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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

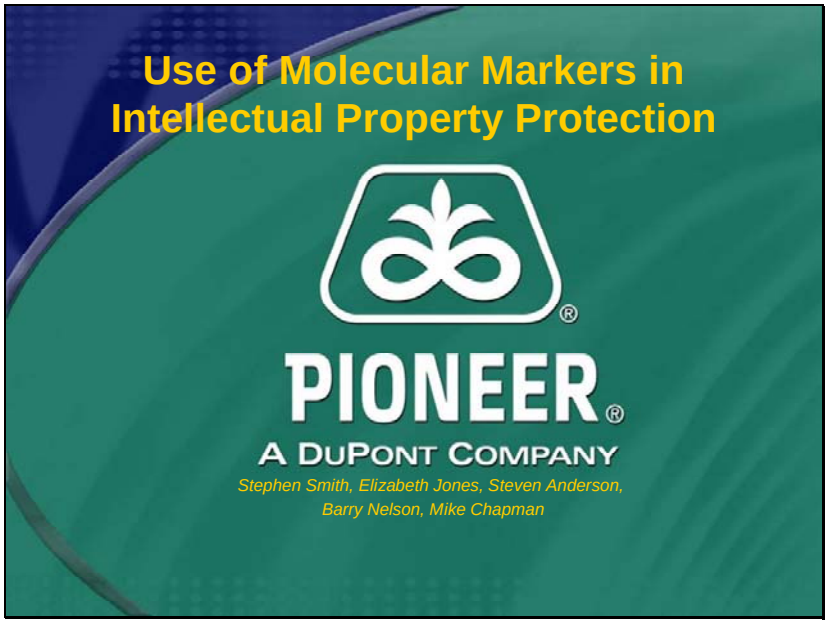
AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR MAIZE

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



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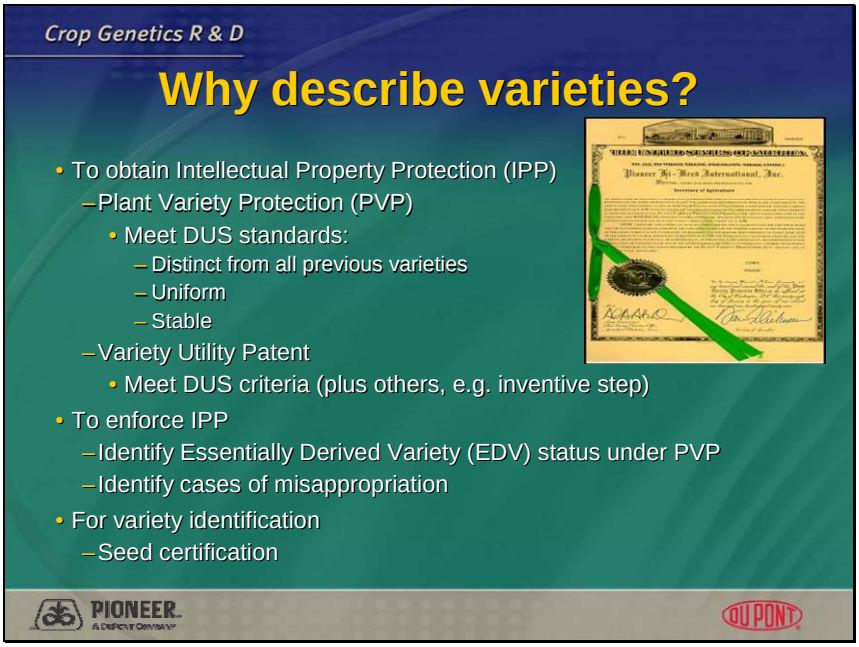

Slide 3

Crop Genetics R & D

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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Slide 4

Crop Genetics R & D

Morphology characteristics

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

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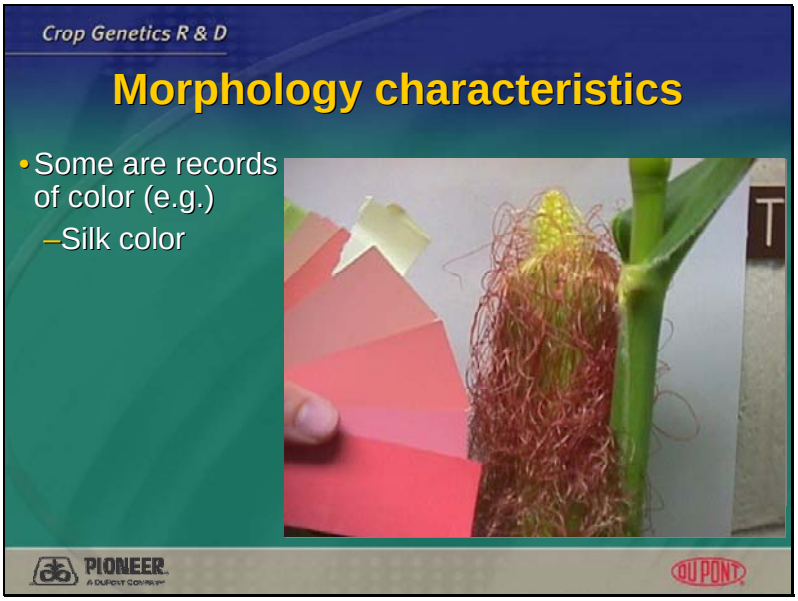

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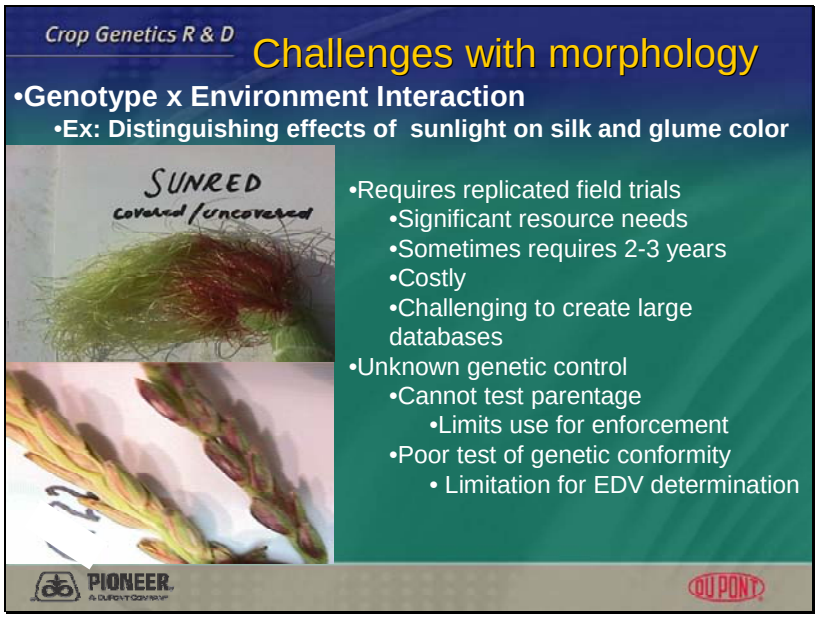

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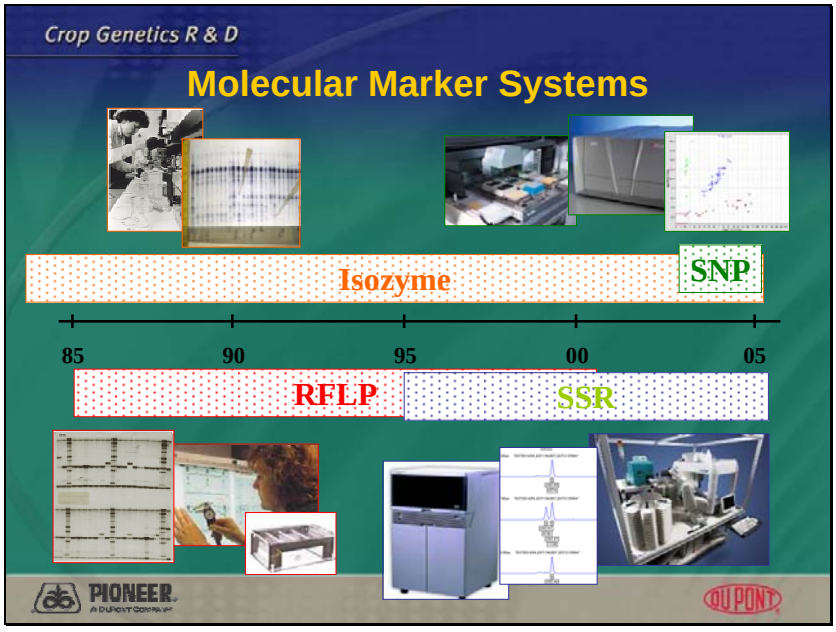

During the last 25 years there has been a rapid evolution of technologies from isozymes (still in use today—for example, an important component of the French GEVES authority approach to DUS testing) to Simple Sequence Repeats (SSRs) and, most recently to Single Nulceotide Polymorphisms (SNPs)s. The speed 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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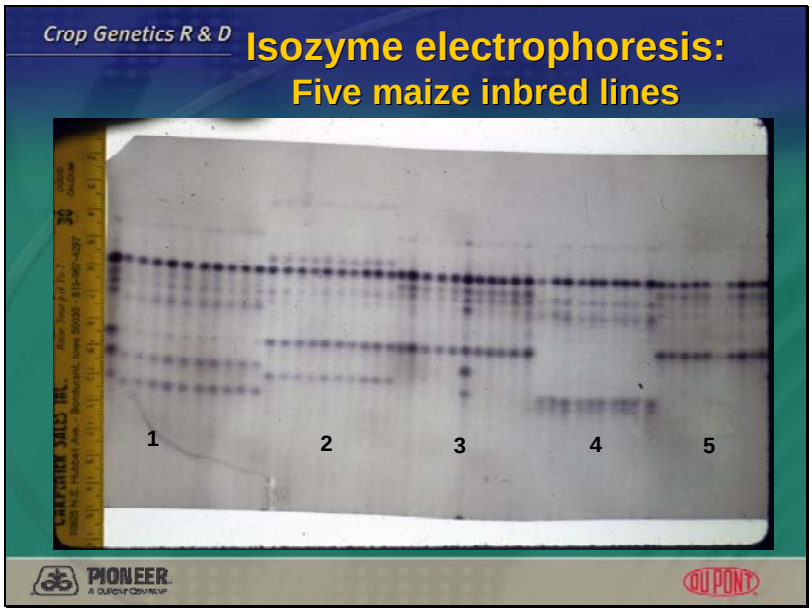

Here is an example of an isozyme. Four of 5 different inbred lines (10 individual plants of each) of maize can be distinguished by this isozyme system, Malate Dehydrogenase, MDH. Read the profiles as vertical rows of bands; controls are placed to the left adjacent to the ruler and in the centre.

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

Slide 9

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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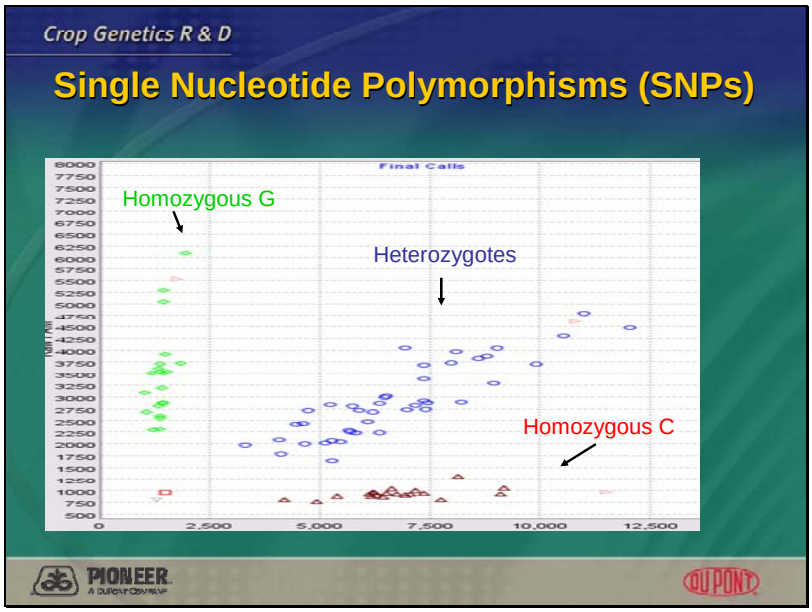

Here a segregating population of maize individuals is being interrogated as to whether they are homozygous for the G allele (green) or homozygous for the C allele (red) or heterozygous (blue). By the time profiles from 30 or more SNP loci are interrogated each maize inbred essentially has a fingerprint.

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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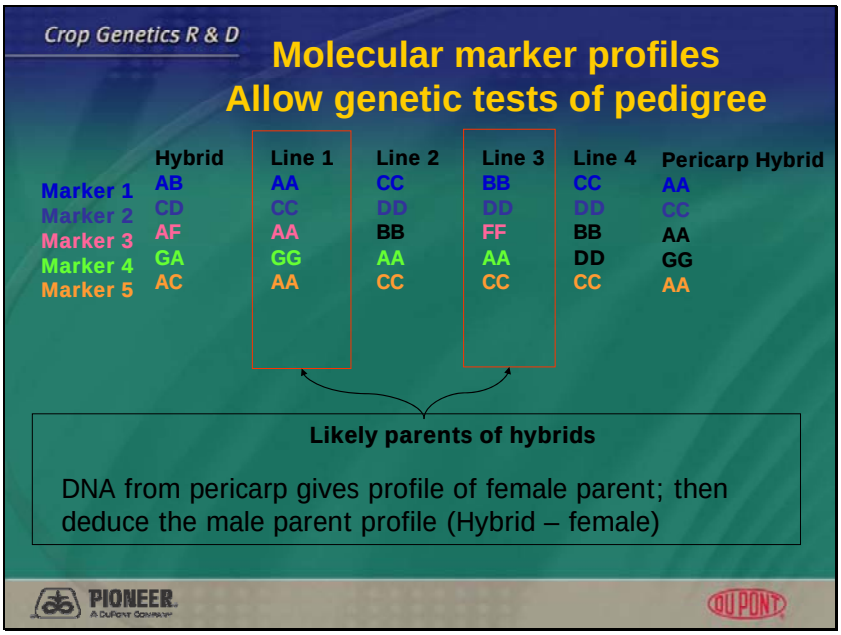

Here is one example of how marker data can be interpreted. These analyses form the foundation for determining parentage of inbred lines and of hybrids.

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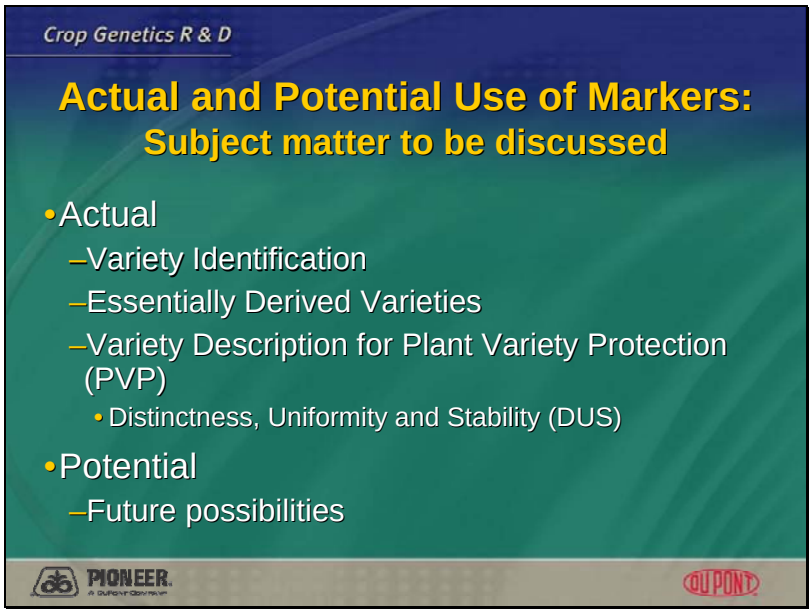

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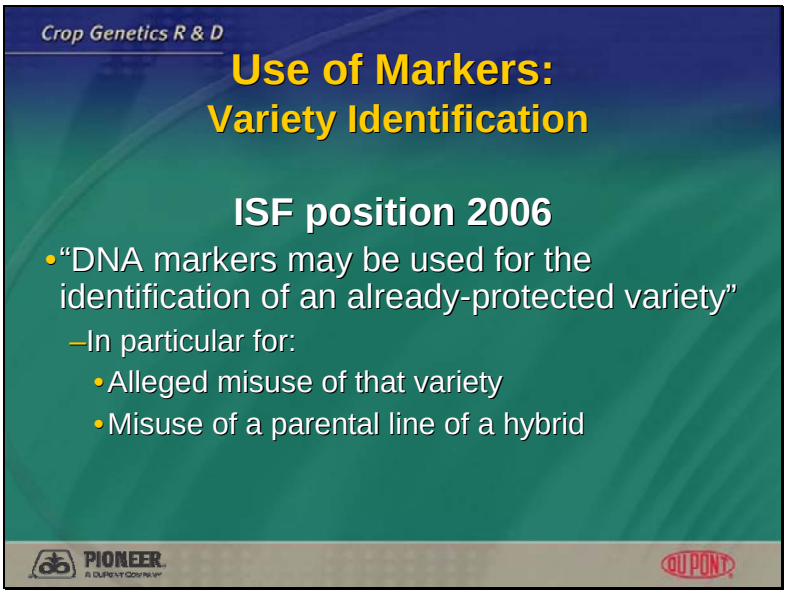

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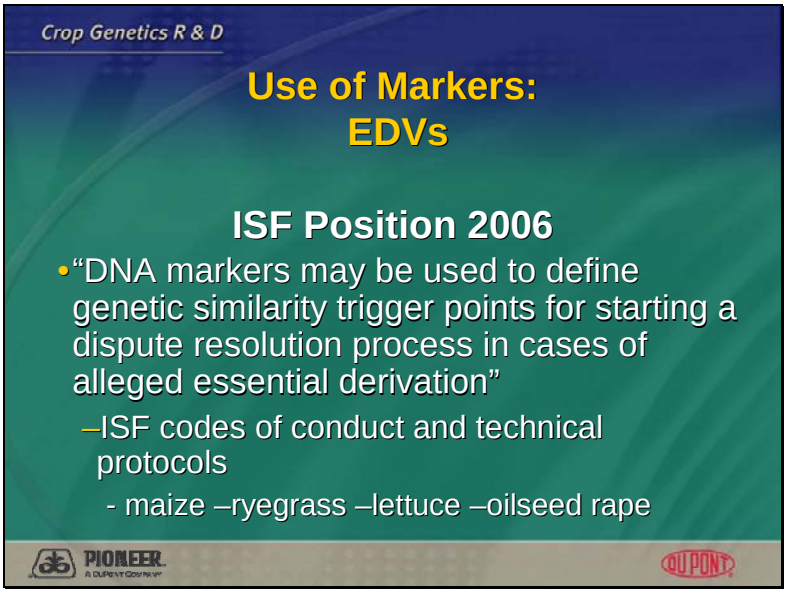

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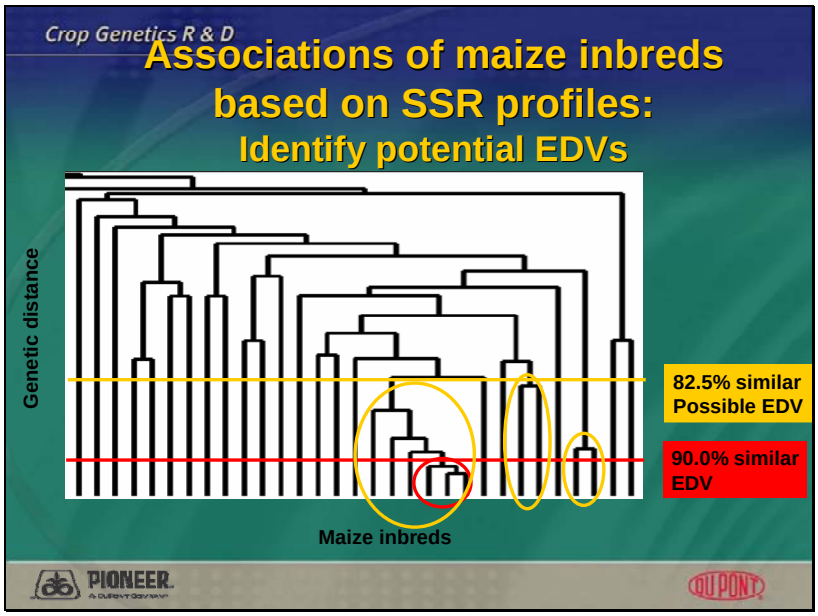

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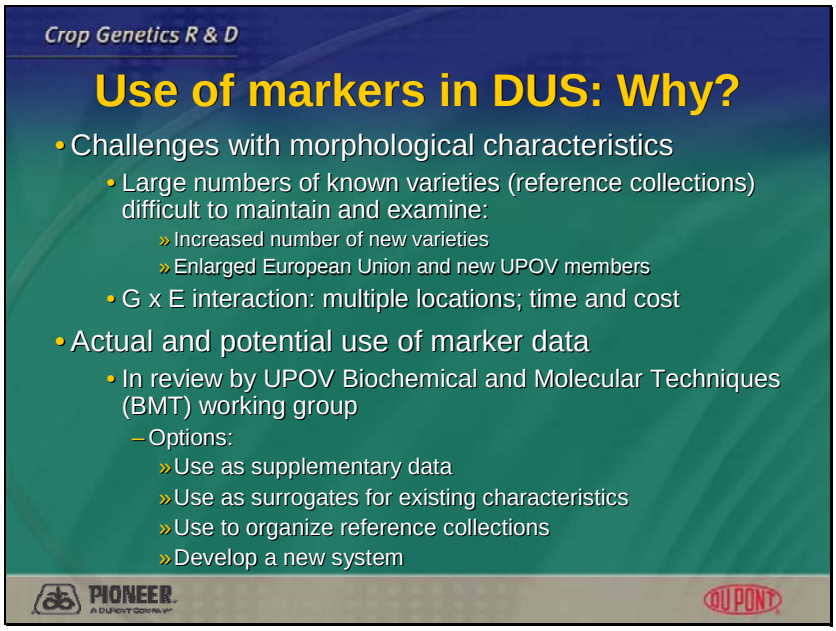

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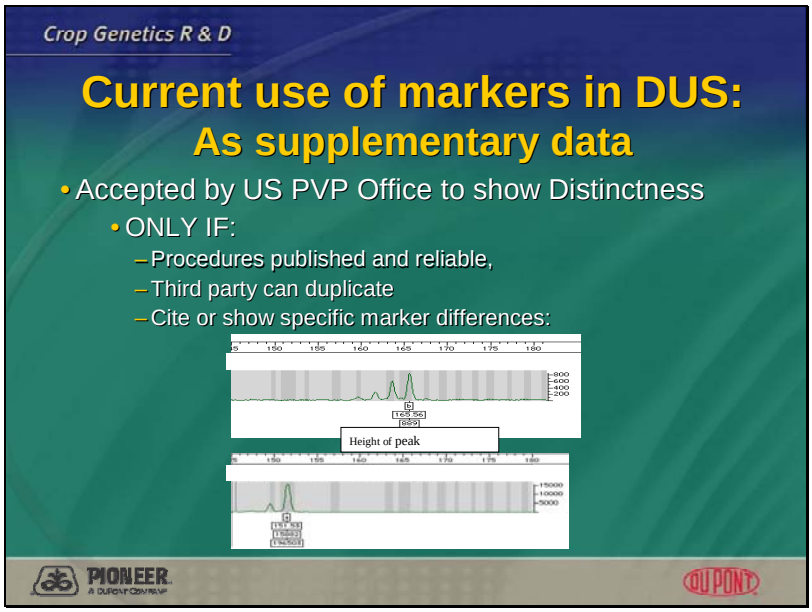

Supplementary data provided to PVP Office in circumstances where none of the regular traits show sufficient degree of distinctness or statistical confidence

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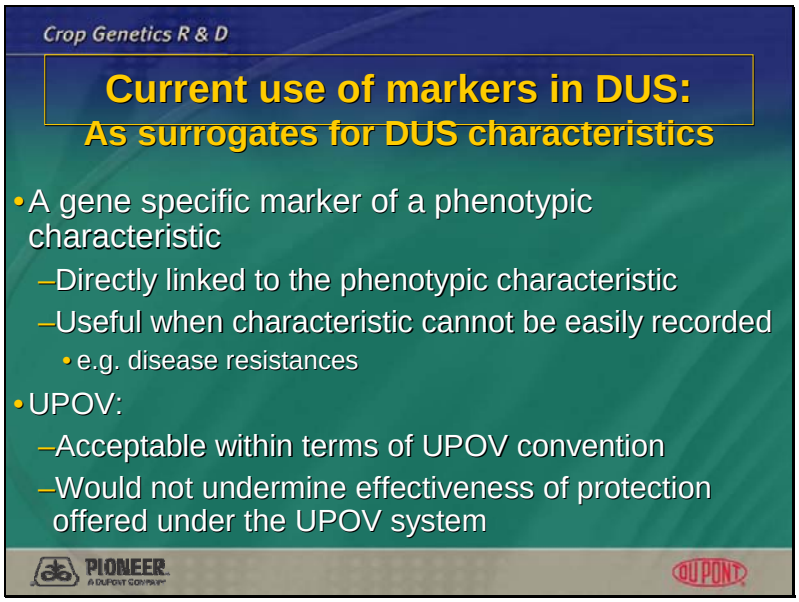

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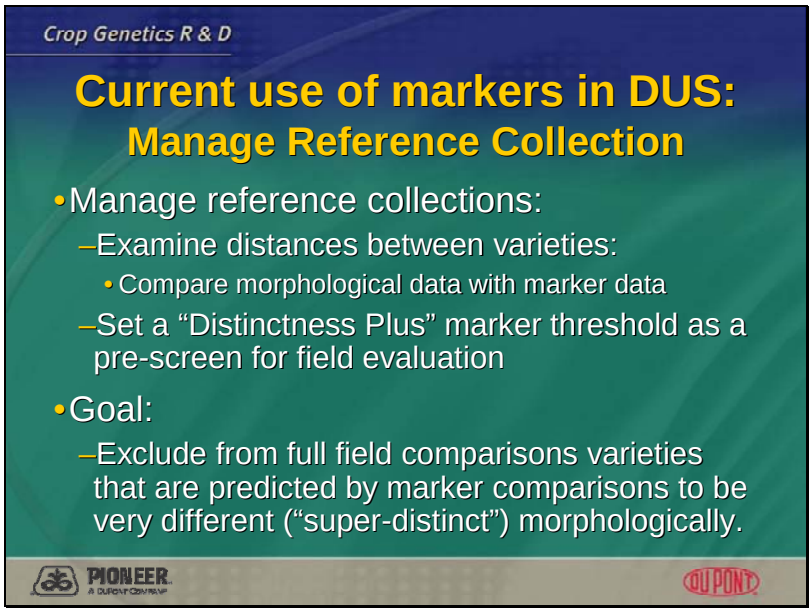

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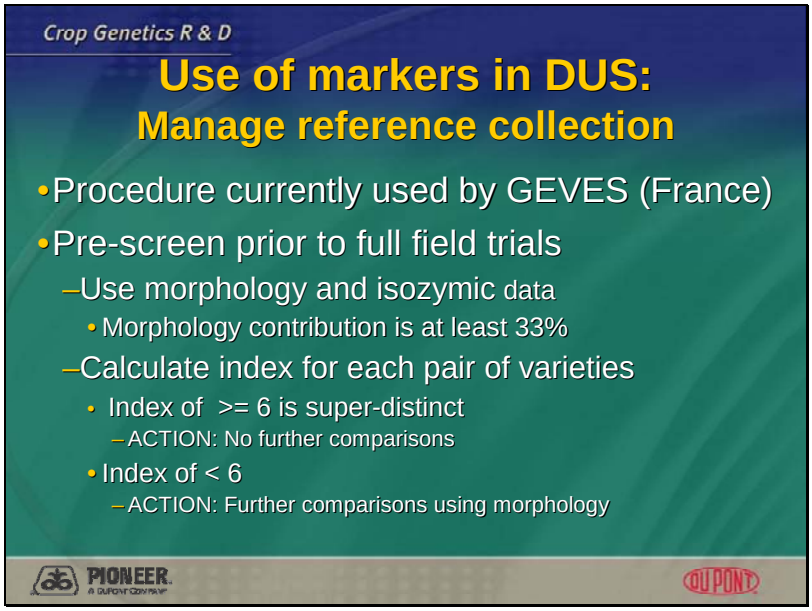

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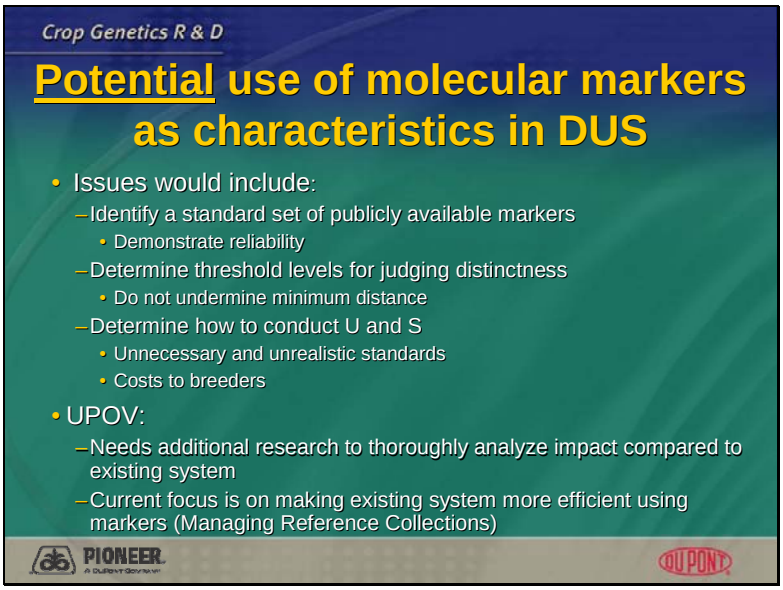
Crop Genetics R & D
Potential use of molecular markers as characteristics in DUS
• Issues would include:
– Identify a standard set of publicly available markers
• Demonstrate reliability
– Determine threshold levels for judging distinctness
• Do not undermine minimum distance
– Determine how to conduct 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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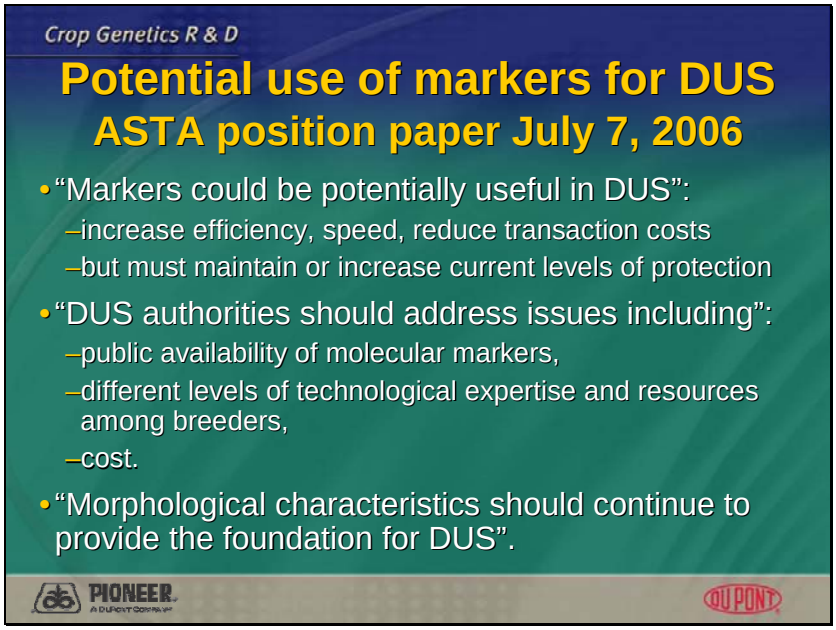

ASTA Position Paper on the Use of Molecular Markers in DUS Testing

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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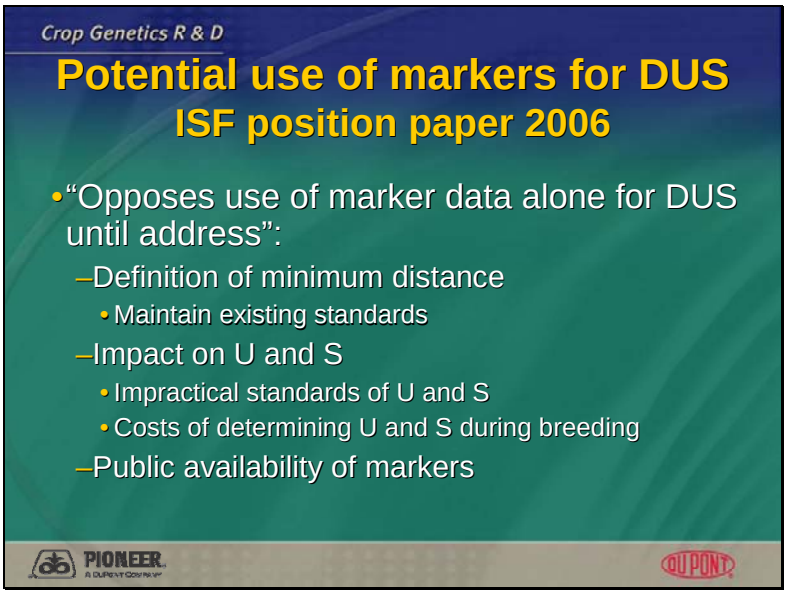

The ASTA and ISF positions are very closely aligned; fully aligned on the challenges and questions that must be satisfactorily addressed.

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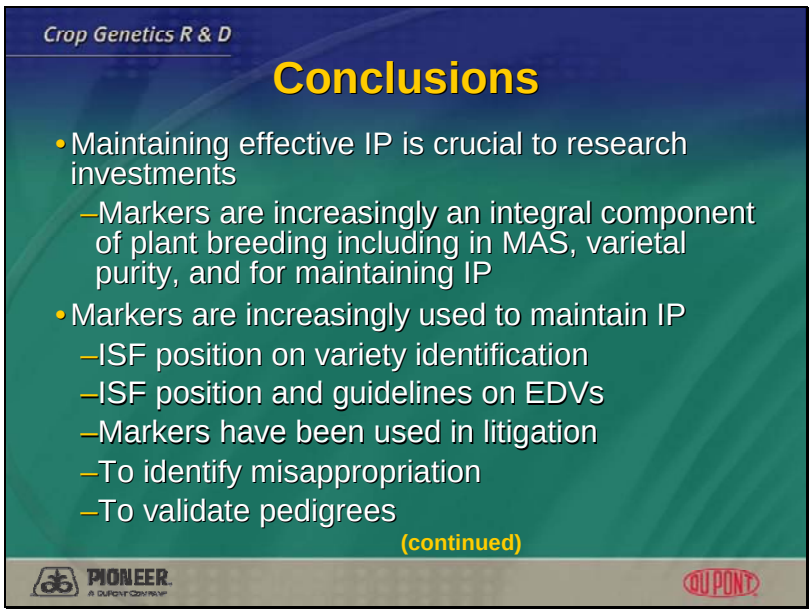

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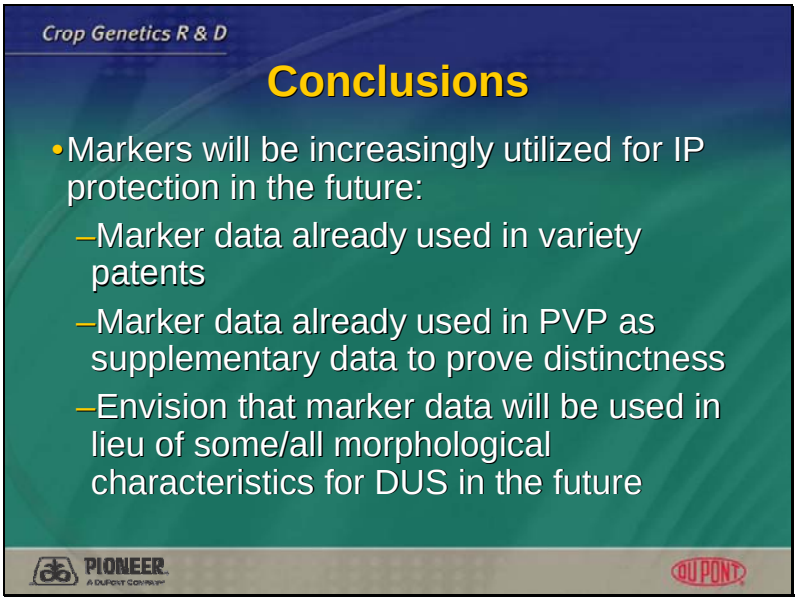

Envision that molecular marker data will be used in lieu of some/all morphological characteristics for DUS PVP authorities gaining experience using molecular marker data to manage reference collections.

UPOV is actively engaged in examining data and implications.

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

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