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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

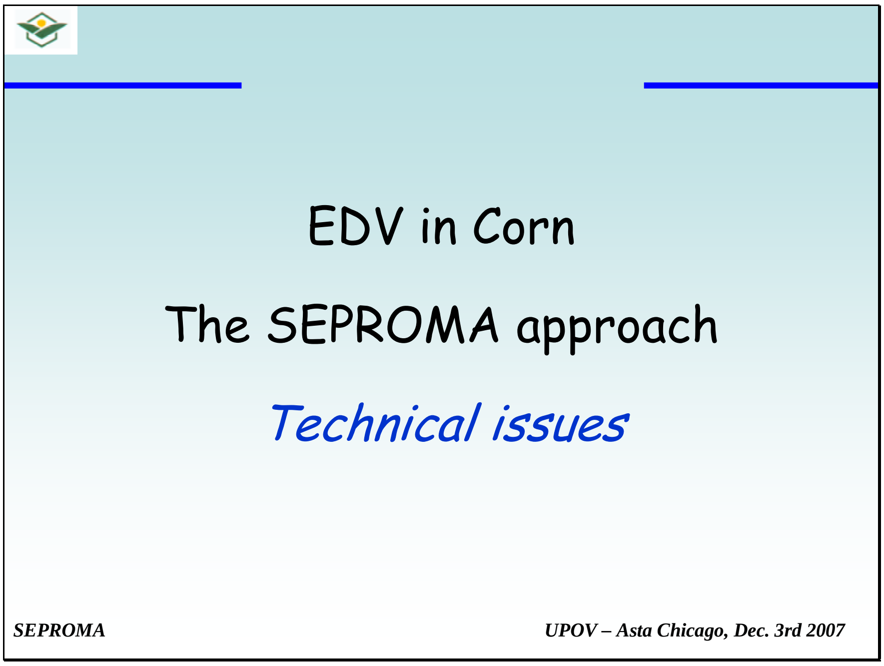
Chicago, United States of America, December 3, 2007

EDV IN CORN:

THE SEPROMA APPROACH – TECHNICAL ISSUES

Document prepared by experts from SEPROMA

Slide 1



EDV in Corn

The SEPROMA approach

Technical issues

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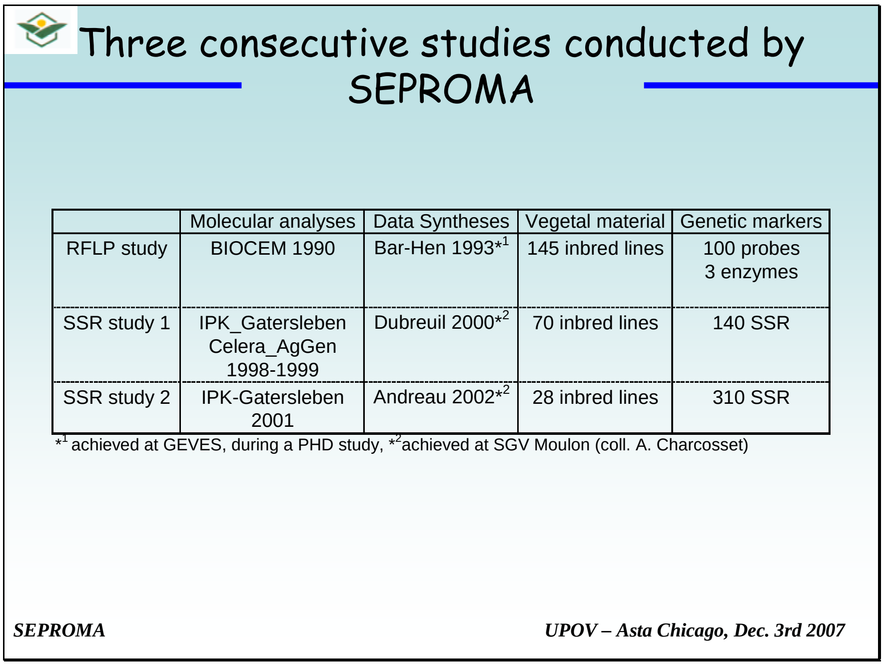
Slide 2



Three consecutive studies conducted by SEPROMA

	Molecular analyses	Data Syntheses	Vegetal material	Genetic markers
RFLP study	BIOCEM 1990	Bar-Hen 1993*[1]	145 inbred lines	100 probes 3 enzymes
SSR study 1	IPK_Gatersleben Celera_AgGen 1998-1999	Dubreuil 2000*[2]	70 inbred lines	140 SSR
SSR study 2	IPK-Gatersleben 2001	Andreau 2002*[2]	28 inbred lines	310 SSR

*[1] achieved at GEVES, during a PHD study, *[2] achieved at SGV Moulon (coll. A. Charcosset)

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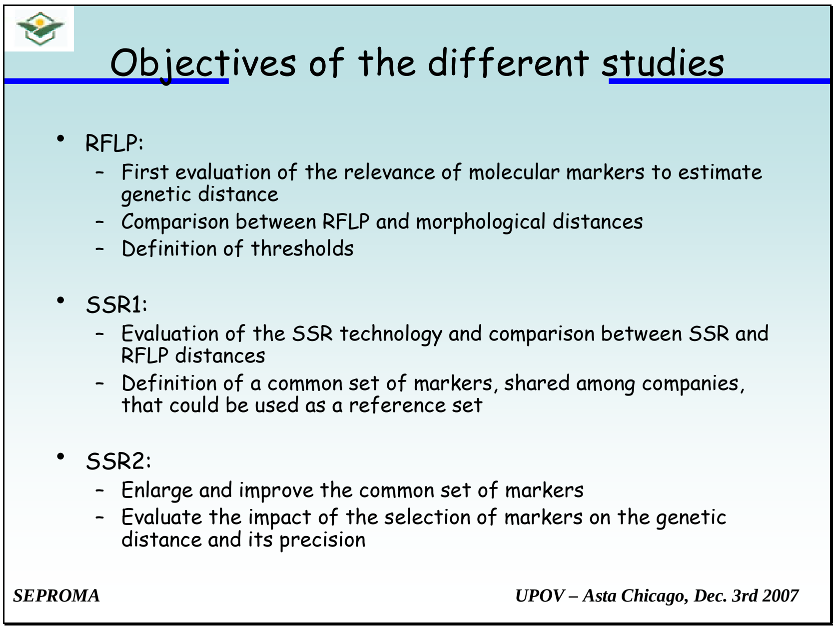
Slide 3



Objectives of the different studies

- RFLP:
  - First evaluation of the relevance of molecular markers to estimate genetic distance
  - Comparison between RFLP and morphological distances
  - Definition of thresholds
- SSR1:
  - Evaluation of the SSR technology and comparison between SSR and RFLP distances
  - Definition of a common set of markers, shared among companies, that could be used as a reference set
- SSR2:
  - Enlarge and improve the common set of markers
  - Evaluate the impact of the selection of markers on the genetic distance and its precision

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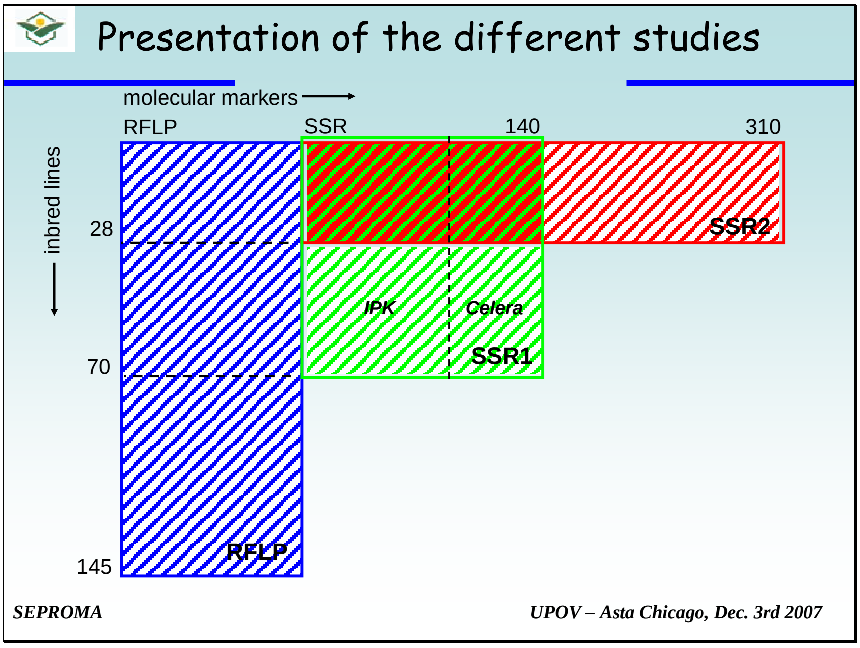

Presentation of the different studies

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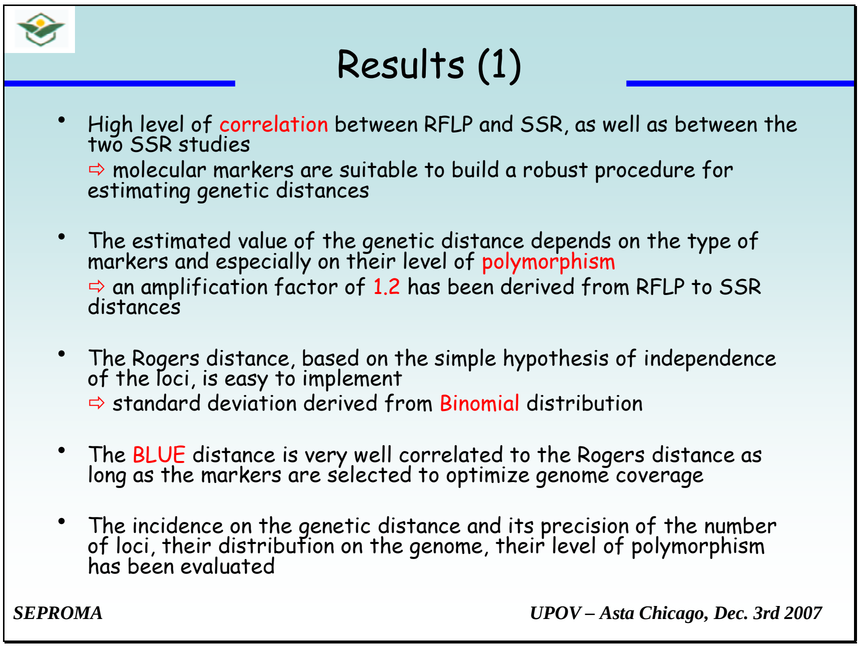

Results (1)

- High level of correlation between RFLP and SSR, as well as between the two SSR studies
  ⇨ molecular markers are suitable to build a robust procedure for estimating genetic distances
- The estimated value of the genetic distance depends on the type of markers and especially on their level of polymorphism
  ⇨ an amplification factor of 1.2 has been derived from RFLP to SSR distances
- The Rogers distance, based on the simple hypothesis of independence of the loci, is easy to implement
  ⇨ standard deviation derived from Binomial distribution
- The BLUE distance is very well correlated to the Rogers distance as long as the markers are selected to optimize genome coverage
- The incidence on the genetic distance and its precision of the number of loci, their distribution on the genome, their level of polymorphism has been evaluated

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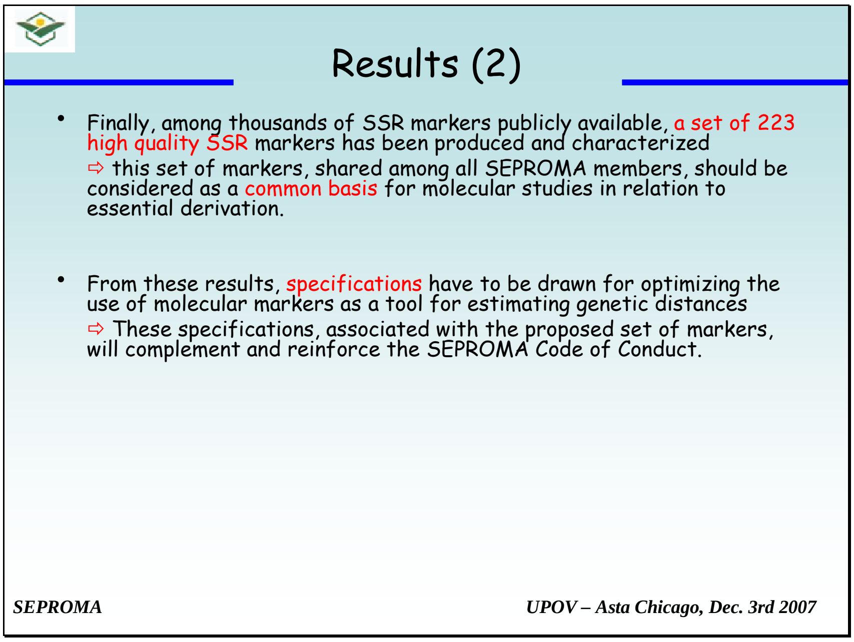

Results (2)

- Finally, among thousands of SSR markers publicly available, a set of 223 high quality SSR markers has been produced and characterized
  ⇨ this set of markers, shared among all SEPROMA members, should be considered as a common basis for molecular studies in relation to essential derivation.
- From these results, specifications have to be drawn for optimizing the use of molecular markers as a tool for estimating genetic distances
  ⇨ These specifications, associated with the proposed set of markers, will complement and reinforce the SEPROMA Code of Conduct.

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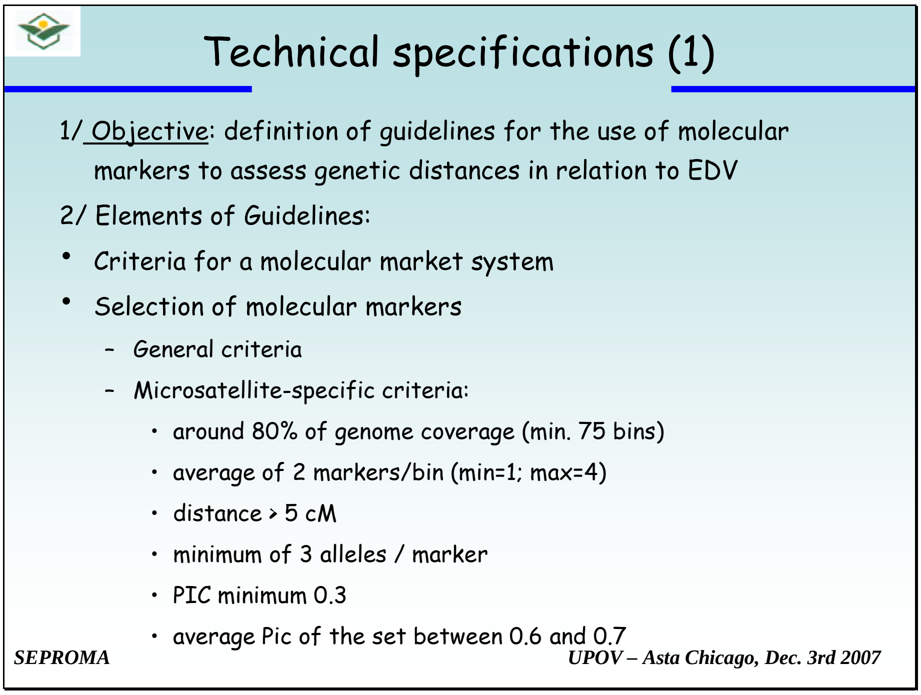

Technical specifications (1)

1/ Objective: definition of guidelines for the use of molecular markers to assess genetic distances in relation to EDV

2/ Elements of Guidelines:

- Criteria for a molecular market system
- Selection of molecular markers
  - General criteria
  - Microsatellite-specific criteria:
    - around 80% of genome coverage (min. 75 bins)
    - average of 2 markers/bin (min=1; max=4)
    - distance > 5 cM
    - minimum of 3 alleles / marker
    - PIC minimum 0.3
    - average Pic of the set between 0.6 and 0.7

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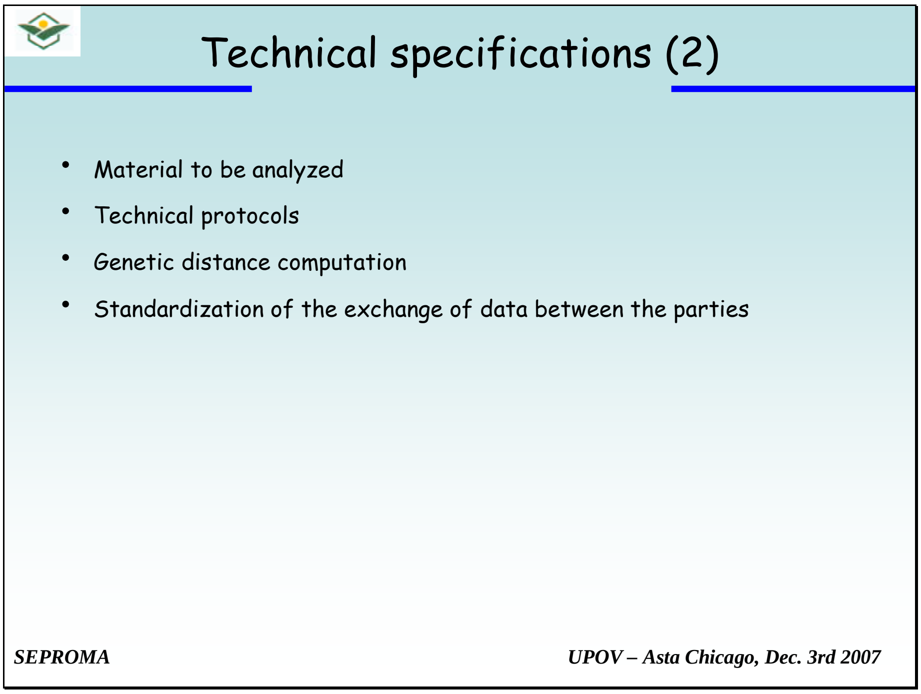

Technical specifications (2)

- Material to be analyzed
- Technical protocols
- Genetic distance computation
- Standardization of the exchange of data between the parties

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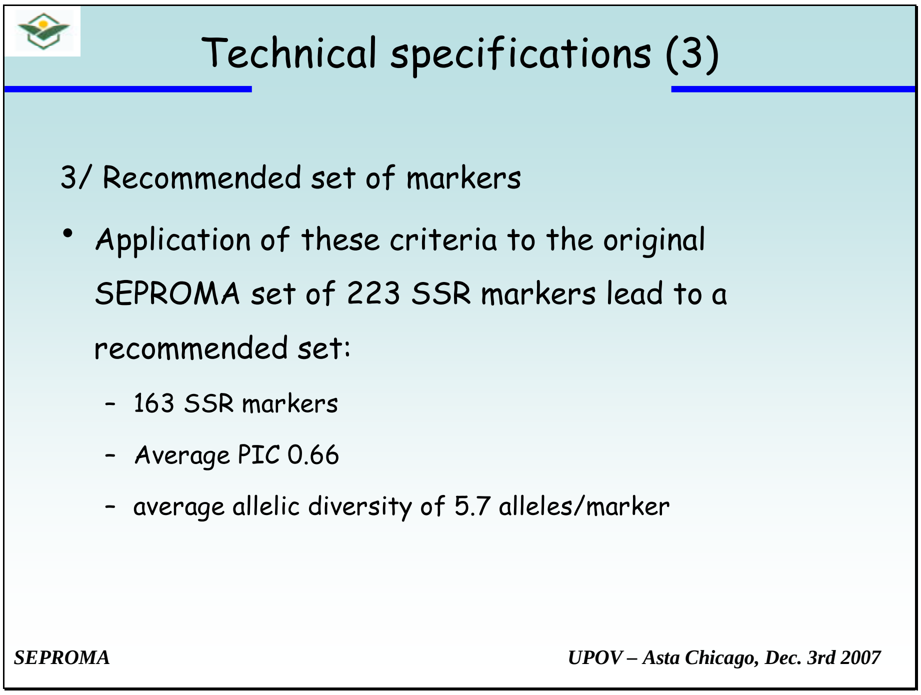

Technical specifications (3)

3/ Recommended set of markers

- Application of these criteria to the original SEPROMA set of 223 SSR markers lead to a recommended set:
  - 163 SSR markers
  - Average PIC 0.66
  - average allelic diversity of 5.7 alleles/marker

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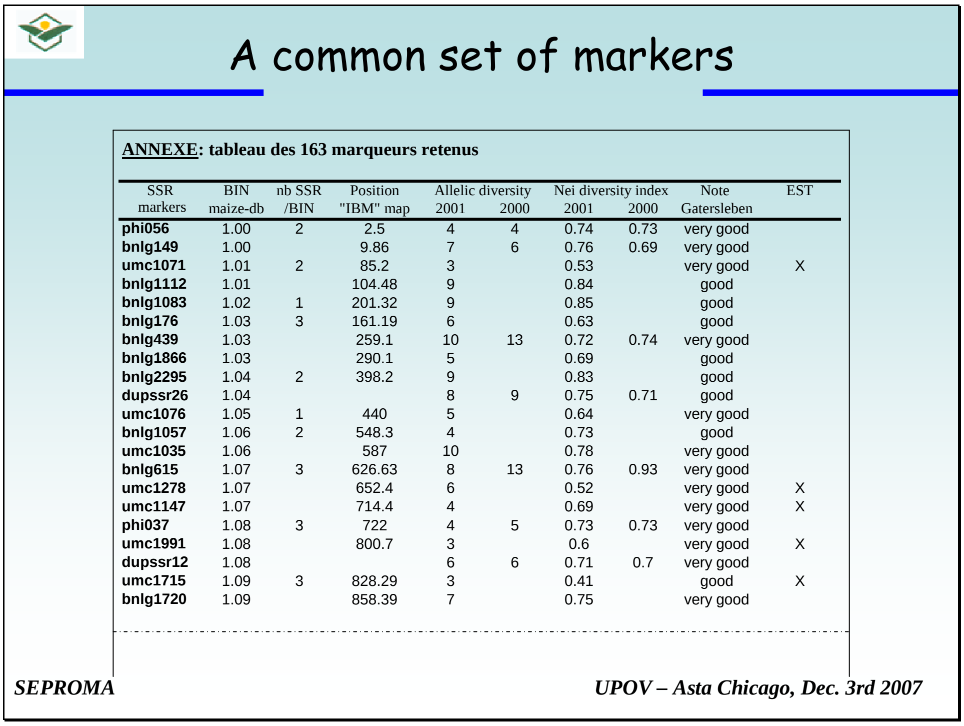

A common set of markers

<u>ANNEXE</u>: tableau des 163 marqueurs retenus

SSR markers	BIN maize-db	nb SSR /BIN	Position "IBM" map	Allelic diversity 2001	Allelic diversity 2000	Nei diversity index 2001	Nei diversity index 2000	Note Gatersleben	EST
phi056	1.00	2	2.5	4	4	0.74	0.73	very good	
bnlg149	1.00		9.86	7	6	0.76	0.69	very good	
umc1071	1.01	2	85.2	3		0.53		very good	X
bnlg1112	1.01		104.48	9		0.84		good	
bnlg1083	1.02	1	201.32	9		0.85		good	
bnlg176	1.03	3	161.19	6		0.63		good	
bnlg439	1.03		259.1	10	13	0.72	0.74	very good	
bnlg1866	1.03		290.1	5		0.69		good	
bnlg2295	1.04	2	398.2	9		0.83		good	
dupssr26	1.04			8	9	0.75	0.71	good	
umc1076	1.05	1	440	5		0.64		very good	
bnlg1057	1.06	2	548.3	4		0.73		good	
umc1035	1.06		587	10		0.78		very good	
bnlg615	1.07	3	626.63	8	13	0.76	0.93	very good	
umc1278	1.07		652.4	6		0.52		very good	X
umc1147	1.07		714.4	4		0.69		very good	X
phi037	1.08	3	722	4	5	0.73	0.73	very good	
umc1991	1.08		800.7	3		0.6		very good	X
dupssr12	1.08			6	6	0.71	0.7	very good	
umc1715	1.09	3	828.29	3		0.41		good	X
bnlg1720	1.09		858.39	7		0.75		very good	

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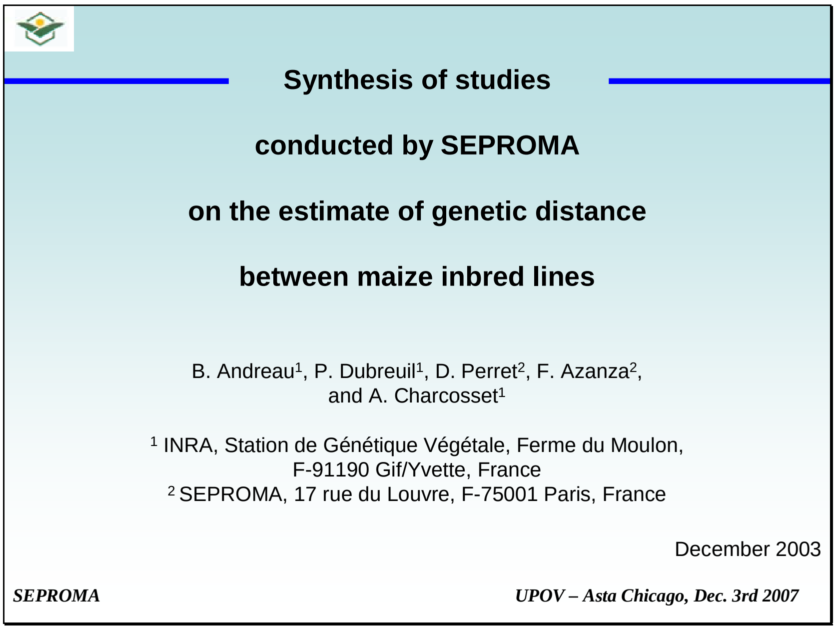

Synthesis of studies conducted by SEPROMA on the estimate of genetic distance between maize inbred lines

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and A. Charcosset[1]

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F-91190 Gif/Yvette, France
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December 2003

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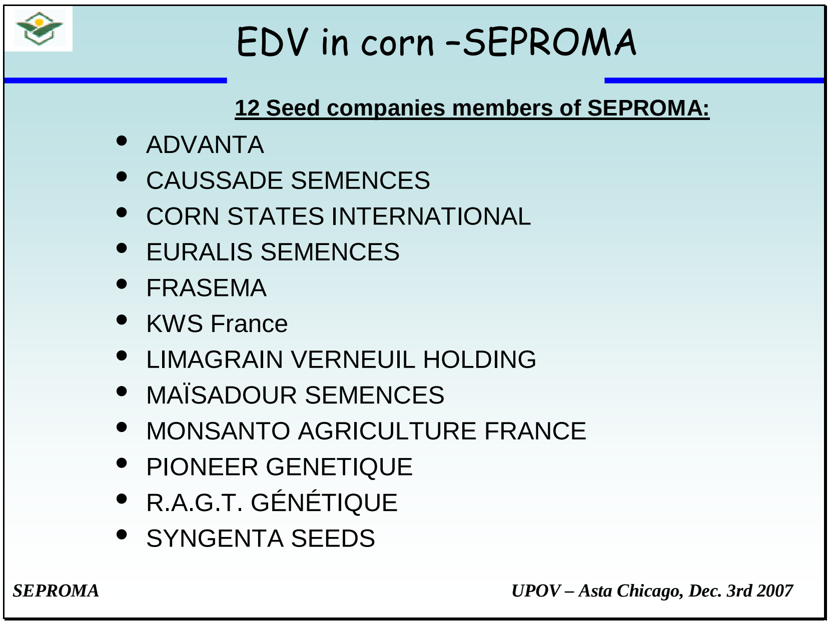

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12 Seed companies members of SEPROMA:

- ADVANTA
- CAUSSADE SEMENCES
- CORN STATES INTERNATIONAL
- EURALIS SEMENCES
- FRASEMA
- KWS France
- LIMAGRAIN VERNEUIL HOLDING
- MAÏSADOUR SEMENCES
- MONSANTO AGRICULTURE FRANCE
- PIONEER GENETIQUE
- R.A.G.T. GÉNÉTIQUE
- SYNGENTA SEEDS

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