics provided were insufficient to prove distinctness and then additional characteristic data was provided to confirm distinctness. The soybean molecular method was performed on these varieties after protection was granted

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PVPO Soybean Examinations Now

Here is our plan moving forward:

- We are taking our time to determine the best use of the molecular method as a confirmatory tool
- PVPO will continue meeting with other UPOV countries to gather comments and feedback on the method
- We will continue to examine soybean applications using our current procedures
- We will look for ideal cases to test the molecular method
- When a current case is identified we will contact the applicant to work through the procedure with them
- The method will only be used for confirmation
- We will provide future presentations on the progress of the method assessment

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Meeting Summary

Thank you

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