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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

***AD HOC* CROP SUBGROUP ON MOLECULAR TECHNIQUES
FOR MAIZE**

Second Session

Chicago, United States of America, December 3, 2007

USE OF MOLECULAR MARKERS IN INTELLECTUAL PROPERTY PROTECTION

Document prepared by experts from the United States of America

Slide 1

Use of Molecular Markers in Intellectual Property Protection

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Why describe varieties?

- To obtain Intellectual Property Protection (IPP)
 - Plant Variety Protection (PVP)
 - Meet DUS standards:
 - Distinct from all previous varieties
 - Uniform
 - Stable
 - Variety Utility Patent
 - Meet DUS criteria (plus others, e.g. inventive step)
- To enforce IPP
 - Identify Essentially Derived Variety (EDV) status under PVP
 - Identify cases of misappropriation
- For variety identification
 - Seed certification



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Variety

Description compared to Identification

- Description is the first step
 - Test if the new variety meets DUS criteria
 - A new variety is only established when DUS criteria are met
 - Morphological characteristics are the primary descriptors
- Tests of varietal identity or pedigree occur AFTER a variety has been initially described for DUS.
 - Test of varietal identity or pedigree use:
 - Molecular Marker data
 - And/Or
 - Morphological data
 - And/Or
 - Pedigree records

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Morphology characteristics

- Some are measured (e.g.)
 - Ear length



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Morphology characteristics

- Some are records of color (e.g.)
 - Silk color




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Challenges with morphology

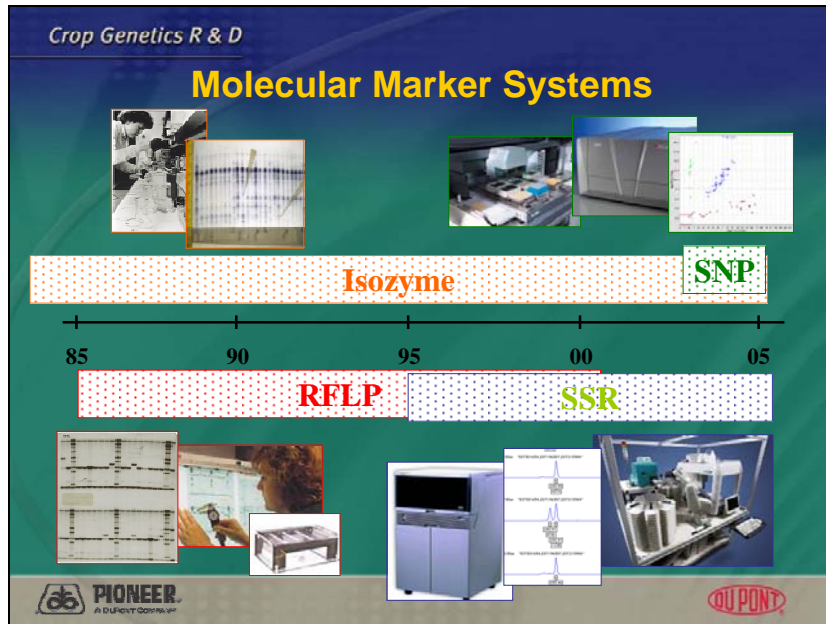
- Genotype x Environment Interaction
 - Ex: Distinguishing effects of sunlight on silk and glume color



- Requires replicated field trials
 - Significant resource needs
 - Sometimes requires 2-3 years
 - Costly
 - Challenging to create large databases
- Unknown genetic control
 - Cannot test parentage
 - Limits use for enforcement
 - Poor test of genetic conformity
 - Limitation for EDV determination

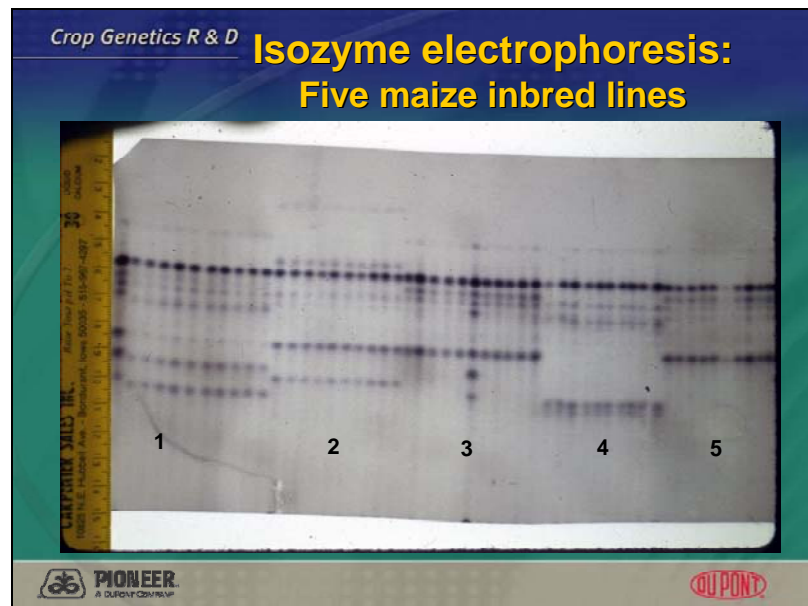
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During the last 25 years there has been a rapid evolution of technologies from isozymes (still in use today—for example, an important component of the French GEVES authority approach to DUS testing) to Simple Sequence Repeats (SSRs) and, most recently to Single Nucleotide Polymorphisms (SNPs). The speed of these changes and the fluidity of the markers has itself lead to difficulties in deciding upon one system and the development of standardized procedures. However, SNP loci appear to be the method of choice because one cannot go to a higher level of detail than the level of the individual nucleotide. And although platforms for interrogating SNP loci will likely continue to change the SNPs data that have already been obtained using older platforms will remain valid.

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Here is an example of an isozyme. Four of 5 different inbred lines (10 individual plants of each) of maize can be distinguished by this isozyme system, Malate Dehydrogenase, MDH. Read the profiles as vertical rows of bands; controls are placed to the left adjacent to the ruler and in the centre.

STRENGTHS

Reports expressed gene products

LIMITATIONS

Not 100% unique inbred identification (approx 85%)

Not sufficient genomic coverage for EDV determination

Not usually associated with specific DUS morphology or agronomic traits

Old technology (1970s and 1980s), relatively slow and cumbersome, increasingly difficult to find laboratories able to run isozymes

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Simple Sequence repeat

Read these SSR profiles of maize inbred lines as vertical rows of bands. Red bands are internal controls to calibrate molecular weights. You can see the upper cluster of blue bands denote show inbred lines have 4 different alleles at that SSR locus. By the time data from 30 or more loci have been recorded the SSR profiles provide what is essentially a fingerprint.

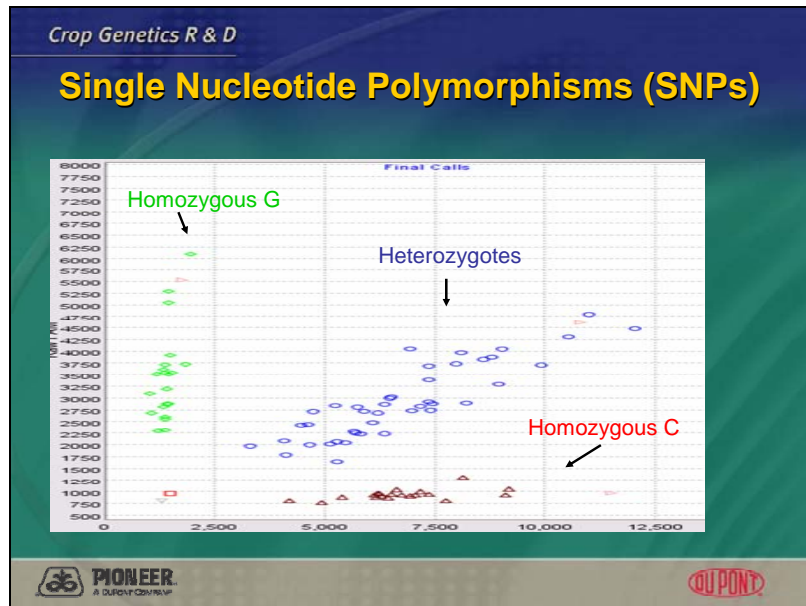
STRENGTHS

100% unique inbred identification for many crops (except for single gene conversions)
Sufficient genomic coverage for EDV determination

LIMITATIONS

Not usually associated with specific DUS morphological characteristics
Relatively resource and time consuming
Challenging to compare profiles across different laboratories
Technology being replaced by SNPs for many crops

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Here a segregating population of maize individuals is being interrogated as to whether they are homozygous for the G allele (green) or homozygous for the C allele (red) or heterozygous (blue). By the time profiles from 30 or more SNP loci are interrogated each maize inbred essentially has a fingerprint.

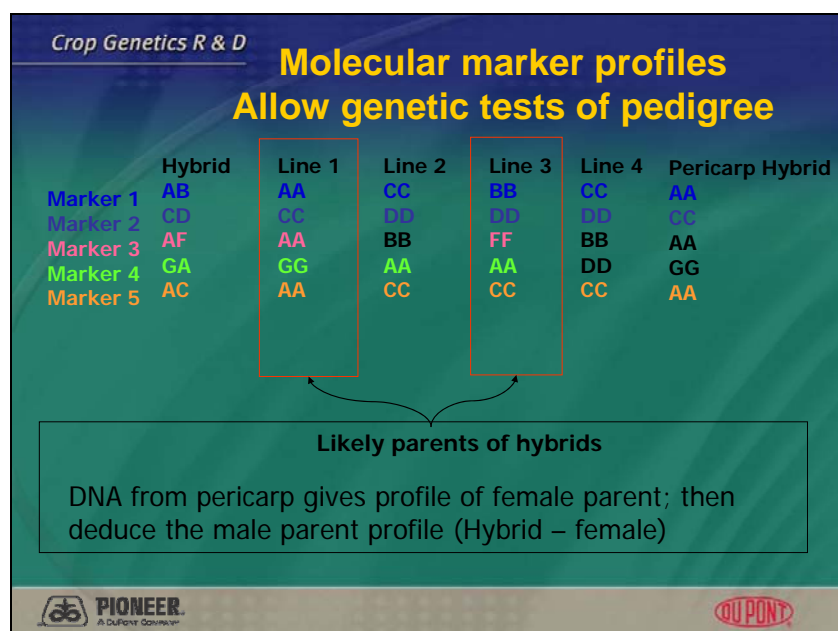
STRENGTHS

- 100% unique inbred identification for many crops (except for single gene conversions)
- Sufficient genomic coverage for EDV determination
- Comparable among different laboratories

LIMITATIONS

- Not usually associated with specific DUS morphological characteristics

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Here is one example of how marker data can be interpreted. These analyses form the foundation for determining parentage of inbred lines and of hybrids.

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Actual and Potential Use of Markers: Subject matter to be discussed

- Actual
 - Variety Identification
 - Essentially Derived Varieties
 - Variety Description for Plant Variety Protection (PVP)
 - Distinctness, Uniformity and Stability (DUS)
- Potential
 - Future possibilities

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Use of Markers: Variety Identification

ISF position 2006

- “DNA markers may be used for the identification of an already-protected variety”
 - In particular for:
 - Alleged misuse of that variety
 - Misuse of a parental line of a hybrid

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Use of Markers: EDVs

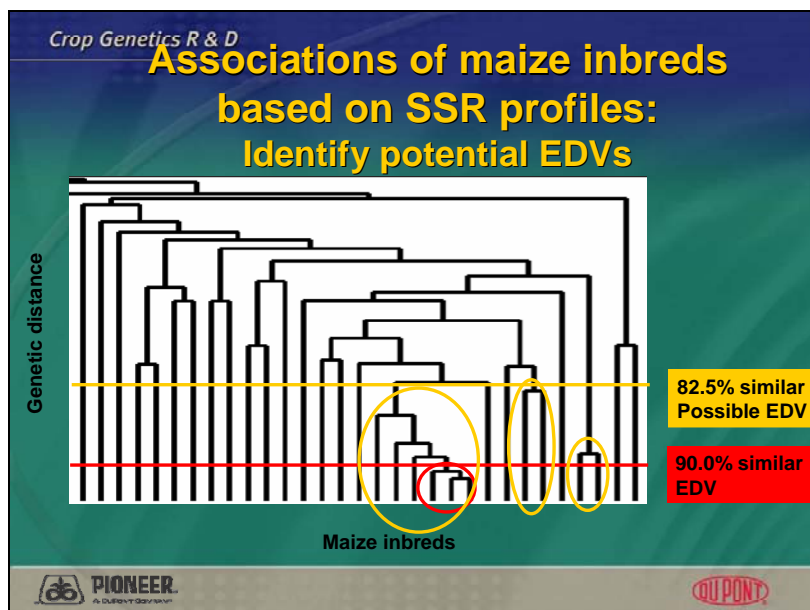
ISF Position 2006

- “DNA markers may be used to define genetic similarity trigger points for starting a dispute resolution process in cases of alleged essential derivation”
 - ISF codes of conduct and technical protocols
 - maize –ryegrass –lettuce –oilseed rape

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Use of markers in DUS: Why?

- Challenges with morphological characteristics
 - Large numbers of known varieties (reference collections) difficult to maintain and examine:
 - » Increased number of new varieties
 - » Enlarged European Union and new UPOV members
 - G x E interaction: multiple locations; time and cost
- Actual and potential use of marker data
 - In review by UPOV Biochemical and Molecular Techniques (BMT) working group
 - Options:
 - » Use as supplementary data
 - » Use as surrogates for existing characteristics
 - » Use to organize reference collections
 - » Develop a new system

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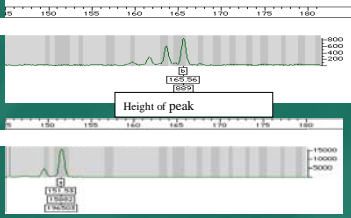
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Current use of markers in DUS: As supplementary data

- Accepted by US PVP Office to show Distinctness
 - ONLY IF:
 - Procedures published and reliable,
 - Third party can duplicate
 - Cite or show specific marker differences:



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Supplementary data provided to PVP Office in circumstances where none of the regular traits show sufficient degree of distinctness or statistical confidence

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Current use of markers in DUS: As surrogates for DUS characteristics

- A gene specific marker of a phenotypic characteristic
 - Directly linked to the phenotypic characteristic
 - Useful when characteristic cannot be easily recorded
 - e.g. disease resistances
- UPOV:
 - Acceptable within terms of UPOV convention
 - Would not undermine effectiveness of protection offered under the UPOV system

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Current use of markers in DUS: Manage Reference Collection

- Manage reference collections:
 - Examine distances between varieties:
 - Compare morphological data with marker data
 - Set a “Distinctness Plus” marker threshold as a pre-screen for field evaluation
- Goal:
 - Exclude from full field comparisons varieties that are predicted by marker comparisons to be very different (“super-distinct”) morphologically.

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Use of markers in DUS: Manage reference collection

- Procedure currently used by GEVES (France)
- Pre-screen prior to full field trials
 - Use morphology and isozymic data
 - Morphology contribution is at least 33%
 - Calculate index for each pair of varieties
 - Index of ≥ 6 is super-distinct
 - ACTION: No further comparisons
 - Index of < 6
 - ACTION: Further comparisons using morphology

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Potential use of molecular markers as characteristics in DUS

- Issues would include:
 - Identify a standard set of publicly available markers
 - Demonstrate reliability
 - Determine threshold levels for judging distinctness
 - Do not undermine minimum distance
 - Determine how to conduct U and S
 - Unnecessary and unrealistic standards
 - Costs to breeders
- UPOV:
 - Needs additional research to thoroughly analyze impact compared to existing system
 - Current focus is on making existing system more efficient using markers (Managing Reference Collections)

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Potential use of markers for DUS ASTA position paper July 7, 2006

- “Markers could be potentially useful in DUS”:
 - increase efficiency, speed, reduce transaction costs
 - but must maintain or increase current levels of protection
- “DUS authorities should address issues including”:
 - public availability of molecular markers,
 - different levels of technological expertise and resources among breeders,
 - cost.
- “Morphological characteristics should continue to provide the foundation for DUS”.

ASTA Position Paper on the Use of Molecular Markers in DUS Testing

July 7, 2006

ASTA supports changes that increase the efficiency, speed, informativeness, and/or reduce transaction costs of the current DUS procedures while maintaining, or increasing, current levels of protection afforded by Plant Variety Protection (PVP). ASTA currently believes that morphological characteristics should continue to provide the foundation for DUS. There are a number of reasons for this belief, including familiarity and experience with morphological traits.

The use of molecular markers in plant breeding and plant variety identification has increased considerably in many crop species. Progress has been made in terms of technologies, cost, species and informativeness. ASTA believes that DUS testing authorities should begin considering and addressing issues that could arise, if molecular marker data would in the future, be incorporated into the DUS system.

Member companies foresee the potential usefulness of marker applications for DUS. However, there are a number of issues that will need to be addressed to facilitate the utilization of molecular markers in DUS. Further studies should be conducted to address issues such as: public availability of informative markers, differences between crop species for availability of markers, levels of technological expertise and resources between breeders, the extent to which markers need to define the phenotype to be useful for DUS, and cost, amongst others.


Until these studies are conducted, ASTA believes that morphological characteristics should remain the foundation for determining DUS.


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Potential use of markers for DUS ISF position paper 2006

- “Opposes use of marker data alone for DUS until address”:
 - Definition of minimum distance
 - Maintain existing standards
 - Impact on U and S
 - Impractical standards of U and S
 - Costs of determining U and S during breeding
 - Public availability of markers

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The ASTA and ISF positions are very closely aligned; fully aligned on the challenges and questions that must be satisfactorily addressed.


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Conclusions

- Maintaining effective IP is crucial to research investments
 - Markers are increasingly an integral component of plant breeding including in MAS, varietal purity, and for maintaining IP
- Markers are increasingly used to maintain IP
 - ISF position on variety identification
 - ISF position and guidelines on EDVs
 - Markers have been used in litigation
 - To identify misappropriation
 - To validate pedigrees

(continued)

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The slide features a dark blue header with the text 'Crop Genetics R & D' in white. Below the header, the word 'Conclusions' is written in a large, bold, yellow font. The main content is a bulleted list in white text on a dark green background. At the bottom of the slide, there is a grey bar containing the 'PIONEER A DUPONT COMPANY' logo on the left and the 'DU PONT' logo on the right.

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Conclusions

- Markers will be increasingly utilized for IP protection in the future:
 - Marker data already used in variety patents
 - Marker data already used in PVP as supplementary data to prove distinctness
 - Envision that marker data will be used in lieu of some/all morphological characteristics for DUS in the future

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Envision that molecular marker data will be used in lieu of some/all morphological characteristics for DUS PVP authorities gaining experience using molecular marker data to manage reference collections.

UPOV is actively engaged in examining data and implications.

Seed associations have determined issues of concern and closely monitor developments.

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