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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

AD HOC CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR MAIZE

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EXAMINING CHARACTERISTIC SETS FOR DUS TESTING:
INTRODUCTION TO A WORK IN PROGRESS

Document prepared by experts from the United Kingdom and Pioneer Hi-Bred International Inc., USA

Slide 1

Examining characteristic sets for DUS testing: Introduction to a work in progress

John Law (ex NIAB, UK).

Stephen Smith, Elizabeth Jones, Steven R. Anderson, Barry K. Nelson, Enver Mulaosmanovic and Jean Donnenwirth (Pioneer Hi-Bred International Inc., U.S.A.).

Slide 2

Phase 1: Morphological Data

- Characteristics
  - 34 morphological and 14 isozyme characteristics in UPOV Test Guidelines.
    - But US PVP system lists 59 phenotypic characteristics (includes most of the UPOV set)
- Complexities
  - Different characteristics sets European Union (EU) and United States of America (US)
  - Genotype x Environment interaction
  - Often complex or unknown genetic control
  - Translating continuous to discontinuous data
    - 5 note (FR) or 9 note systems
    - Or treat as continuous (USA)
  - Continuous/5 note/or 9 note approaches impact distinction
  - **Each crop species has its own complexities and characteristics**
  - **GOAL 1: To better understand morphological traits for DUS**

Slide 3

Phase 2: Marker data

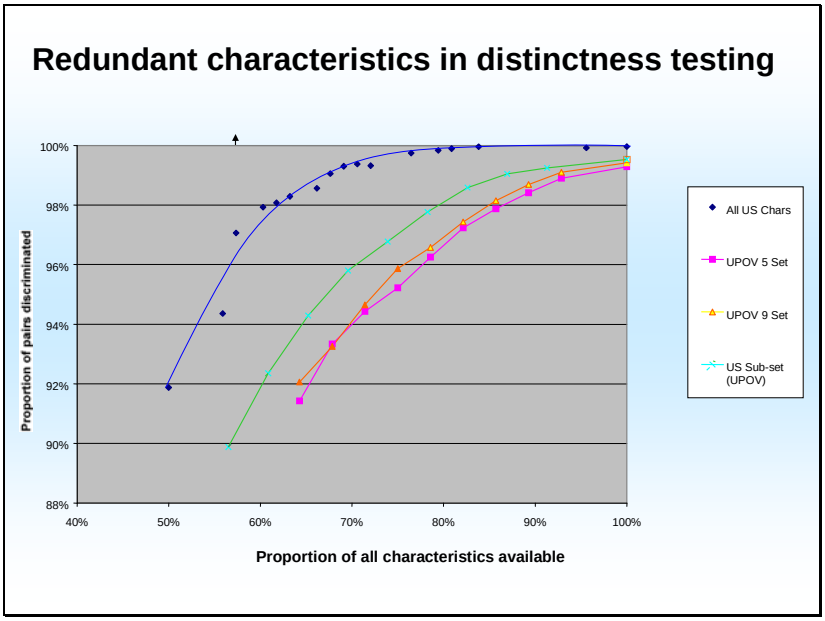
- Concerns
  - Rapid technological change
    - Complicates standardization
  - Avoid “easy” Distinction to undermine IP
    - One-several marker differences/100-1,000 markers clearly not enough
    - NOT facilitate marker based engineered cosmetic changes
  - Costs
    - Avoid added costs during breeding for uniformity and stability
    - Avoid added costs for official testing
- Why consider?
  - Potential to increase cost effectiveness
  - Standardisation using publicly available markers with known genetic control will increasingly become the norm
- Likely need to consider on a crop by crop basis
- Utilise sound scientific data

Slide 4

Materials and Methods

- Morphological data
  - 686 Inbreds, 223 Hybrids described in US
    - 1998-2005; three sites per year
    - 59 morphological characteristics
  - 150 Inbreds described in the EU using EU PVP data
- Marker data for 50% US inbreds and all EU inbreds
  - 400 publicly available SSR markers
  - Approx. 700 publicly available SNP loci (forthcoming)
- Methods
  - Rank characteristics and markers for discrimination
  - Measure discrimination power of characteristic combinations (pairs presented here)
  - Measure repeatability of morphological characteristics (not presented here)
  - Determine genetic control of morphological characteristics (by association mapping, not presented here)
  - Explore models of using marker data (Phase II)

Slide 5



1) Not all morphological characteristics are required to provide Discrimination/Distinctness.
For example, the US approach uses 59 morphological characteristics; 100% distinction among inbreds is provided using about 80% of those characteristics.
The EU trait set (34 morphological characteristics plus 14 isozyme characteristics) provides less ability to discriminate.
Question: Is the US approach being too discriminative; i.e. not providing a sufficient level of IP??

2) How the same morphological data are treated has an effect.
The green line shows a US data subset equal to the EU BUT treated as continuous data.
The orange line shows the same data characterized into one of 9 classes per each characteristic
The pink line shows the same data characterized into one of 5 classes per each characteristic
So:
a) there are differences in the discrimination power depending how the morphological data are recorded
b) treating the data as continuous data provides greater precision.

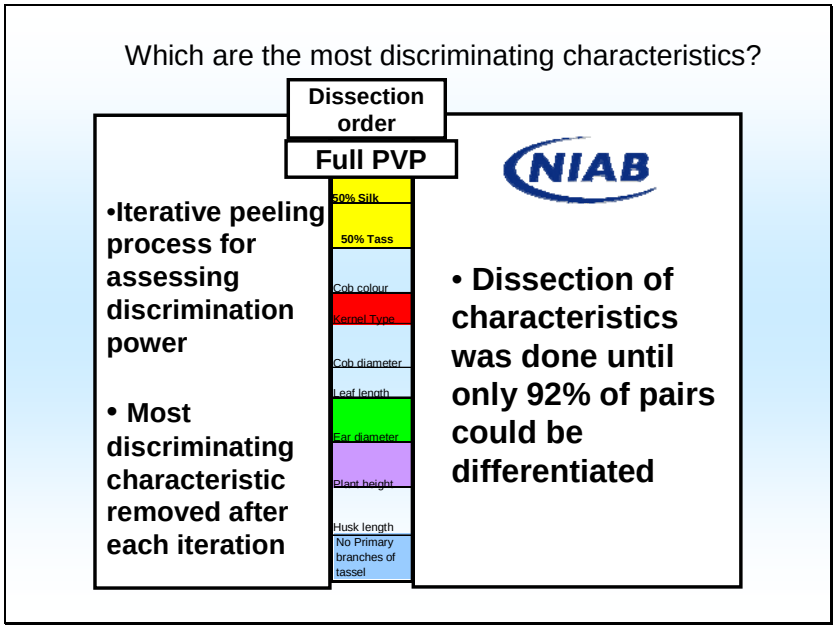
Conclusions:

Morphological data represent complex characteristics and there are different approaches—a standardized approach among different agencies has not yet been achieved.

There is a loss of discrimination power if you reduce the precision with which you measure the phenotype.

There appears to be a degree of redundancy in the character set in that a level of discrimination - say 98% - can be achieved with 80% (or less) of the available characters.

Slide 6



Look at the four columns under Dissection order.

For example, under “full PVP”-50% silk was the most discriminating characteristic among all inbreds

Then when data for that characteristic was excluded, 50% tassel was the next most discriminating characteristic among all inbreds.

Then when data for that characteristic also was excluded the next most discriminating characteristics was Cob colour

This peeling process was continued to find the next most highest discriminating characteristic.

This process was repeated for each of the remaining data sets-UPOV 9 note, UPOV 5 note and US subset but treated as continuous data.

Conclusions:

Discrimination power of each characteristic is rather complex
It depends upon which characteristic set is being used: e.g. the US “full PVP” set or the EU set

AND it depends upon how the morphological data are recorded: as continuous data, or as 5-note discontinuous data or as 9-note discontinuous data.

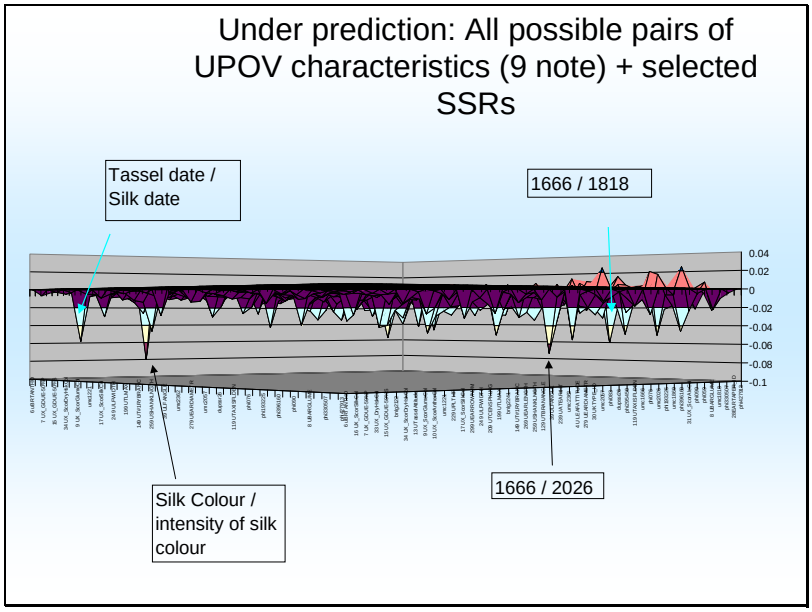
Further research will repeat these analyses for inbreds within each of several maturity groups.

Which characteristics are the most discriminating depends somewhat on how the make up of the entire set of characteristics and how the data are recorded

Even the characteristic set you choose seems dependent on how you measure the phenotypes and analyze the data.

This slide is supposed to show lack of consistency in the order of characteristics identified as important for D.

Slide 7



This slide shows an example of examining the discrimination power of pairs of characteristics.

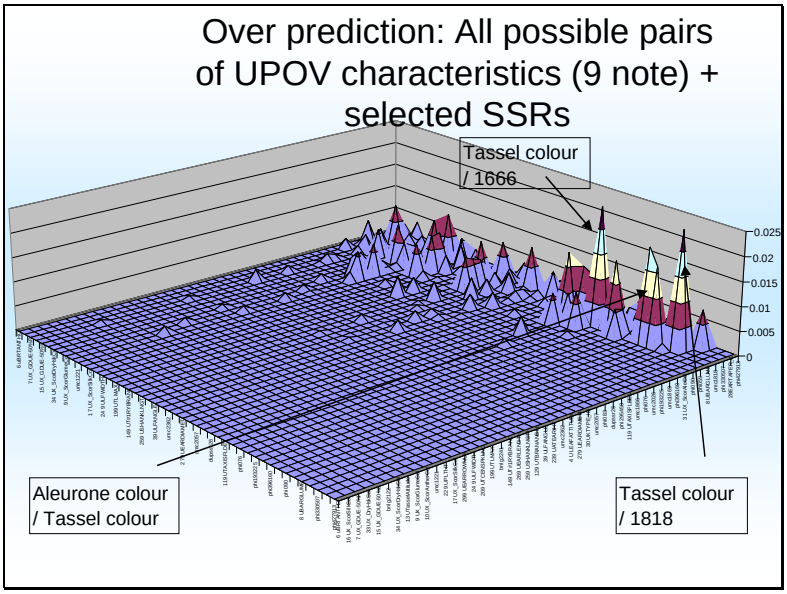
Characteristics, here including both morphology and markers, are arrayed along the horizontal *x* and *y* axes

Discrimination power is along the vertical axis.

Here are highlighted pairs of characteristics that relatively underperform in providing distinction.

Peaks projecting downwards show pairs of characteristics that are relatively underperforming at discrimination (for example tassel date with silk date, or silk colour with intensity of silk colour, or marker 1666 with marker 2026, or marker 1666 with marker 1818). So perhaps it is unnecessary to collect or to use both tassel data AND silk date for example.

Slide 8



Here are highlighted pairs of characteristics that relatively overperform at discrimination.

For example, aleurone colour with tassel colour, or tassel colour with marker 1666, or tassel colour with marker 1818.

Note that 1666 and 1818 both complement Tassel colour –BUT the previous slide showed that the pair of markers 1666 with 1818 relatively underperformed at distinction—i.e. They individually gave very similar information regarding discrimination among the inbreds—SO only one of those markers is really useful for discrimination.

Slide 9

A work in progress

- Now
  - Initial exploration of data and methodologies
  - Measure repeatability of morphological characteristics
  - Measure discrimination of morphological characteristics
    - In relation to contrasting systems
      - US, EU
      - Continuous or discrete data, and number of classes
- Future:
  - Obtain more data
    - SSR data for those inbreds
    - SNP data are forthcoming
  - Examine models of incorporating marker data
    - As pre-screen to morphology: managing reference collections
    - As a component with morphological characteristics
    - Markers alone
    - Being always aware of concerns expressed by stakeholders
  - **Recognizing maize represents just ONE model species**
  - **For other crops likely requires a crop species by crop species approach**

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