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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

STANDARDS FOR HELPING TO DETERMINE EDV STATUS IN MAIZE (ZEA MAYS L.) USING SSR'S AND FUTURE PROSPECTS USING SNP'S

Document prepared by experts from the United States of America

Standards for helping to determine EDV status in maize using SSRs and future prospects using SNPs

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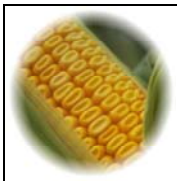
American Seed Trade Association
Corn Variety Identification Sub-Committee

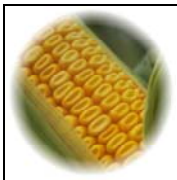
Selection and evaluation of panels of SSR loci

- ASTA (American Seed Trade Association) have selected a set of 285 SSR loci for EDV
  - Selection based on:
    - Genetic conformity
    - Expected Heterozygosity (informative)
    - Genome coverage
    - Scoring ease using an inexpensive assay
  - 150 sub-set (Core Set 1) for efficiency, additional 135 use as needed.
  - Published in Crop Science
    - Kahler, A.L., J.L. Kahler, S.A. Thompson, R.S. Ferriss, E.S. Jones, B.K. Nelson, M.A. Mikel, and S. Smith. 2010. **North American Study on Essential Derivation in Maize: II. Selection and Evaluation of a Panel of Simple Sequence Repeat Loci**. Crop Sci 50: 486-503.
  - 285 marker set posted on ASTA web site (www.amseed.org)
- UFS (French Maize Breeders, formerly SEPROMA)
  - selected a set of 223 SSR with a core set of 163 for EDV



Evaluation of SNPs for genetic similarity in maize

- Maize breeders are moving to SNPs
  - Throughput
  - Cost efficiencies
- Necessitates the need to evaluate SNP utility in EDV
- Objective
  - Evaluate SNP's for potential use in EDV
    - Considered a "primary step" before EDV thresholds could be determined
      - Genetic associations with pedigree relatedness and SSRs
      - Number of SNPs necessary



Inbreds Used

- 98 inbreds selected by ASTA members
  - Public sources
    - Public material
    - Off-PVP
  - Same material used in SSR panel evaluation
- 30 inbred sub-set profiled with SEPROMAs 163 core set
  - Reduced inbred set due to cost
  - Maintained diversity of 98 inbred set.

Marker Sets -SSR Sets and SNP Assay

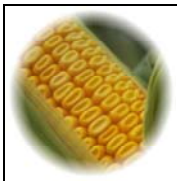
SSRs
- 150 ASTA public set
- 163 SEPROMA SSRs

SNPs
- Started with 768 public set
  - Profiled by Pioneer
    - Aliquots of same DNA used for published SSR panel
    - Illumina GoldenGate® assay
  - Data subject to quality control steps
    - Thresholds for both inbreds and markers
      - 10% hets
      - 70% data coverage
    - 80 of the 98 inbreds used in analysis
    - 26 of 30 SEPROMA sub-set

Marker Sets -SNP Set Selections

6 Subsets evaluated
- 601 Set
  - Passed quality control
- 447 Set
  - Removed unmapped and expected heterozygosity = 0
- 306 Set
  - Removed loci mapped to same location, retaining highest expected heterozygosity
- 204 Set
  - Removed loci mapped within 10 cM of another, while maintaining genome coverage & previous expected heterozygosity average

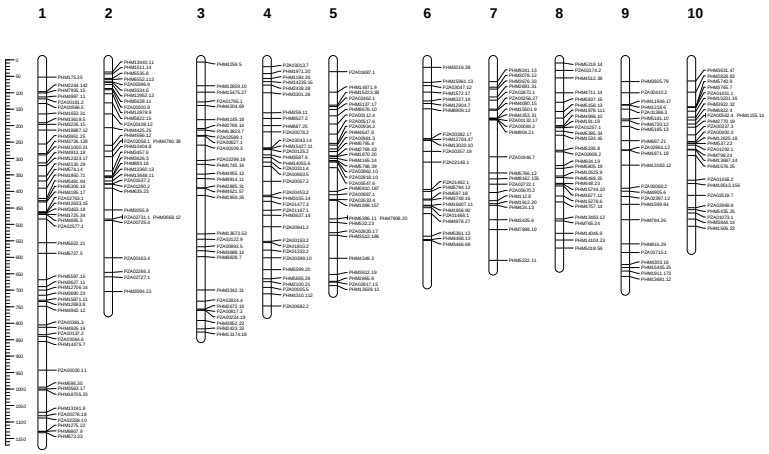
Maintain genome coverage (with increasing intervals between loci) & previous expected heterozygosity average
- 83 Set
- 42 Set

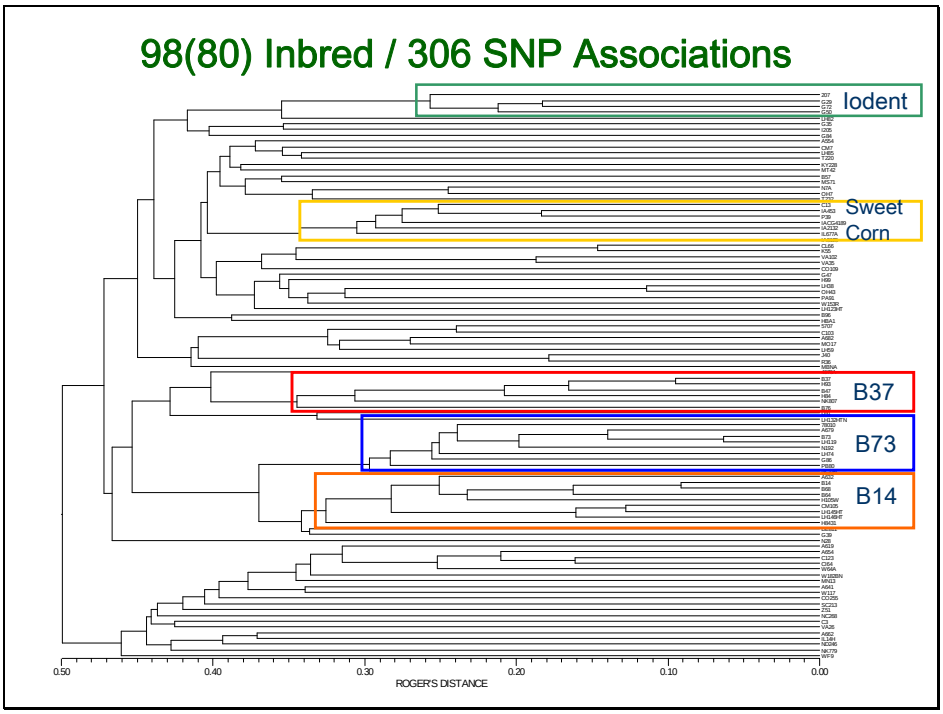


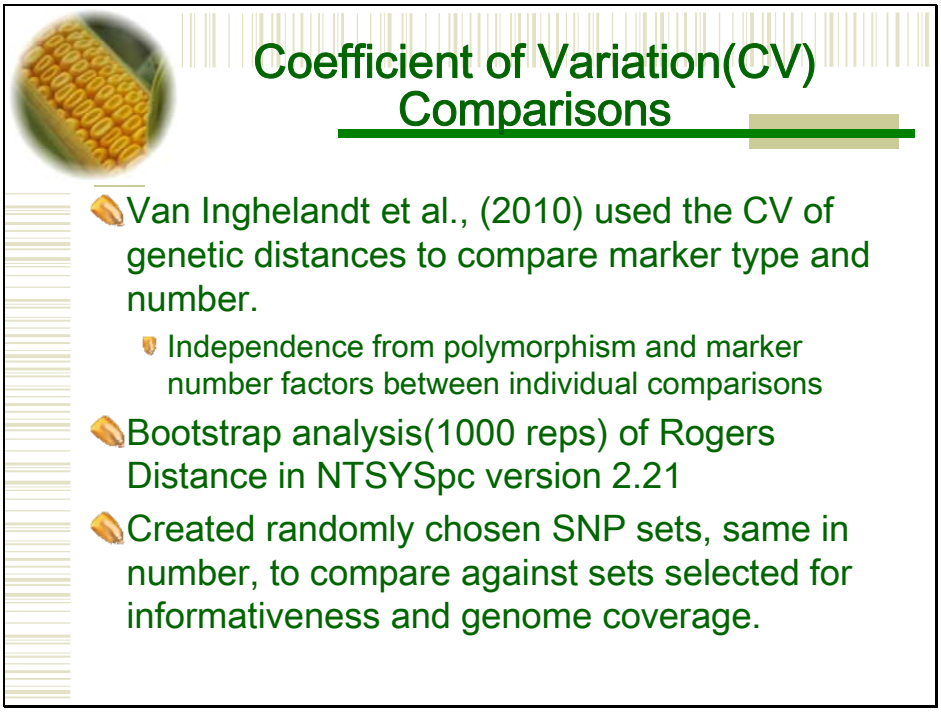
Marker Sets
-Expected Het. & Genome Coverage

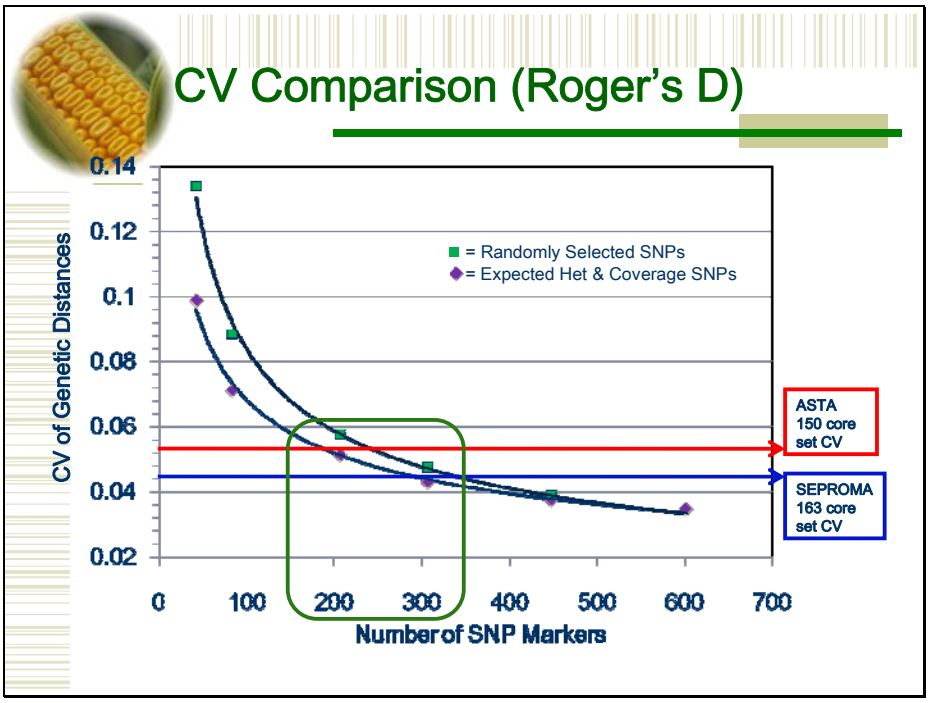
Marker Set	Average (range) expected heterozygosity for 80 inbred set	Average (range) expected heterozygosity for 26 inbred subset	Genome coverage (%)	Average (range) marker distances (cM)
150 ASTA SSR	**0.53** (0.03 – 0.79)	**0.49** (0.00 – 0.80)	**89.0**	**47.2** (0-196.0)
163 SEPROMA SSR	NA	**0.65** (0.00 – 0.88)	**88.2**	**44.1** (0-206.1)
601 SNP	**0.35** (0.00-0.50)	**0.34** (0.00-0.50)	**88.4**	**15.1** (0-112.8)
447 SNP	**0.38** (0.03-0.50)	**0.37** (0.00-0.50)	**88.4**	**15.1** (0-112.8)
306 SNP	**0.41** (0.03-0.50)	**0.39** (0.00-0.50)	**88.0**	**22.3** (0.2-117.8)
204 SNP	**0.41** (0.03-0.50)	**0.40** (0.00-0.50)	**87.2**	**33.7** (6.4-117.8)
83 SNP	**0.48** (0.30-0.5)	**0.46** (0.15-0.50)	**83.5**	**89.5** (13.8-240.3)
42 SNP	**0.48** (0.34-0.5)	**0.47** (0.28-0.50)	**74.6**	**186.7** (44.6-408.8)

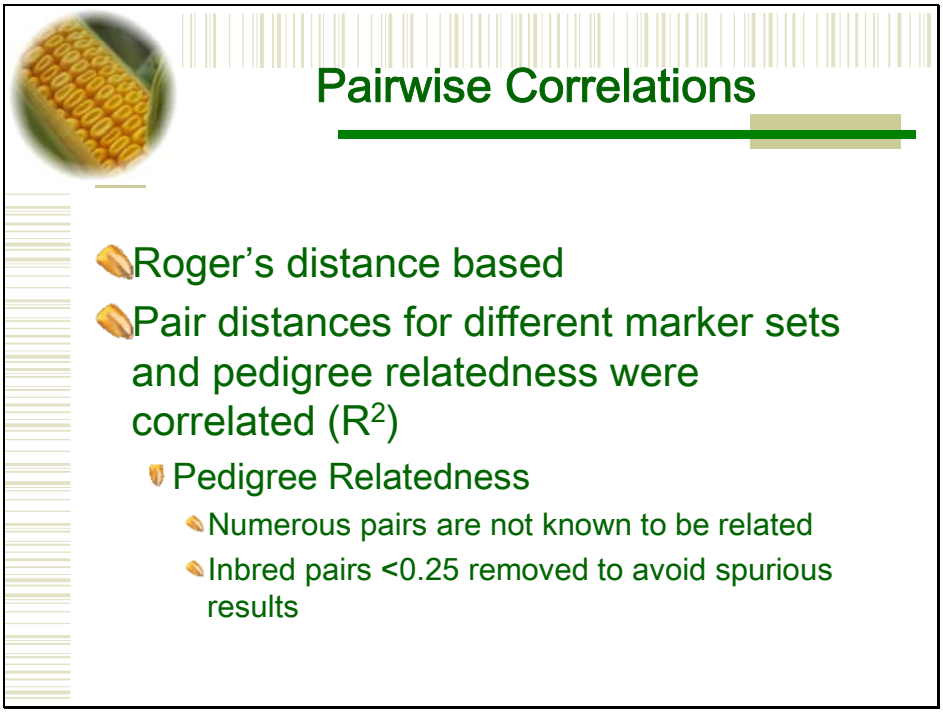
Map Coverage for 306 SNP Marker Set Based on IBM2 2008 Map




98(80) Inbred / 306 SNP Associations
Iodent
Sweet Corn
B37
B73
B14
0.50
0.40
0.30
0.20
0.10
0.00
ROGER'S DISTANCE


Coefficient of Variation(CV) Comparisons
Van Inghelandt et al., (2010) used the CV of genetic distances to compare marker type and number.
Independence from polymorphism and marker number factors between individual comparisons
Bootstrap analysis(1000 reps) of Rogers Distance in NTSYSpc version 2.21
Created randomly chosen SNP sets, same in number, to compare against sets selected for informativeness and genome coverage.


CV Comparison (Roger's D)
CV of Genetic Distances
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
100
200
300
400
500
600
700
Number of SNP Markers
= Randomly Selected SNPs
= Expected Het & Coverage SNPs
ASTA 150 core set CV
SEPROMA 163 core set CV


Pairwise Correlations
Roger's distance based
Pair distances for different marker sets and pedigree relatedness were correlated (R^2)
Pedigree Relatedness
Numerous pairs are not known to be related
Inbred pairs <0.25 removed to avoid spurious results

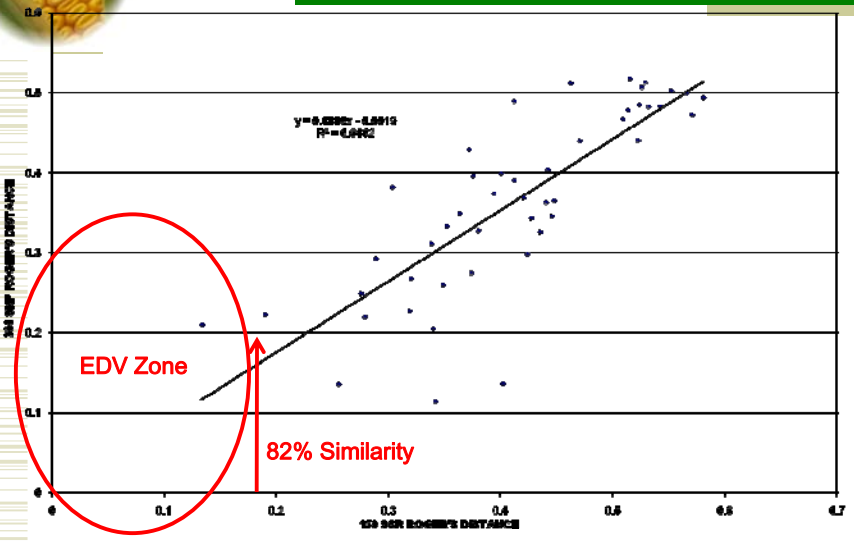
Pairwise Correlations (R^2)

80 inbreds

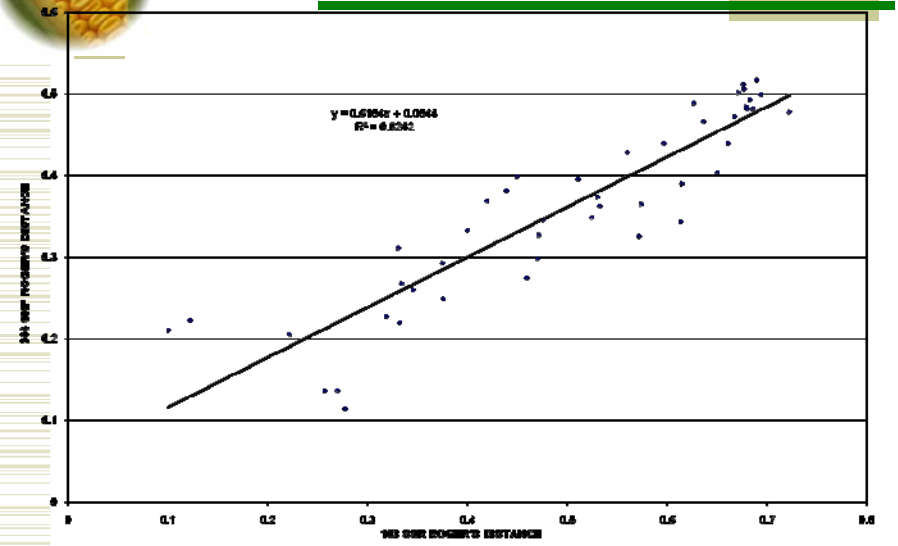
	Pedigree Relatedness	150 ASTA SSR	163 SEPROMA SSR	601 SNP	447 SNP	306 SNP	204 SNP
Pedigree Relatedness		*0.38*	*NA*	*0.44*	*0.46*	*0.46*	*0.46*
150 ASTA SSR	0.42		*NA*	*0.55*	*0.58*	*0.61*	*0.59*
163 SEPROMA SSR	0.66	0.8		*NA*	*NA*	*NA*	*NA*
601 SNP	0.49	0.56	0.75		*0.98*	*0.96*	*0.92*
447 SNP	0.52	0.61	0.8	0.98		*0.98*	*0.95*
306 SNP	0.53	0.65	0.82	0.97	0.99		*0.97*
204 SNP	0.56	0.66	0.82	0.95	0.97	0.98	

26 inbreds

150 ASTA SSR's / 306 SNP's

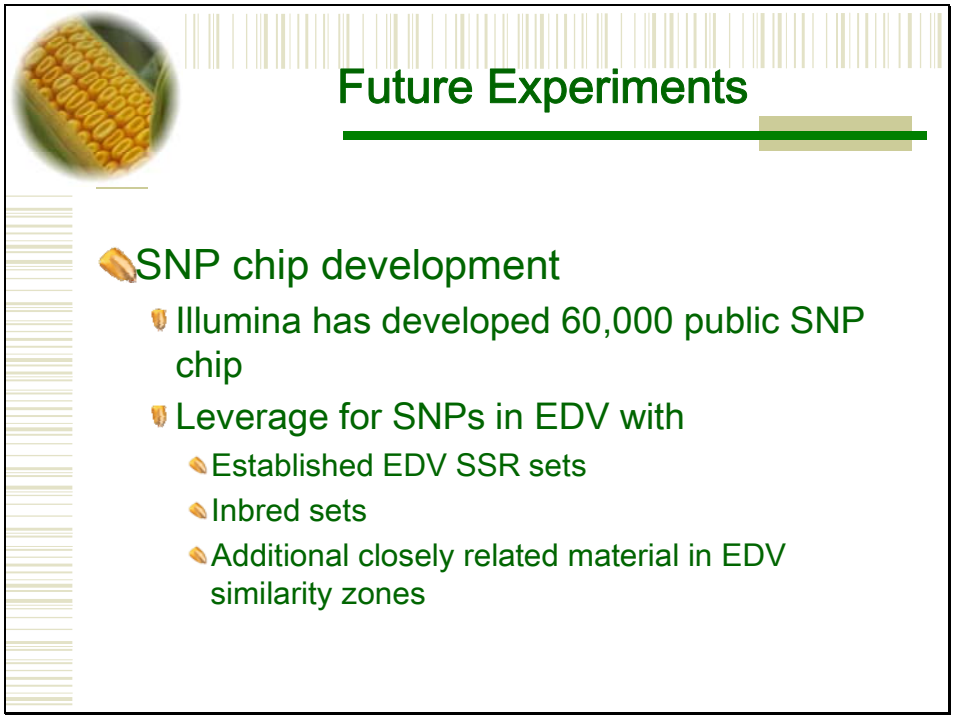


163 SEPROMA SSR's / 306 SNP's



Summary

- SNPs will be useful in EDV determination
  - Demonstrate high discriminating power
  - Accurately reflect inbred associations with known pedigrees
- SNP sets selected for informativeness and genome coverage had lower CVs than randomly chosen sets of the same number of SNPs per set
- 204 and 306 SNP sets
  - Gave equivalent CVs to the 150 ASTA and 163 SEPROMA SSR sets
  - Had the best correlations to pedigree relatedness and SSR distances



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