Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular

BMT/17/17 Add.

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ADDENDUM TO THE UNITED STATES MOLECULAR MARKER WORKING GROUP: BACKGROUND FOR THE USE OF DNA MARKERS IN DUS

Document prepared by experts from the Seed Association of the Americas (SAA)

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The Annex to this document contains a copy of a presentation on “The United States Molecular Marker Working Group: Background for the use of DNA markers in DUS”, prepared by experts from the Seed Association of the Americas (SAA), to be made at the seventeenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (BMT).

[Annex follows]

ANNEX

THE UNITED STATES MOLECULAR MARKER WORKING GROUP: BACKGROUND FOR THE USE OF DNA MARKERS IN DUS

Presentation prepared by experts from the Seed Association of the Americas (SAA)

The United States Molecular Marker Working Group: Background on DNA Markers in DUS

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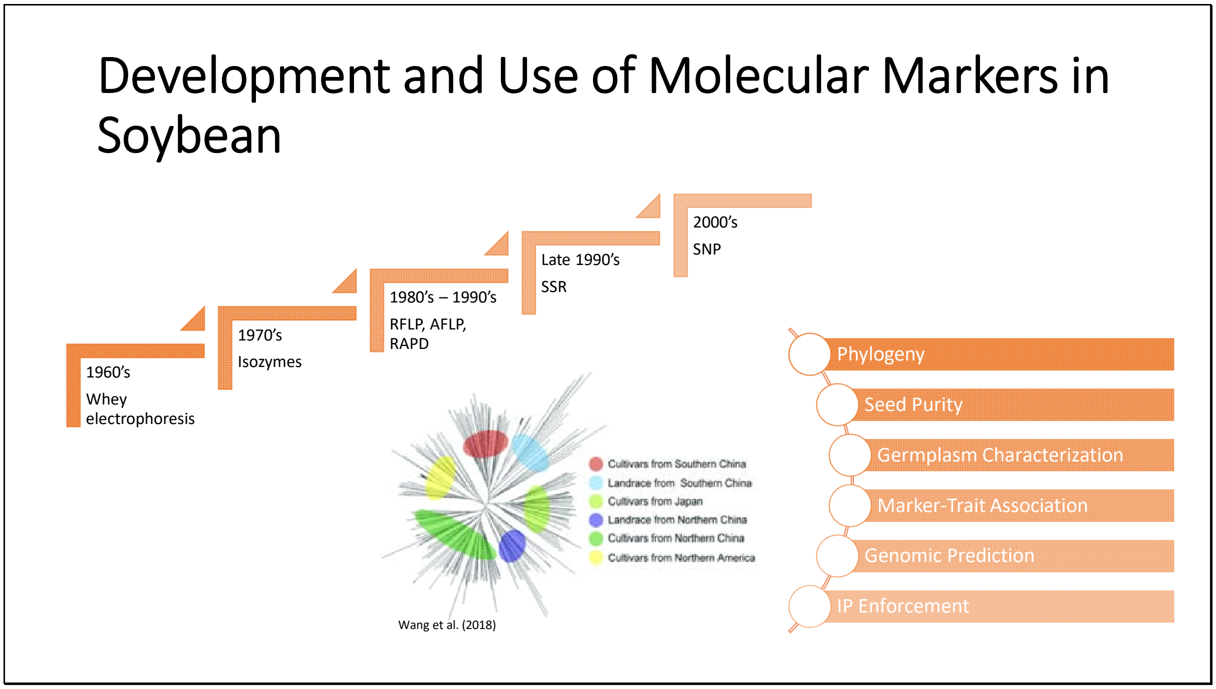
Soybean Cultivation and Improvement

- Soybean [Glycine max (L.) Merr.] was introduced into North America as a forage crop in the 18th century.
- 1930's transition to grain crop.



Development and Use of Molecular Markers in Soybean

1960's
Whey electrophoresis

1970's
Isozymes

1980's – 1990's
RFLP, AFLP, RAPD

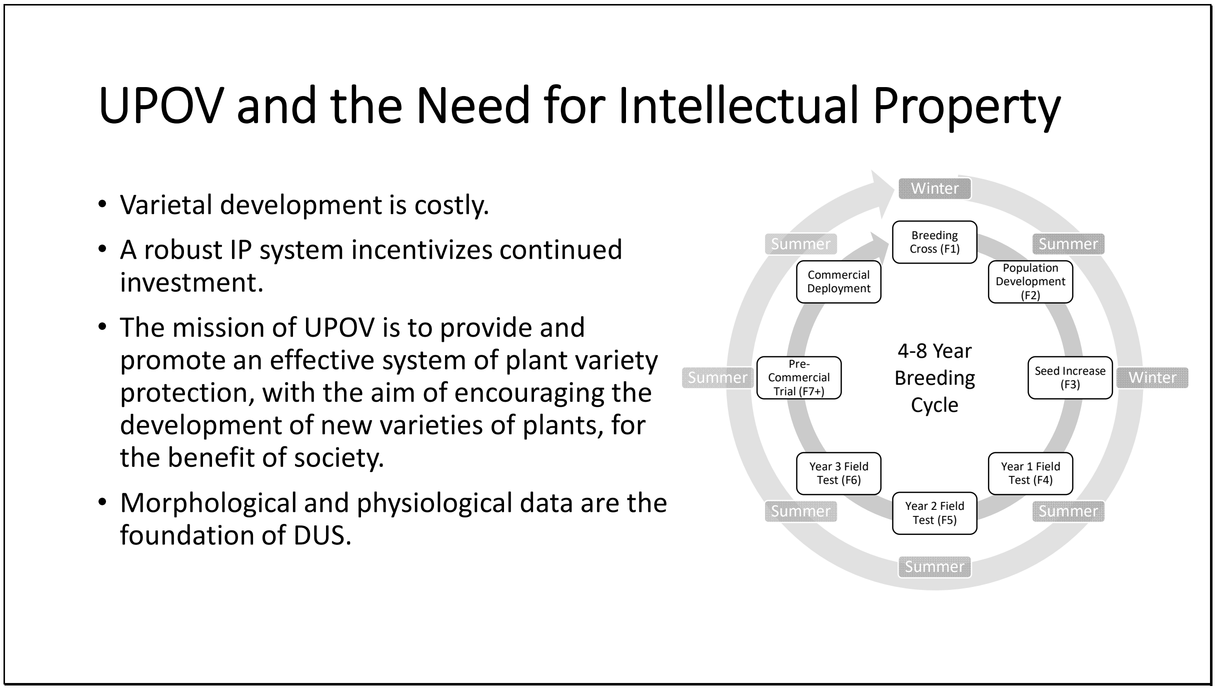
Late 1990's
SSR

2000's
SNP

● Cultivars from Southern China
● Landrace from Southern China
● Cultivars from Japan
● Landrace from Northern China
● Cultivars from Northern China
● Cultivars from Northern America

Wang et al. (2018)

- Phylogeny
- Seed Purity
- Germplasm Characterization
- Marker-Trait Association
- Genomic Prediction
- IP Enforcement



UPOV and the Need for Intellectual Property

- Varietal development is costly.
- A robust IP system incentivizes continued investment.
- The mission of UPOV is to provide and promote an effective system of plant variety protection, with the aim of encouraging the development of new varieties of plants, for the benefit of society.
- Morphological and physiological data are the foundation of DUS.

Approved Uses of Molecular Marker Data

- When there is a reliable link between the marker and the characteristic. (document TGP/15/1)
- Combined with morphological characteristics for management of reference collections. (document TGP/15/1)
- Isozymes as complement to morphology in annex to DUS technical guidelines for soybean. (document TG/80/6)
- Measure genotypic conformity as a means to helping determine EDV status. (ISF 2012)
- As “supporting evidence” when morphological differences are small but not sufficient (proposal). (document TWA/28/17)

Markers as Supporting Evidence

- Proposal from French experts where differences exist but none sufficient for establishing distinctness. (document TWA/28/17)
- Markers as another tool.
- Flax example, France and Belgium field trials, 1992/1993:

N° UPOV TG/57/6	F-1992	F-1993	B-1992	B-1993	Remark
1) Plant: natural height	- 2,6 cm	- 1,8 cm	+ 9,4 cm	- 1,3 cm	Minimum distance (5 cm) achieved once and inconsistency of differences.
2) Stem: length	+ 1,0 cm	+ 2,2 cm	+ 4,9 cm	+ 0,5 cm	Minimum distance (5 cm) never achieved but consistency of small differences.
3) Flower: size of corolla	0	0	0	0	No difference
4) Sepal: dotting	+2	+1	+4	+2	Characteristic highly influenced by environment. Minimum distance of 4 achieved once and consistency of small differences.
5) Petal: color of crown	0	0	0	0	No difference
6) Petal: color of corola	0	0	0	0	No difference
7) Petal: longitudinal folding	0	0	0	0	No difference
8) Stamen: color of distal part	0	0	0	0	No difference
9) Anther: color	0	0	0	0	No difference
10) Style: color	0	0	0	0	No difference
11) Boll: size	+2	+1	+1	+1	Minimum difference (2) achieved once and consistency of small differences.
12) Boll: ciliation of false septa	0	0	0	0	No difference
13) Seed: weight per 1000 seeds	+ 0,2 g	+ 0,4 g	+ 0,6 g	+ 0,4 g	Minimum distance (0,5 g) achieved once and consistency of small differences.
14) Seed: color	0	0	0	0	No difference
15) Time of beginning of flowering	- 1 day	+ 1 day	+ 2 days	+ 1 day	Minimum distance (3 days) never achevived and inconsistency of differences.

Recognizing Potential Pitfalls

Pitfall

- Distinctness via one or a few base pair polymorphisms.
- Unreasonable uniformity requirements across entire genome.
- Increase in costs of breeding, seed increase, and purity assessment.

Safeguard

- Sufficiently large SNP set.
  - Many hundreds or few thousands.
- Proper within and between varietal uniformity thresholds.
  - Examine genotypes for varieties which have already been granted varietal status based on morphologically expressed characteristics.

Inter and Intra-Variety Heterogeneity

Inter-varietal heterogeneity must be factored when establishing distinctness thresholds.
“Subject to the variation that may be expected from the particular features of propagation” (UPOV Convention Article 8)

Examples

i)Genetic variation underlying the expression of agronomic traits was observed within F5–derived lines (Byth and Weber, 1968).

i)Maize inbred lines of doubled haploid derivation were found to accumulate variation in agronomic traits via mutation (Sprague et al. 1960; Russell et al. 1963).

i)Continued response to selection was observed in maize after more than 90 generations (Dudley and Lambert, 2004).

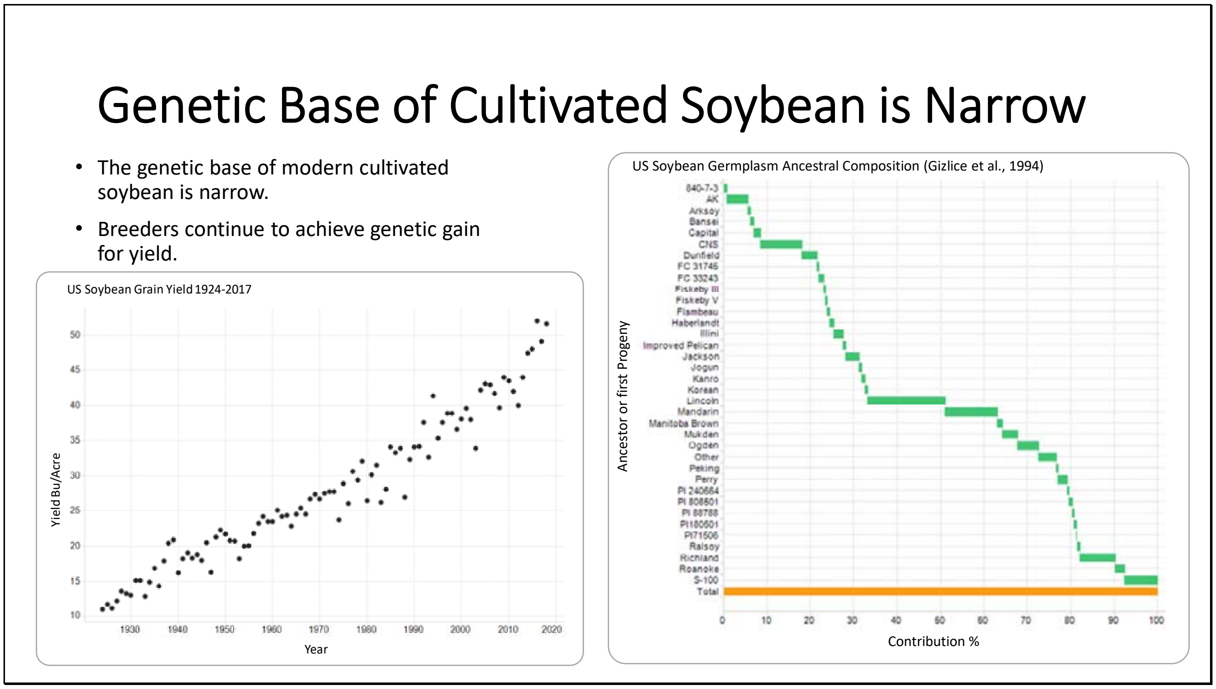
i)Residual diversity for agronomic traits was found within each of the soybean varieties “Benning”, “Haskell”, and “Cook” (Fasoula and Boerma, 2007)

i)Residual SNP variation has been found in the soybean variety “Williams 82” though most of the genome is fixed. (Haun et al. 2011).

i)Rasmusson and Phillips (1997) reported generation of de novo variation in elite germplasm pools.

i)McClintock (1984) suggested that environmental stress may be a trigger of genomic change allowing for continued adaptation under selection.

i)Due to heterosis in soybean, selections can retain heterozygosity in genomic regions under selection (Fasoula and Boerma 2007)

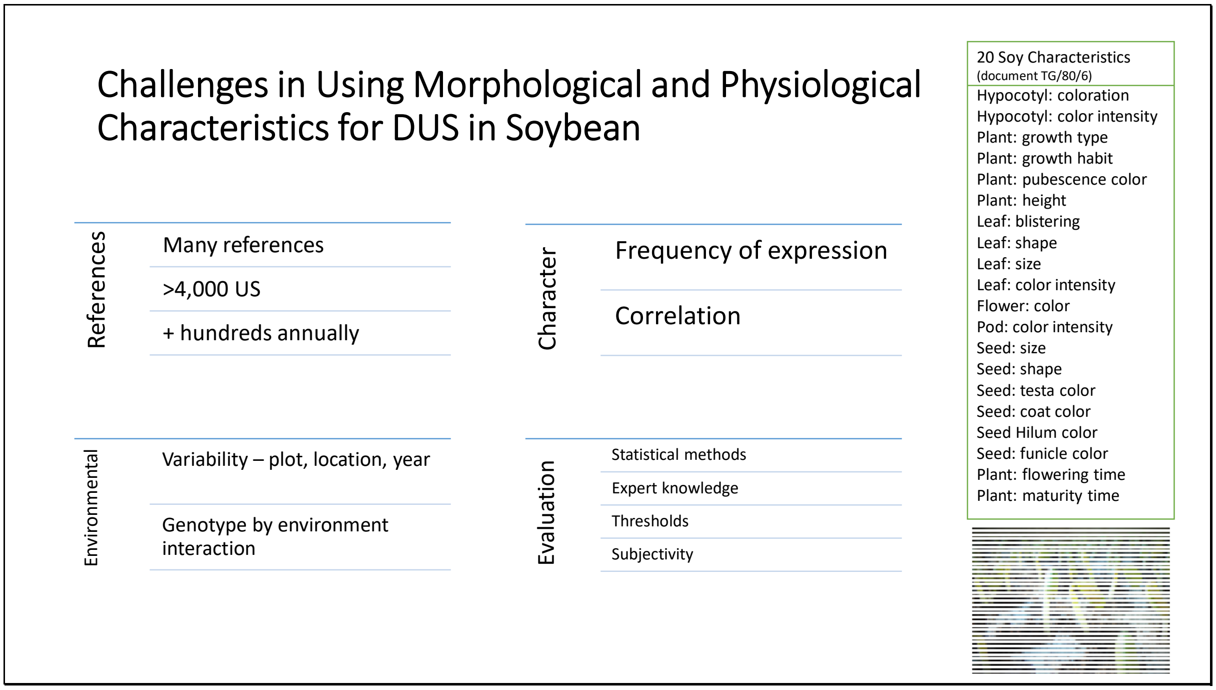


Genetic Base of Cultivated Soybean is Narrow

- The genetic base of modern cultivated soybean is narrow.
- Breeders continue to achieve genetic gain for yield.

US Soybean Grain Yield 1924-2017

US Soybean Germplasm Ancestral Composition (Gizlice et al., 1994)



Challenges in Using Morphological and Physiological Characteristics for DUS in Soybean

References
- Many references
- >4,000 US
- + hundreds annually

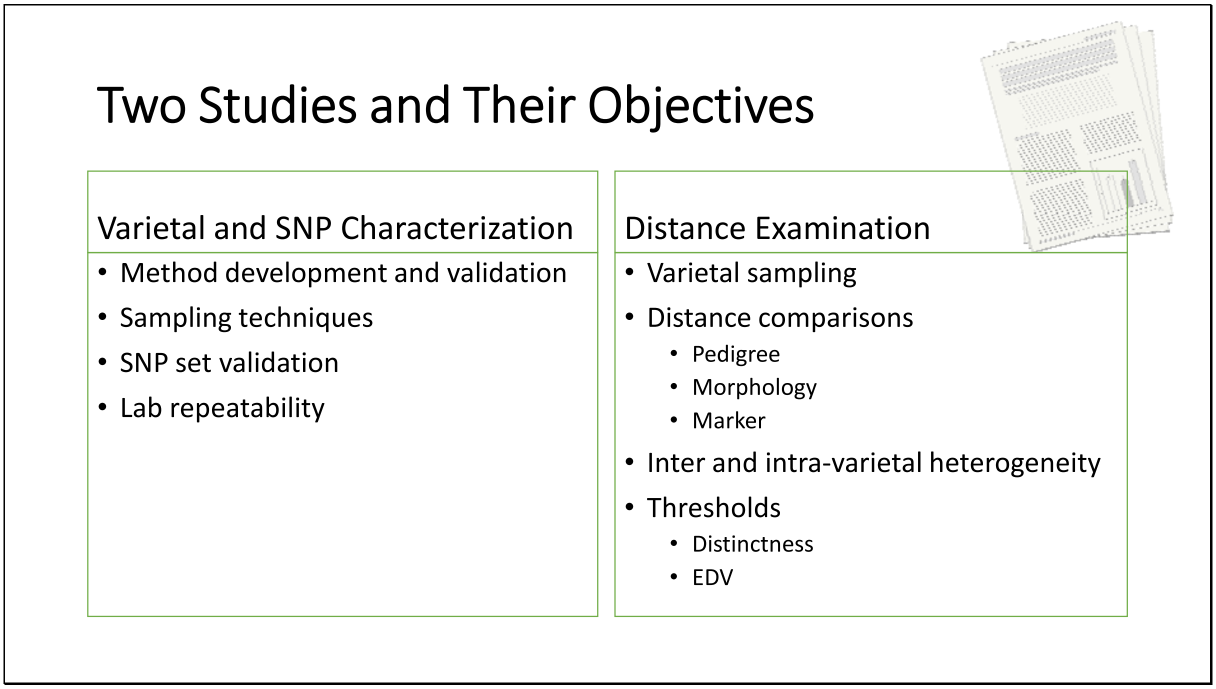
Character
- Frequency of expression
- Correlation

Environmental
- Variability – plot, location, year
- Genotype by environment interaction

Evaluation
- Statistical methods
- Expert knowledge
- Thresholds
- Subjectivity

20 Soy Characteristics
(document TG/80/6)

- Hypocotyl: coloration
- Hypocotyl: color intensity
- Plant: growth type
- Plant: growth habit
- Plant: pubescence color
- Plant: height
- Leaf: blistering
- Leaf: shape
- Leaf: size
- Leaf: color intensity
- Flower: color
- Pod: color intensity
- Seed: size
- Seed: shape
- Seed: testa color
- Seed: coat color
- Seed Hilum color
- Seed: funicle color
- Plant: flowering time
- Plant: maturity time



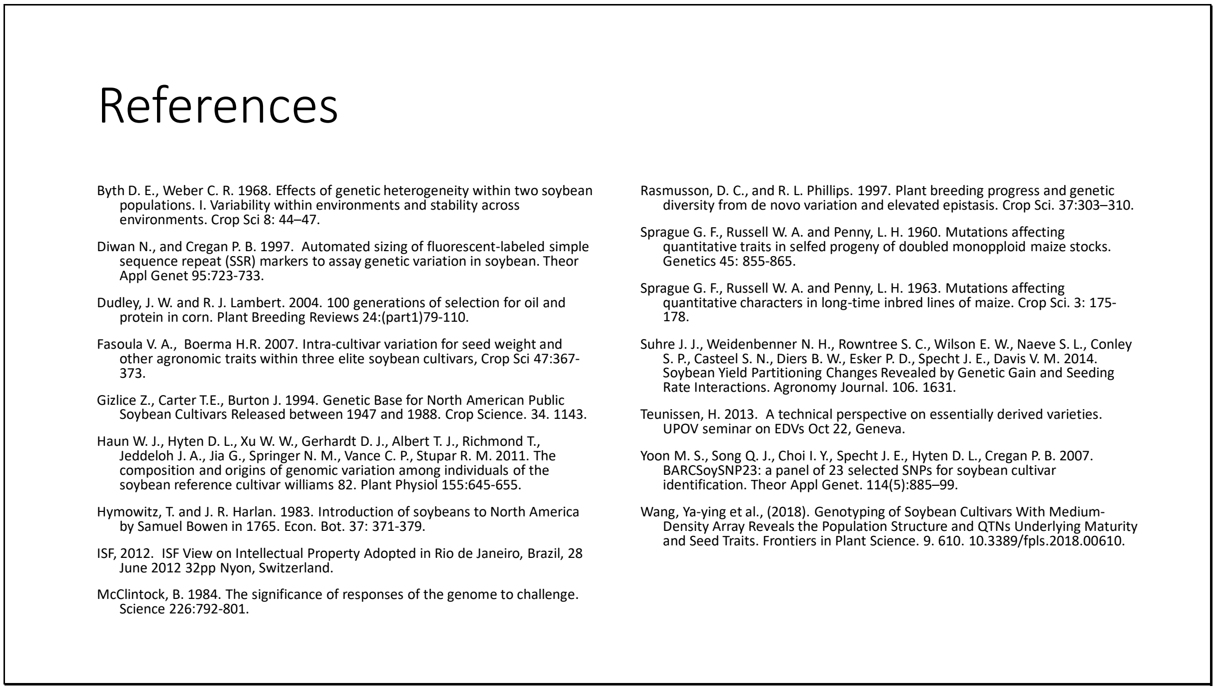
Two Studies and Their Objectives

Varietal and SNP Characterization

- Method development and validation
- Sampling techniques
- SNP set validation
- Lab repeatability

Distance Examination

- Varietal sampling
- Distance comparisons
  - Pedigree
  - Morphology
  - Marker
- Inter and intra-varietal heterogeneity
- Thresholds
  - Distinctness
  - EDV



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