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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

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

Twelfth Session
Ottawa, Canada, May 11 to 13, 2010

ADDENDUM

THE USE OF MOLECULAR METHODS FOR DETERMINING DISTINCTNESS WITHIN U.S. PVP

Document prepared by an expert from the United States of America

U.S. Update

- Completed
  - ARIPO Regional Training on UPOV Convention in Dar Es Salaam, Tanzania; February 23-26, 2010
  - UPOV Train the Trainers Program in Alexandria, Virgina May 3-7, 2010
- Planning
  - OAPI Regional Program in Cameroon; July 6-9, 2010

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Incoming PVP Applications - Top 10 Crops

FY2009		FY2008		FY2007	
Corn	175	Corn	110	Corn	100
Soy bean	74	Soybean	55	Soybean	68
Wheat	54	Wheat	48	Cotton	53
Fescue	26	Lettuce	28	Wheat	45
Potato	26	Kentucky Bluegrass	25	Lettuce	29
Lettuce	23	Potato	15	Ryegrass	29
Bean	16	Cotton	12	Beans	20
Cotton	15	Ryegrass	10	Potato	11
Kentucky Bluegrass	8	Oat	9	Zinnia	11
Rice	7	Peanut	9	Pea	9
Others	68	Others	91	Others	80
Total	492	Total	412	Total	455

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U.S. PLANT VARIETY PROTECTION

The use of molecular methods for determining distinctness within U.S. PVP

USDA PVPO Overview

- History
  - Formed in 1971 after the passage of the Plant Variety Protection Act (passed December 24, 1970) with 3 staff members
  - First PVP certificate issued in 1973
- Location
  - Located on the 4th floor of the National Agricultural Library on the grounds of the Beltsville Agricultural Research Center (ARS facility) in Beltsville, Maryland.

Plant Variety Protection Office (PVPO)

- The Staff
  - 1 Commissioner
  - 5 PVP Examiners
  - 1 QA PVP Examiner
  - 2 Associate PVP Examiners
  - 2 Information Technology Specialist
  - 3 PVP Program Analyst
  - 1 Student Intern
  - Total: 14 Full Time + 1 Part Time

US PVP Molecular Techniques

The PVPO will accept differences using molecular techniques (DNA fingerprinting) only if:

- The molecular marker locus is publicly disclosed and cited (cites to URLs such as Soybase or MaizeGDB are acceptable);
- the molecular marker locus is clearly identified;
- the specific differentiating data is cited;

US PVP Molecular Techniques

The PVPO will accept differences using molecular techniques (DNA fingerprinting) only if:

- if photographic copies are provided, they contain sufficient resolution of scientific publishable quality gels or other molecular data with sufficient resolution and labeling to resolve the individual data in question are provided;
- •the molecular marker locus can be detected by a third party.

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US PVP Molecular Techniques

- For example, in the case of:
  - SNPs - the locus is defined by the SNP sequence showing the substitution or insertion-deletion polymorphisms (InDels)
  - SSRs - the locus may be defined by primer pairs or sequence
  - AFLPs - the locus is defined by primer pairs
  - RAPDs - the locus is defined by primer pairs

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US PVP - Molecular Markers:

- Must be treated the same as other methods used to establish distinctness (morphology and physiology)
- Must meet the quality controls in place for appropriate supporting evidence
  - For example, when used to establish distinctness, the molecular data must prove that the difference is present in all individuals of the varieties and can be relied upon to prove the distinctness to anyone who performs the tests

Current PVP Use of Molecular Markers

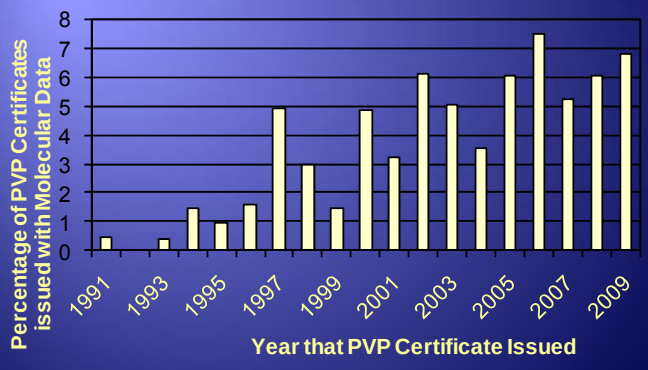
- Differentiate a new variety from a few older varieties that are retrieved by the computer search
  - Often faster than doing grow-out trials to establish morphological differences

Current PVP Use of Molecular Markers

- Establish that the application variety is different from the most similar comparison varieties
  - Often done when a gene has been inserted in the new variety and its presence or absence makes the variety distinct from other varieties

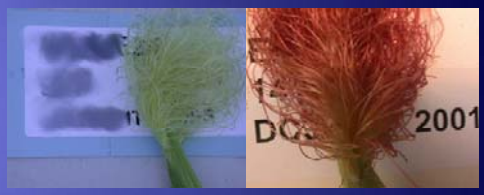
Biomolecular Data Usage Summary

Number of Certificates Issued with Molecular Data cited by the Examiner as a Primary Distinguishing Trait



PVP Application
Visual Information Provided by PVP Applicants

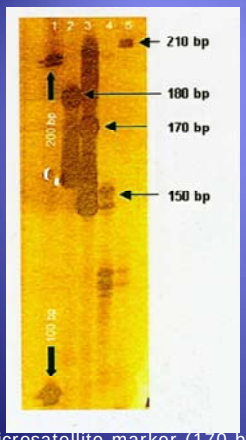
King Henry(PV#9600323) - Top; Tall Guzmaine – Below



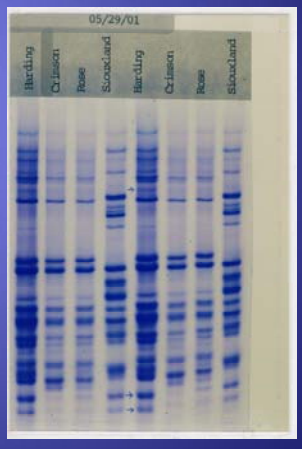
PHHBP (PV#9300108) vs. PH5W4 (200100244) corn

Tropicana Bright Eye Vinca (PV#9200109) vs. Tropicana Blush

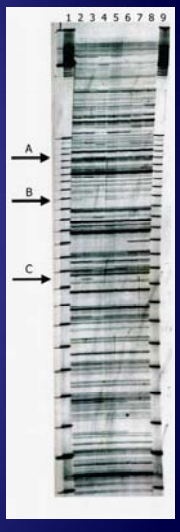
Examples of PVP Biochemical & Molecular Information Utilized



Microsatellite marker (170 bp) present for the subject variety in lane #3 vs. absent in most similar variety (lane 4)



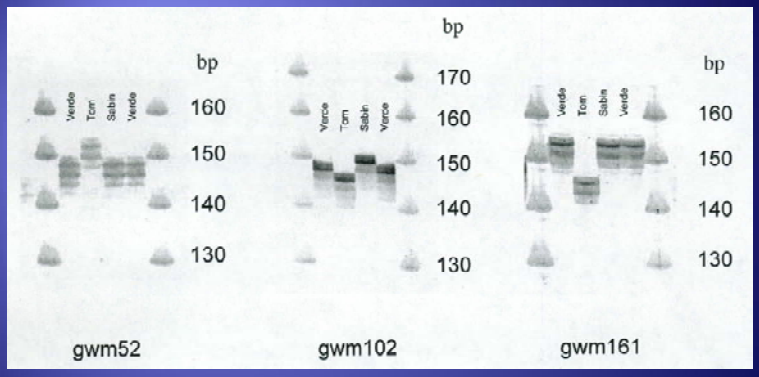
PAGE banding pattern difference



AFLP Markers

Examples – PVP Molecular Evidence Provided

Wheat PV# 200900236

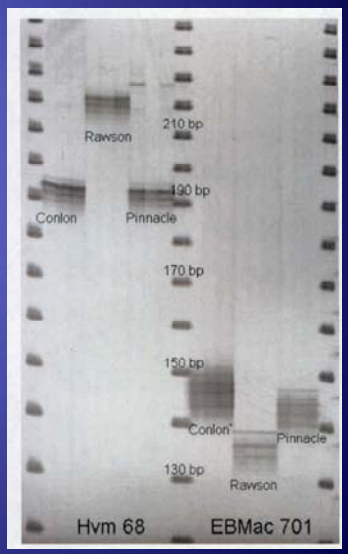


- 'Tom' is most similar to 'Verde'; however, 'Tom' can be differentiated from 'Verde' using the simple sequence repeat (SSR) marker Xgwm52. This marker amplified a fragment of approximately 152 base pairs (bp) from 'Tom' and a fragment of approximately 148 bp from 'Verde'. In addition, 'Tom' differs from 'Verde' using the SSR marker Xgwm102. This marker amplified a fragment of approximately 147 bp from 'Tom' and a fragment of approximately 149 bp from 'Verde'. Also, 'Tom' differs from 'Verde' using the SSR marker Xgwm161. This marker amplified a fragment of approximately 146 bp from 'Tom' and a fragment of approximately 154 bp from 'Verde'.

Examples – PVP Molecular Evidence Provided

Barley PV# 200800160

'Rawson' is most similar to 'Conlon' and 'Pinnacle'; however, 'Rawson' can be differentiated from 'Conlon' and 'Pinnacle' with simple sequence repeat (SSR) markers. Using the Scottish Crop Research Institute (SCRI) Hvm 68 marker, 'Rawson' has a 218 base pair (bp) band that is not found in 'Conlon' and 'Pinnacle'. In addition, using the SCRI EBMac 710 marker, 'Rawson' has a 137 base pair (bp) band that is not found in 'Conlon' and 'Pinnacle'.

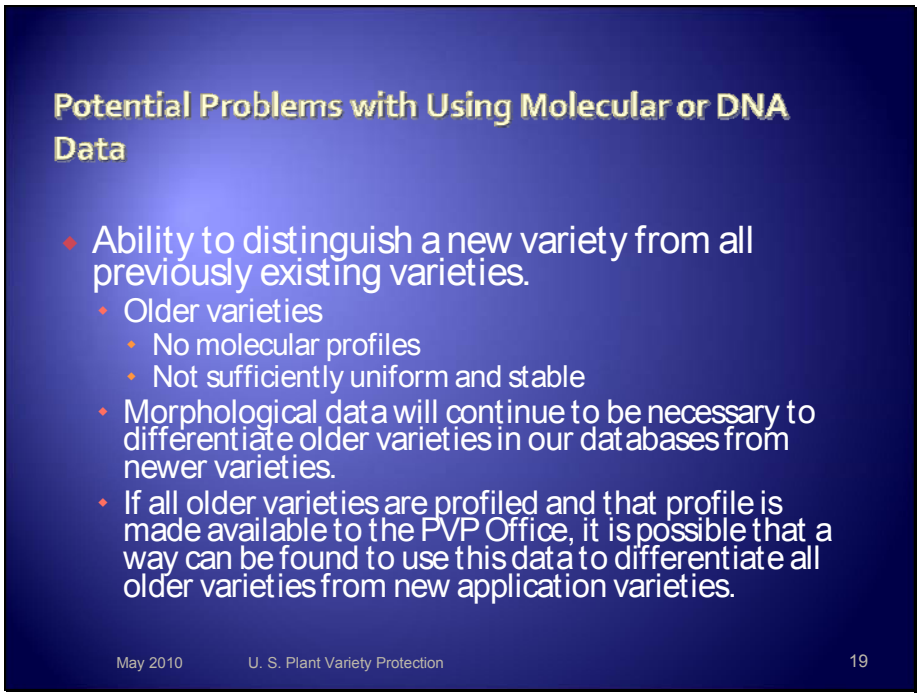


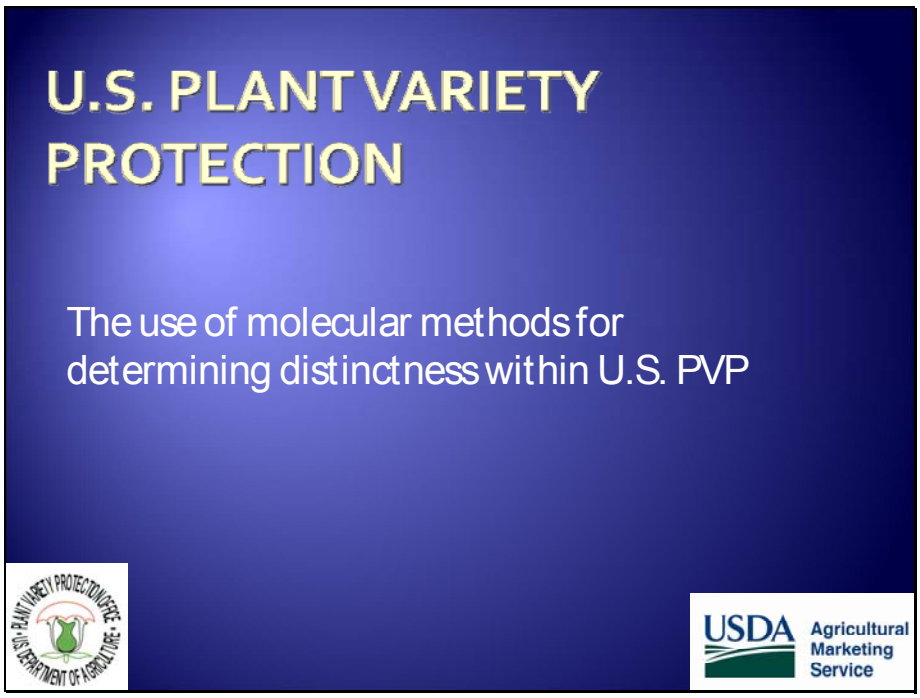
U.S. PVP
Issues on Using Molecular Data

- Use of molecular data must be treated the same as other methods used to establish distinctness.
- Tests - Need to use published procedures and reagents that are available to everyone who wants to perform the tests.
- Distinct Difference – Must be uniform and stable
  - Show that the tests were done on more than one individual
  - Two or more generations.

Potential Problems with Using Molecular or DNA Data

- Repeated tests
  - Show differences exist between all individuals of the varieties
  - Differences do not change over time.
- Issue of the sole basis for distinctness





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