



BMT/13/24 Add.

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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

**CONSTRUCTION OF A MOLECULAR DATABASE FOR SOYBEAN VARIETY
IDENTIFICATION IN BRAZIL**

Document prepared by experts from Brazil



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

BMT 13/24:
**Construction of a Molecular Database for
Soybean Variety Identification in Brazil**

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Ivan Schuster



Soybean characterization

- ▶ Elite commercial varieties
 - Narrow genetic base
- ▶ Registration and plant variety protection
 - Morphological descriptors
 - Low variability
 - Difficult to differentiate
 - Molecular characterization
 - High variability at molecular level
 - Easy to distinguish varieties
 - Correct identification – Statistically demonstrated
 - Molecular Database with allele frequencies

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Objective

- To characterize a set of 32 soybean varieties from Coodetec
 - Microsatellite markers
 - Genotyped in automated sequencer (ABI 3130xl)
- To obtain allelic frequencies of a set of 48 microsatellite markers
- To estimate the minimum number of markers to identify precisely each variety.

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Material and Methods

- 32 soybean varieties from Coodetec

CD 201	CD212RR	CD 223AP
CD 202	CD213RR	CD 224
CD 203	CD214RR	CD 225RR
CD 204	CD215	CD 226RR
CD 205	CD216	CD 227
CD 206	CD217	CD 228
CD 207	CD218	CD 229RR
CD 208	CD219RR	CD 230RR
CD 209	CDFAPA220	CD 231RR
CD 210	CD221	CD 232
CD 211	CD222	

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Material and Methods



► 48 microsatellite markers

Marker	Type	Label	L. G.	Marker	Type	Label	L. G.
Sat_085	di	6FAM	C1	Satt302	tri	VIC	H
Sat_141	di	6FAM	G	Satt303	tri	NED	G
Sat_168	di	VIC	G	Satt307	tri	6FAM	C2
Sat_294	di	NED	A2	Satt309	tri	6FAM	G
Satt020	tri	6FAM	B2	Satt311	tri	NED	D2
Satt030	tri	6FAM	F	Satt335	tri	NED	F
Satt070	tri	NED	B2	Satt352	tri	NED	G
Satt079	tri	VIC	C2	Satt358	tri	PET	O
Satt080	tri	PET	N	Satt371	tri	PET	C2
Satt114	tri	NED	F	Satt386	tri	VIC	D2
Satt173	tri	6FAM	O	Satt406	tri	6FAM	J
Satt175	tri	PET	M	Satt417	tri	VIC	K
Satt177	tri	PET	A2	Satt426	tri	VIC	B1
Satt181	tri	NED	H	Satt431	tri	VIC	J
Satt184	tri	PET	D1a	Satt464	tri	PET	D2
Satt191	tri	6FAM	G	Satt485	tri	NED	N
Satt197	tri	VIC	B1	Satt540	tri	NED	M
Satt200	tri	PET	A1	Satt545	tri	6FAM	A1
Satt216	tri	NED	D1b	Satt579	tri	PET	D1b
Satt231	tri	VIC	E	Satt600	tri	VIC	D1b
Satt233	tri	NED	A2	Satt663	tri	VIC	F
Satt253	tri	PET	H	Satt685	tri	VIC	E
Satt285	tri	NED	J	Satt703	tri	VIC	D1b
Satt301	tri	NED	D2	Satt728	tri	NED	M

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Material and Methods



► PCR

- Final Volume: 20 µL
- 30 ng DNA
- 3 mM MgCl₂
- 1x buffer (2 mM de Tris, 5 mM de KCl)
- 250 µM dNTP
- 0,4 µM primer (sense and antisense)
- 1 unit *Taq DNA polymerase*

► Amplification

- 1 cycle initial denaturation
 - 94° C – 3 min
- 35 cycles amplification
 - 94° C – 30 sec
 - 55° C – 30 sec
 - 72° C – 45 sec
- 1 cycle final extension
 - 72° C – 20 min

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Material and Methods

- ▶ Proof and counterproof
 - Made in different days
 - Two independent DNA extraction
 - Two independent PCR and electrophoresis
- ▶ Analysis
 - Reproducibility
 - Differences in allele sizes in proof and counterproof assay.

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Results

Varieties	Sat_085		Sat_141	
	P	CP	P	CP
CD 201	174.56	174.40	183.19	183.00
CD 202	200.45	200.37	183.33	183.16
CD 203	200.46	200.24	183.27	183.06
CD 204	174.31	174.72	183.18	183.04
CD 205	174.54	174.37	183.18	183.07
CD 206	174.42	174.20	183.16	183.02
CD 207	174.60	173.91	182.40	182.98
CD 208	200.35	200.37	183.23	183.01
CD 209	174.59	174.42	183.25	183.11
CD 210	174.51	174.53	203.55	202.65
CD 211	174.48	174.36	183.24	183.03
CD 212RR	174.42	174.43	183.37	182.57
CD 213RR	174.38	174.25	205.75	204.87
CD 214RR	174.63	174.16	183.02	183.06
CD 215	200.12	200.12	183.09	182.96

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Results

Varieties	Sat_085	Sat_141
	Diference	Diference
CD 201	0.16	0.19
CD 202	0.08	0.17
CD 203	0.22	0.21
CD 204	0.41	0.14
CD 205	0.17	0.11
CD 206	0.22 0.22	0.14
CD 207	0.69	0.58 0.31
CD 208	0.02	0.22
CD 209	0.17	0.14
CD 210	0.02	0.90 0.65
CD 211	0.12	0.21
CD 212RR	0.01	0.80 0.70
CD 213RR	0.13	0.88 0.45
CD 214RR	0.47	0.04
CD 215	0.00	0.13

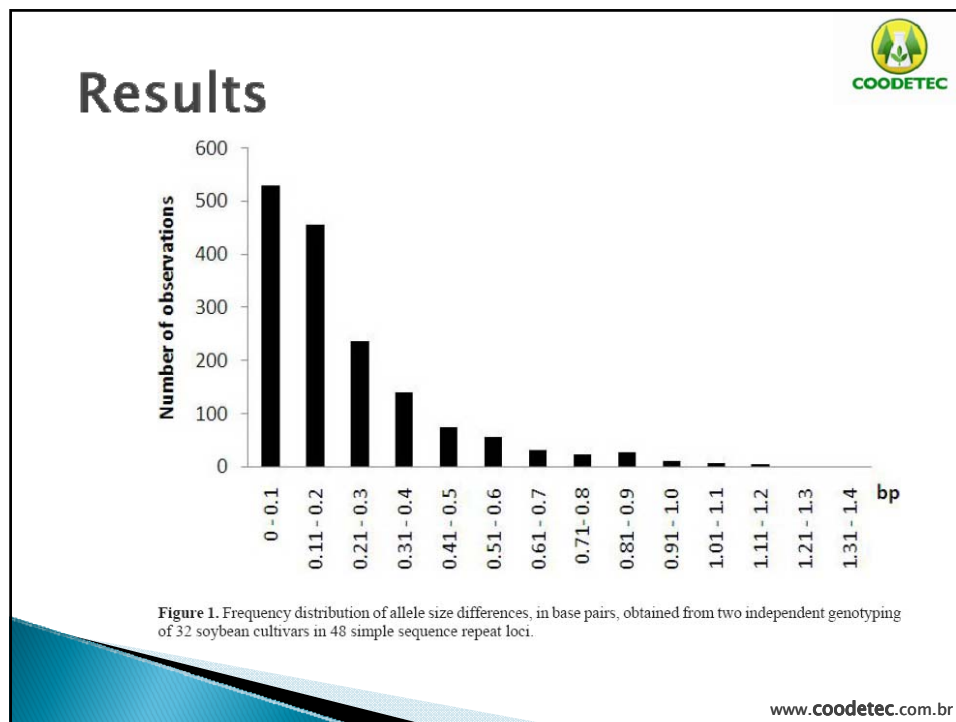
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Results

- ▶ 32 varieties x 48 primers = 1536 paired data points
- ▶ Diference between allele size in proof and counterproof assay:
 - 0 – 1.32bp. Average 0.22bp
- ▶ Standar Deviation
 - 0 – 0.93bp

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Results

Molecular profiles

Varieties	Sat_085	Sat_141	Sat_168	Sat_294	Satt020
CD 201	174	183	155	256	101
CD 202	200	183	177	206	101
CD 203	200	183	155	256	101
CD 204	174	183	155	206	101
CD 205	174	183	155	206	119
CD 206	174 200	183	155	206	119
CD 207	174	183 235	169	206	101
CD 208	200	183	155	206	101
CD 209	174	183	155	206	119
CD 210	174	203 235	169	206	101
CD 211	174	183	155	206	101
CD 212RR	174	183 205	155 169	256	101 119
CD 213RR	174	205 235	169	186 206	119
CD 214RR	174	183	155	256	101
CD 215	200	183	177	206	101


COODETEC

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Results



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	
CD FAPA 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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Results



Varieties	Satt177		Satt181		Satt184		Satt191		Satt197		Satt200		Satt216		Satt231		Satt233		Satt253		Satt285		Satt301	
	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	122		207		186		225		182		228		192		226		199		152		204		244	
CD 202	113		198		186		228		185		246		222		226		199		152		240		199	
CD 203	113		207		171		228		185		246		156		226		199		152		240		262	
CD 204	107		198		150		225		185		228		156		220		199		137		204		199	
CD 205	110		216		141		207		134		228		192		226		187		155		240		244	
CD 206	113		177		150		225		185		228		156		220		187		137		204		259	
CD 207	110		216		141		207		188		246		192		220		187		155		240		244	
CD 208	122		207		186		228		182		228		192		226		199		152		204		244	
CD 209	110		177		150		207		134		228		192		220	226	187		137		240		244	
CD 210	110		216		141		225		182		228		192		220		208		137		240		247	
CD 211	122		198		150		207		185		246		192		220		199		137		204		244	
CD 212RR	113		207		141		228		188		246		168		226		187		137	152	204		199	
CD 213RR	113		207		150		228		188		228	246	222		220		187	208	137		204		199	
CD 214RR	122		207		150		225		182		228		192		226		199		152		240		244	247
CD 215	110		177		186		204		182		246		192		238		199		137	152	204		262	
CD 216	110		216		141		207	228	134		228		192		226		187		152		240		244	
CD 217	122		207		150		189		173		228		222		220		199		137		204		199	
CD 218	110		198		150		228		185		246		192		220	226	187		155		240		199	
CD 219RR	122		207		150		225		185		228		192		220		199		152		204		244	
CD FAPA 220	110		216		141		225		134		228		156		223		187		137		240		244	
CD 221	110		177		141		225		182		246		192		220		187		155		204		262	
CD 222	107		198	216	141		207	225	185	188	228	249	138		220		199		137	155	204	240	199	
CD 223AP	110		207		186		228		182		228		171		226		199		152		240		244	
CD 224	110		207		141		207		182		246		192		226		199		137		204	240	244	
CD 225RR	122		216		150		228		173		246		192		226		187	199	152		204		244	
CD 226RR	122		207		186		228		182		228		192		226		199		152		204		244	
CD 227	107		198		150		207		185		228		156		226		199		137		204		244	
CD 228	110		216		171		207		134		246		189		226		187		155		240		244	
CD 229RR	110		216		141		207		173		246		192	222	226		187		137		204		244	
CD 230RR	113		216		150		204		134		246		192		226		187		137		204		244	
CD 231RR	113		207		141		204		173		246		192		226		187		137		240		199	
CD 232	110	113	207		150		225		134		228		192		220		187		155		240		259	

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Results

Varieties	Satt302		Satt303		Satt307		Satt309		Satt311		Satt335		Satt352		Satt358		Satt371		Satt386		Satt406		Satt417	
	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	257		237		163		130		187		159		182		161		251		199		242		325	
CD 202	206		255		172		124		199		159		191		203		275		196		242		325	
CD 203	257		255		163		124		199		150		182		203		251		196		323		325	
CD 204	206		255		172		130		232		159		191		194		275		166		242	323	283	
CD 205	206		246		184		130		232		165		185		194		275		199		242		325	
CD 206	206		255		172		130		232		150		191		194		275		166		323		283	
CD 207	206		237		184		124		232		150		182		194		275		166		242		325	
CD 208	257		237		163		130		187		159		182		161		251		199		242		325	
CD 209	206		246		184		130		232		165		185		194		275		199		242		325	
CD 210	206		255		184		124		187		150		191		194		251		166		242		325	
CD 211	206		255		172		130		232		165		191		194		275		199		242		325	
CD 212RR	257		237		172		124	130	187		150	165	182		194		275		166	199	242		325	
CD 213RR	206	257	237		172		124		187		165		182		194		275		199		242	323	325	
CD 214RR	257		237		163		130		187	199	150		182		161		251		166		242		325	
CD 215	206		255		172		124		232		150		191		194		275		199		242		325	
CD 216	257		246		163	184	130		232		165		185		194		251		199		242		325	
CD 217	206		222		184		133		232		159		167		203		260		199		245		283	
CD 218	206		255		172		124		232		159		191		203		275		196		242		325	
CD 219RR	206		255		172		130		232		159		191		194		275		166		242		325	
CD FAPA 220	206		237		172		130		232		159		182		194		275		199		242		325	
CD 221	206		255		184		124		232		150		191		194		251		199		242		325	
CD 222	206		237		184		124	130	232		159		182		194		275		166	199	242	245	283	
CD 223AP	257		246		163		130		232		150		185		194		251		196		242		283	
CD 224	206		246		172		130		232		159		185		161		251		166	199	245		325	
CD 225RR	257		237		163		130		199		159		182		194		275		166		326		325	
CD 226RR	257		237		172		130		199		159		182		161		275		199		242		325	
CD 227	206		255		184		130		187		159		191		194		254		166		242		283	
CD 228	206		246		163		145		232		165		185		194		275		199		242		325	
CD 229RR	206		237	246	184		145		232		159		167	194	194		275		199		323		325	
CD 230RR	206		237		184		130		232		150		194		194		275		199		242		325	
CD 231RR	257		237		184		145		232		159		194		194		275		199		242		325	
CD 232	257		237		184		130		232		150		182		194		275		199		242		325	

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Results

Varieties	Satt426		Satt431		Satt464		Satt485		Satt540		Satt545		Satt579		Satt600		Satt663		Satt685		Satt703		Satt728	
	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	198		187		219		264		148		191		198		203	215	252		185		229	235	149	
CD 202	198		199		189	219	252		154		203		198		215		252		185	215	229		194	
CD 203	198		199		219		252		154		203		174		155		249		185		229		194	
CD 204	198		232		189		240		169		191		174		155		252		218		199		191	
CD 205	219		232		219		264		154		206		174		155		249		185		229		149	
CD 206	198	201	232		219		240	264	169		191		174		155		252		215		199		191	
CD 207	198		232		219		240		148		203		198		215		249		215		229		194	
CD 208	198		187		219		264		148		191		198		215		252		185		229		149	
CD 209	219		232		219		264		154		203		174		155		249		185		229		149	
CD 210	198		187		219		264		148		188		174		155		249		215		199		194	
CD 211	198		232		219		240		169		203		174		155		249		185		199		191	
CD 212RR	198		187	232	189		264		154		203		198		215		213	249	215		229		149	191
CD 213RR	198		187		189		240		148		203		198		215		213		215		229		191	
CD 214RR	198		187	199	219		264		166		191		198		215		252		215		229		149	
CD 215	198		232		219		240		154		203		198		215		213		215		229		194	
CD 216	219		232		219		264		154		203		192		203		249		215		229		149	
CD 217	198		232		189		240		166		191		174		203		249		218		229		194	
CD 218	198		232		189		252		154		203		198		215		252		185		229		149	
CD 219RR	198		232		219		240		169		191		174		155		252		185		199		149	
CD FAPA 220	219		232		219		264		154		203		174		155		249		185		229		191	
CD 221	198		232		219		264		169		188		198		215		249		215		199		194	
CD 222	198		232		189		240	148	169		191	203	174	198	155	215	249	252	215		229		149	
CD 223AP	201		232		219		264		154		203		174		203		249		185		199	229	149	
CD 224	198		232		219		264		148		203		198		215		249		185		229		149	
CD 225RR	201		199		219		264		166		203		192		203		249		215		235		149	
CD 226RR	198		199		219		264		148		203		198		215		252		185		229		149	
CD 227	198		187		219		252		169		191		174		155		252		215		229		191	
CD 228	201		232		219		264		154		203		174		155		252		215		235		149	
CD 229RR	201		232		189	219	264		154		203		174	198	203		249		185		229		149	194
CD 230RR	219		232		219		264		154		203		198		203		249		185		229		194	
CD 231RR	201		232		189		264		154		203		174		155		249		185		229		149	
CD 232	219		232		219		264		169		203		174		155		249		215		199		191	

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Conclusion

- ▶ Genotyping soybean microsatellite markers in capillary gels was highly reproducible.
- ▶ This genotyping system can be used to characterize soybean cultivars.