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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

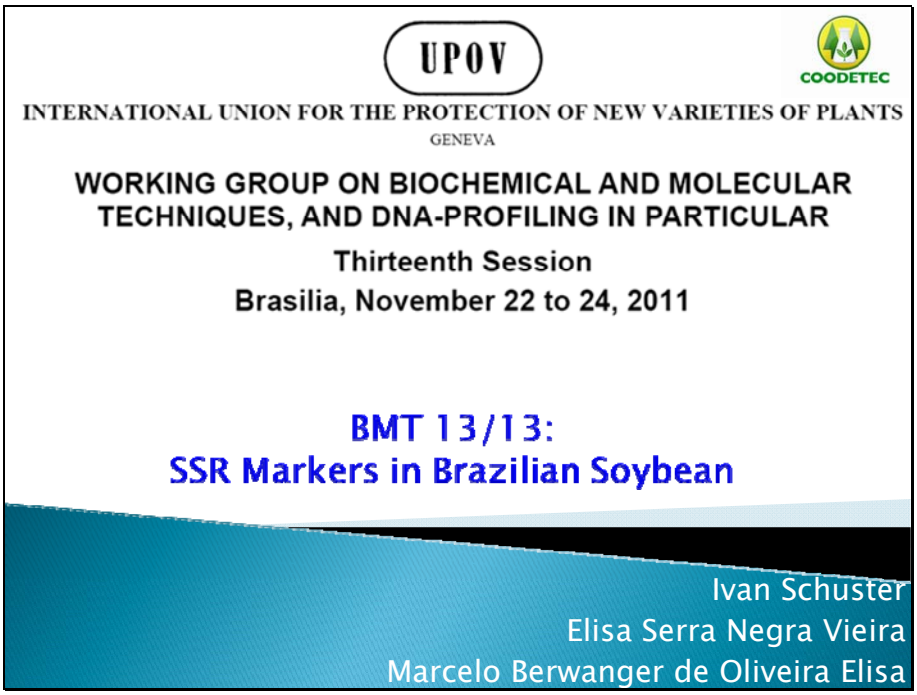
WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES, AND DNA-PROFILING IN PARTICULAR

Thirteenth Session
Brasilia, November 22 to 24, 2011

ADDENDUM

SSR MARKERS IN BRAZILIAN SOYBEAN

Document prepared by experts from Brazil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COODETEC
INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
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Thirteenth Session
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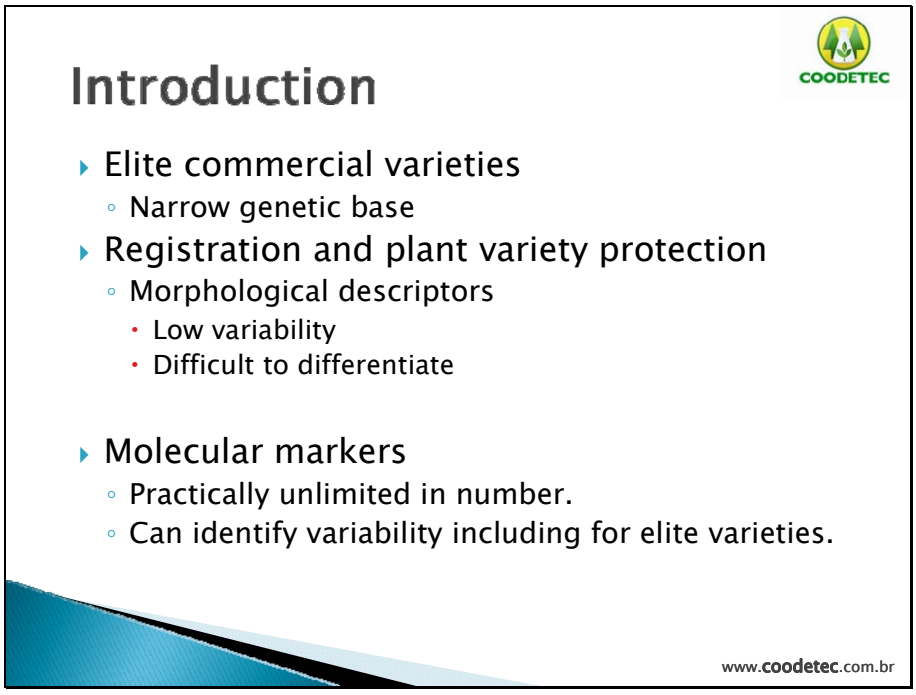
BMT 13/13:
SSR Markers in Brazilian Soybean
Ivan Schuster
Elisa Serra Negra Vieira
Marcelo Berwanger de Oliveira Elisa


COODETEC
Introduction
Elite commercial varieties
Narrow genetic base
Registration and plant variety protection
Morphological descriptors
Low variability
Difficult to differentiate
Molecular markers
Practically unlimited in number.
Can identify variability including for elite varieties.
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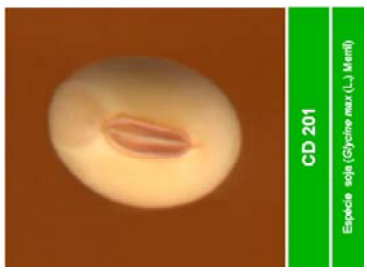
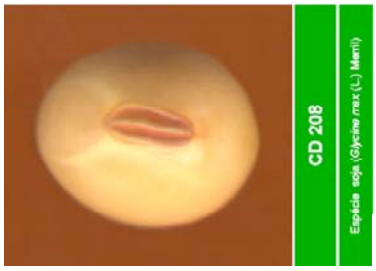
Introduction





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Descriptors

DESCRITORES	CD 201	CD 208
Características da planta		
Cor do hipocótilo	Verde	Verde
Hábitodecrescimento	Determinado	Determinado
Cor da pubescência	Cinza	Cinza
Densidade da pubescência	Normal	Normal
Características da flor		
Cor da flor	Branca	Branca
Características da vagem		
Cor da vagem (sem pubescência)	Marrom clara	Marrom clara
Cor da vagem (com pubescência)	Cinza	Cinza
Características da semente		
Forma	Esférica	Esférica
Cor do tegumento	Amarela	Amarela
Brilhodotegumento	Fosco	Fosco
Cor do hilo	Marrom clara	Marrom clara
Características bioquímicas		
Reação à peroxidase	Positiva	Positiva

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Descriptors

	CD 201	CD 208
Fisiológicos		
Ciclo vegetativo(emergência à floração)	Precoce/médio	Precoce/Médio
Ciclo total	Precoce/médio	Precoce/Médio
Alturadasplantas (na maturação)	Média	Média
Alturadeinserçãovagens inferiores(cm)	15 - 16	11 - 12
Peso de 1000 sementes (g)	Maior ouigual a16	14
Resistênciaaoacamamento	Baixa	Baixa
Resistência à deiscência das vagens	Boa	Boa
Reação a doenças		
Pústula bacteriana	Resistente	Não informado
Crestamentobacteriano	Não informado	Não informado
Cancro da haste	Resistente	Resistente
Podridãovermelha da raiz	Não informado	Não informado
Podridão pardadahaste	Resistente	Não informado
Mosaico comum	Suscetível	Não informado
Nematóide de galha	Resistente	Resistente
Nematóide do cisto	Suscetível	Suscetível
Mancha olho de rã	Resistente	Resistente

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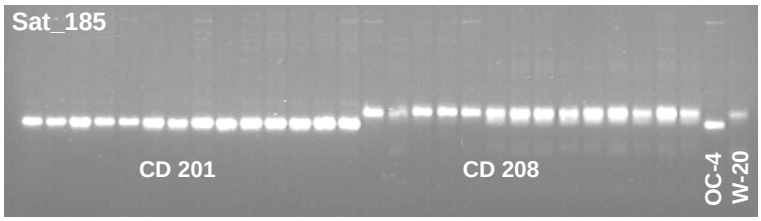
Molecular Markers



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Molecular Markers in Soybean

- Marker Assisted Selection
  - Breeding
- Seed purity certification
  - Genetic seed
  - Seed quality laboratory
    - Commercial production
- Soybean Variety Characterization
  - Plant variety protection enforcement
    - Judicial demand

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Molecular Markers in Plant Variety Protection Enforcement

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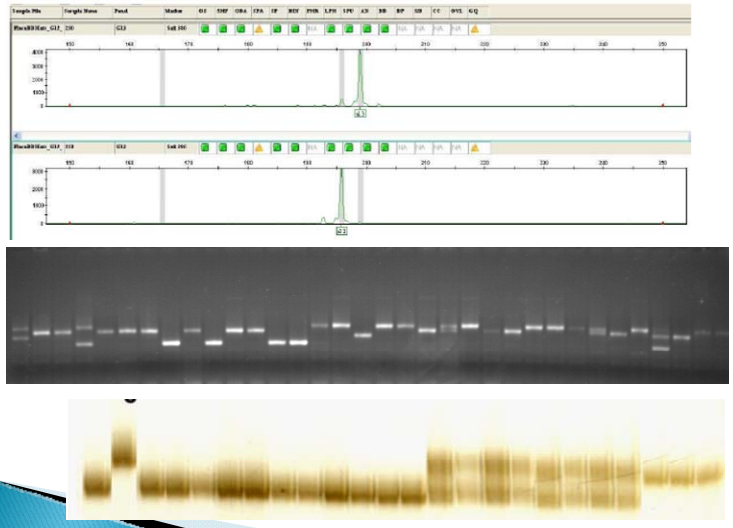
Microsatellite Genotyping



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Genotipyng Soybean SSR in Agarose Gels

- Lower accuracy.
  - Alleles > 10bp difference.
- Sufficient variability can be observed between Brazilian soybean varieties using agarose gels

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Genotipyng Soybean SSR in Agarose Gels

COODETEC

Genetic variability of soybean cultivars obtained through microsatellite markers in agarose gel

Elisa Serra Negra Vieira[1], Ivan Schuster[1], Rosane Bezerra da Silva[2] e Marco Antônio Rott de Oliveira[1]

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Abstract –The objective of this work was to evaluate the genetic variability of soybean cultivars with microsatellite markers selected and characterized for their informativity to be used in agarose gel system. The DNA of 23 soybean cultivars was amplified with 283 microsatellite markers, using a 3% agarose gel systems. The 53 markers with clearly detectable polymorphism in the agarose gels were used to characterize 53 cultivars. In these 53 cultivars, 124 alleles were detected, with an average of 2.34 alleles per locus. Polymorphism information content values varied between 0.16 and 0.66, with an average of 0.47. Allele frequencies were of 0.02 to 0.91, with an average of 0.43. Genetic distance was of 0.02 to 0.73, with an average value of 0.47. The shortest distance was between cultivars CD201 and CD208, and the largest between CD210 and BRSMT Uirapuru. The markers used permitted the identification of all bean 53 cultivars. The soybean microssatellite loci evaluated in agarose gel presented high informativity. It is possible to detect significant variability in the Brazilian soybean germplasm evaluated, even among elite cultivars, when microssatellite molecular markers, selected for their informativity, are used.

Index terms: cultivar characterization, polymorphic information content, genetic diversity, allelic frequency, SSR marker.

Pesq. agropec. bras., Brasília, v.44, n.11, p.1460-1466, nov. 2009

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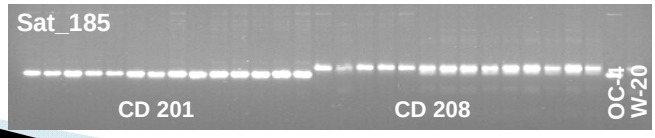
Genotipyng Soybean SSR in Agarose Gels

COODETEC

- 283 markers evaluated in 23 soybean varieties
- 111 polymorphic

Num Alleles	Num. Markers	%
4	2	1.8%
3	25	22.5%
2	84	75.7%

One marker in 283 differentiated CD 201 from CD 208 in agarose gel.



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Genotipyng Soybean SSR in Agarose Gels

- Selecting markers to be used in agarose gels.

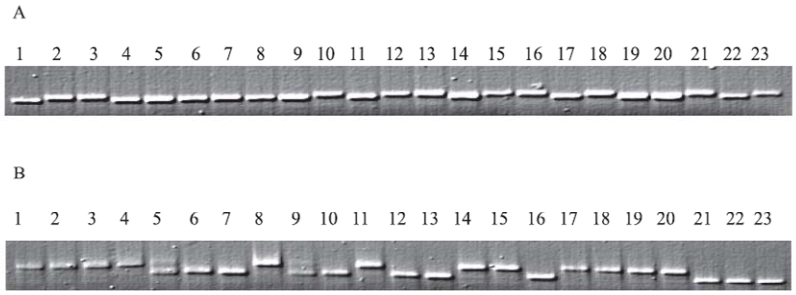

Figura 1. Padrões moleculares obtidos com os locos microssatélites Satt495 (A) e Satt135 (B), em 23 cultivares de soja.

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Genotipyng Soybean SSR in Agarose Gels

- 53 markers were selected for easily genotyping
  - Amplified em 53 soybean varieties

Num Alleles	Num. Markers	%
3	15	28.3%
2	38	71.7%

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Allele frequency

Tabela 2. Iniciadores polimórficos utilizados na avaliação de 53 cultivares de soja, localização no genoma da soja (LG), número de alelos observados, cultivar representativa dos alelos, frequências alélicas e valores de conteúdo de informação polimórfica (CIP).

Iniciadores[1]	LG	Nº de Alelos	Alelos da cultivar exemplo[2]	Frequências alélicas	CIP
Sat_001	D2	3	a: BRS154; b: CD206; c: BRS232	a: 0,49; b: 0,36; c: 0,15	0,61
Sat_099	L	2	a: BRSMG Liderança; b: CD204	a: 0,91; b: 0,09	0,16
Sat_105	I	3	a: BRS134; b: CD216; c: Msoy 8001	a: 0,23; b: 0,42; c: 0,34	0,66
Sat_115	A2	3	a: BRS184; b: CD218; c: BRS133	a: 0,46; b: 0,44; c: 0,10	0,58
Sat_168	G	3	a: MG/BR Conquista; b: CD213RR; c: FT Abyara	a: 0,09; b: 0,22; c: 0,69	0,47
Satt020	B2	2	a: Embrapa 59; b: CD207	a: 0,32; b: 0,68	0,44
Satt094	O	2	a: BRSMG 68 Vencedora; b: CD215	a: 0,62; b: 0,38	0,47
Satt194	C1	2	a: BRS Sambaíba; b: CD214RR	a: 0,50; b: 0,44	0,56
Satt181	H	3	a: CD210; b; CD211; c: Embrapa 48	a: 0,36; b: 0,31; c: 0,34	0,67
Satt200	A1	2	a: BRSMT Uirapuru; b: CD210	a: 0,49; b: 0,51	0,5
Satt216	D1b	4	a: BRSMT Pintado; b: CD208; c: BRSMT Crixás; d: BRS 68 Vencedora	a: 0,29; b: 0,48; c: 0,04; d: 0,19	0,65

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Genotyping Soybean SSR in Capilary Gels

Construction of a molecular database for soybean cultivar identification in Brazil

M.B. Oliveira[1], E.S.N. Vieira[1,2] and I. Schuster[1,2]

Genet. Mol. Res. 9 (2): 705-720 (2010)

http://www.geneticsmr.com//year2010/vol9-2/pdf/gmr706.pdf

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Genotyping Soybean SSR in Capilary Gels

- 48 Soybean SSR markers
- 32 Soybean varieties (all Coodetec)

Num Alleles	Num. Markers	%
7	1	2.1%
6	2	4.2%
5	7	14.6%
4	15	31.3%
3	18	37.5%
2	5	10.4%

Four marker in 48 differentiated CD 201 from CD 208

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Genotyping Soybean SSR in Capilary Gels

Varieties	Sat_085		Sat_141		Sat_168		Sat_294		Satt020		Satt030		Satt070		Satt079		Satt080		Satt114		Satt173		Satt175	
	Al. 1	Al. 2	Al. 1	Al. 2	Al. 1	Al. 2	Al. 1	Al. 2	Al. 1	Al. 2	Al. 1	Al. 2	Al. 1	Al. 2	Al. 1	Al. 2	Al. 1	Al. 2	Al. 1	Al. 2	Al. 1	Al. 2	Al. 1	Al. 2
CD 201	174		183		155		256		101		152		172		149		160		93		251		176	
CD 202	200		183		177		206		101		152		148		125		184		102		251		191	
CD 203	200		183		155		256		101		152		148		143		184		78		251		191	
CD 204	174		183		155		206		101		152		172		125		157		105		206		176	
CD 205	174		183		155		206		119		167		148		125		157		78		251		176	
CD 206	174	200	183		155		206		119		158		148		143		154		78		206		185	
CD 207	174		183	235	169		206		101		167		163		125		157		78		206		191	236
CD 208	200		183		155		206		101		152		172		149		160		93		251		176	
CD 209	174		183		155		206		119		167		148		149		157		93		206	251	176	
CD 210	174		203	235	169		206		101		158		163		149		184		78		251		185	
CD 211	174		183		155		206		101		152		172		125		157		78		263		176	
CD 212RR	174		183	205	155	169	256		101	119	158		148		149		154		105		206		176	
CD 213RR	174		205	235	169		186	206	119		158		148		149		181		93		206		161	
CD 214RR	174		183		155		256		101		152		148		149		160		105		251		176	
CD 215	200		183		177		206		101		152		148		125		184		93		251		191	
CD 216	174		183		155		256		119		167		148		125		157		78		197		167	
CD 217	174		211	235	155		190		101		149		172		125		184		78		206		176	
CD 218	200		183		177		190		125		158		148		125		184		102		206		191	
CD 219RR	174		183		155		206		101		152		172		149		157		93		251		176	
CDFAPA 220	174		183		155		206		101		167		163		143	149	154		78		251		185	
CD 221	200		183		169		222		119		158		148		149		154		78		206		191	
CD 222	174		183		155	169	206		101		167		163	172	125		157		105		206	263	176	191
CD 223AP	174		183		155		256		101		149		163		143		154		78		206		161	
CD 224	174		183		155		206		101		152		163		149		160		93		251		176	
CD 225RR	174		183		155		256		119		161		148		149		181		105		251		176	
CD 226RR	174		183		155		256		101		158		172		149		160		93		251		176	
CD 227	174		183		155		206		101		167		172		125		184		105		206	251	176	
CD 228	174		183		155		206		113		167		175		146		181		105		251		167	
CD 229RR	174		181	183	155		206		119		161		148		125		181		78		251		176	
CD 230RR	174		183		155		206		101		161		148		125		157		78		251		176	
CD 231RR	174		181		155		256		101		167		148		125		181		78		251		176	
CD 232	174		183		155		256		119		167		148		125		157		78		206		185	

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Allele Frequency

Marker	Nº alleles	Allele	Frequency	PIC
Sat_085	2	174	0.80	0.32
		200	0.20	
Sat_141	6	181	0.05	0.31
		183	0.83	
		203	0.02	
		205	0.03	
		211	0.02	
		235	0.06	
Sat_168	3	155	0.75	0.40
		169	0.16	
		177	0.09	
Sat_294	5	186	0.02	0.56
		190	0.06	
		206	0.58	
		222	0.03	
		256	0.31	

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Conclusion

- Microsatellite Markers are highly informative in Brazilian soybean elite cultivars.
- The genotyping method depends of the objective of the molecular analysis:
  - Agarose gel an automatic sequencer can be used to distinguish soybean varieties.
    - Agarose gels can be used in low scale and day by day analysis, using known samples as references.
    - For precise characterization, genotyping in automatic sequencer (capillary gel) is recommended.

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