



Avaliação fenotípica de uma população de *C. canephora* Conilon cultivada em altitude elevada, visando um programa de Seleção Genômica Ampla (SGA) em cafeeiro.

**VIII Simpósio de Pesquisa dos Cafés do Brasil
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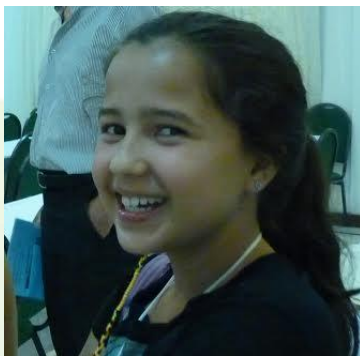
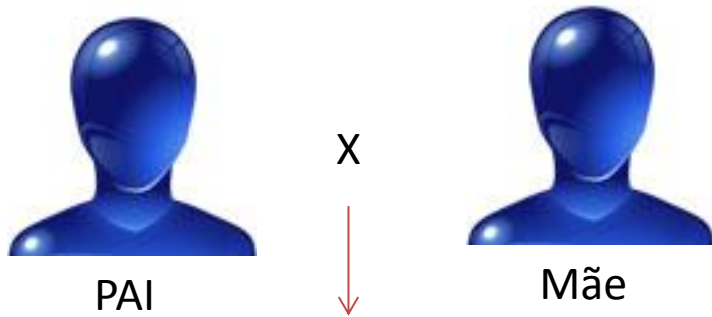
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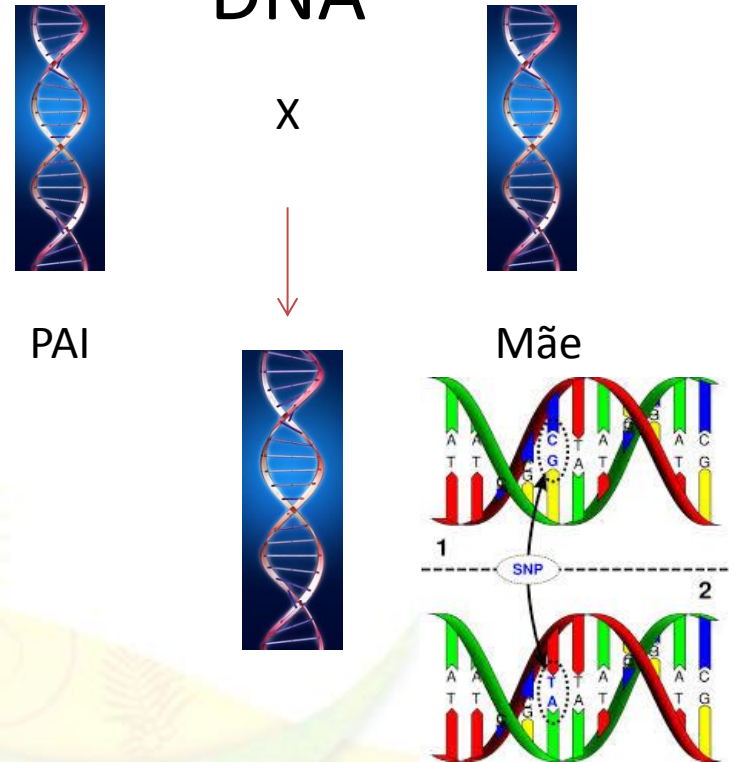


SGA

Fenótipo



DNA



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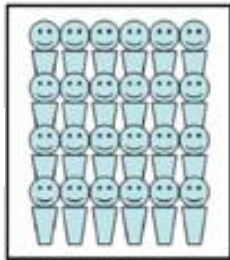
Seleção Genômica Ampla

Fenotipagem

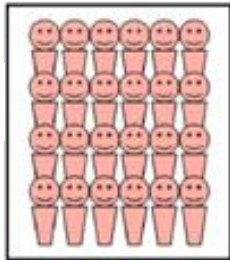
Genotipagem

Mapeamento
(Genoma Referência)

Interesse



Normais



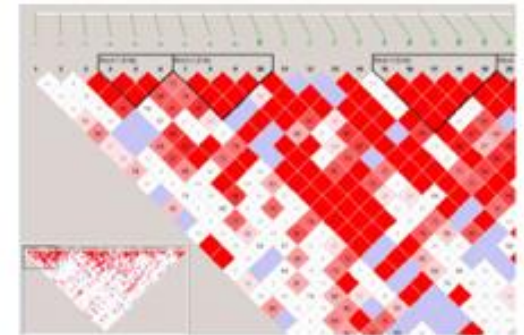
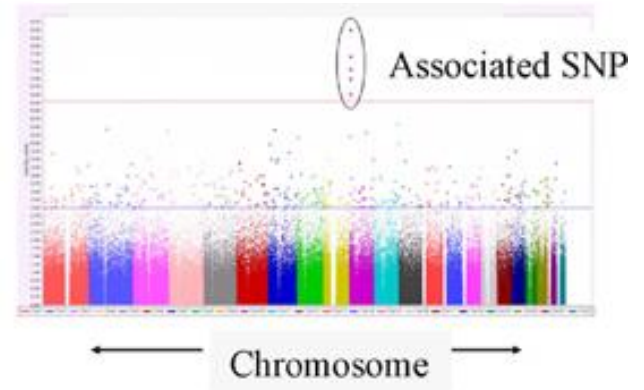
DNA

DNA Chip/GBS



Informação

Análises
Estatísticas



Desequilíbrio de Ligação

$$\begin{aligned}
 W = & |1 - \Phi(\mu_2, 0)| \int_{\Phi^{-1}(\alpha_1/2)}^{\infty} \phi(\mu_1, z_1) dz_1 \\
 & + \int_{\Phi^{-1}(1-\alpha_1/2)}^{\Phi^{-1}(1-\alpha_2/2)} \phi(\mu_1, z_1) [1 - \Phi(\mu_2, \Phi^{-1}\{1 - \frac{\gamma}{4(1-\Phi(z_1))}\})] dz_1 \\
 & + \Phi(\mu_2, 0) \int_{\Phi^{-1}(1-\alpha_2/2)}^{\infty} \phi(\mu_1, z_1) dz_1 + \int_{\Phi^{-1}(1-\alpha_1/2)}^{\Phi^{-1}(1-\alpha_2/2)} \phi(\mu_1, z_1) \Phi(\mu_2, \Phi^{-1}\{1 - \frac{\gamma}{4(1-\Phi(z_1))}\}) dz_1 \\
 & + |1 - \Phi(\mu_2, 0)| \int_{-\infty}^{\Phi^{-1}(\alpha_1/2)} \phi(\mu_1, z_1) dz_1 + \int_{\Phi^{-1}(\alpha_1/2)}^{\Phi^{-1}(\alpha_2/2)} \phi(\mu_1, z_1) [1 - \Phi(\mu_2, \Phi^{-1}\{1 - \frac{\gamma}{4(1-\Phi(z_1))}\})] dz_1 \\
 & + \Phi(\mu_2, 0) \int_{-\infty}^{\Phi^{-1}(\alpha_1/2)} \phi(\mu_1, z_1) dz_1 + \int_{\Phi^{-1}(\alpha_1/2)}^{\Phi^{-1}(\alpha_2/2)} \phi(\mu_1, z_1) \Phi(\mu_2, \Phi^{-1}\{1 - \frac{\gamma}{4(1-\Phi(z_1))}\}) dz_1,
 \end{aligned}$$

(25)



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Esquema de trabalho/ Programa SGA

Material Vegetal

Seleção de indivíduos(1300)
Extração de DNA

Avaliações fenotípicas

Avaliações fisiológicas(Ψ_m)
Avaliações de campo(Produção, Vigor, SP, etc...)
Avaliações de Laboratório (Parâmetros morfológicos e bioquímicos dos frutos)

Genotipagem

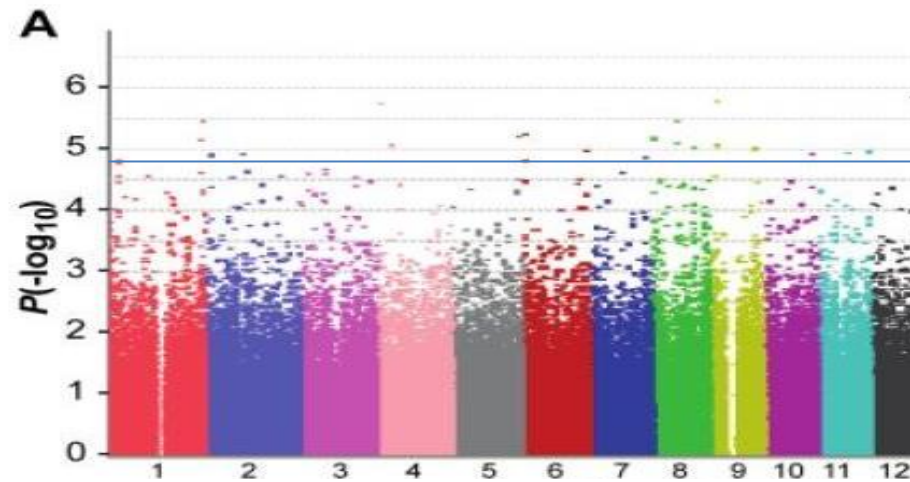
Mapeamento de sequencias (Grupos de diversidade, parentais)
Mineração de SNPs
Genotipagem

Estudos de Associação

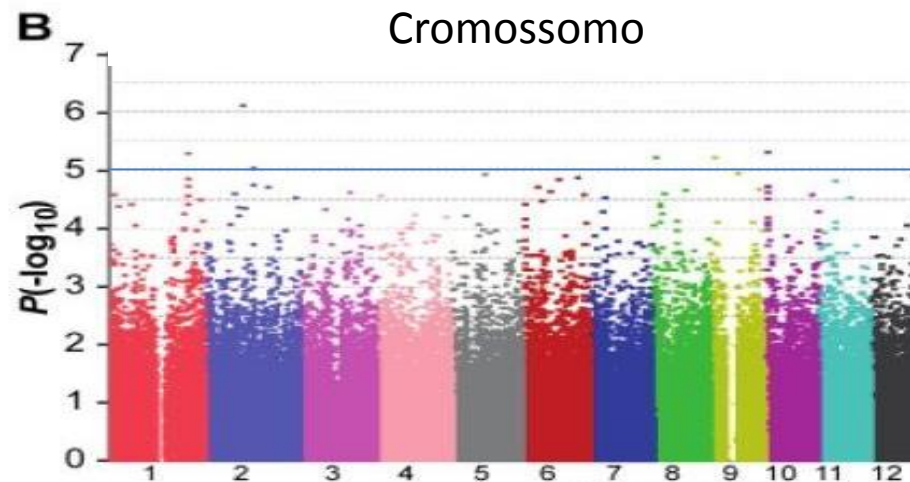
Análises Estatísticas

Seleção Genômica Ampla

Produção



Tolerância à seca



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Coffea canephora



Embrapa Cerrados, Planaltina-DF
(1.175m altitude)



Embrapa

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BRAZILIAN GOVERNMENT
BRASIL



População de Coffea canephora

- População de 3500 indivíduos (Março de 2008)
- Pool de sementes do campo experimental do INCAPER, contendo 48 parentais
- Introdução de híbridos do CIRAD
- (Clone 61/126 (Guinean x SG1+SG2) x A03 (SG1))



Diversidade Fenotípica



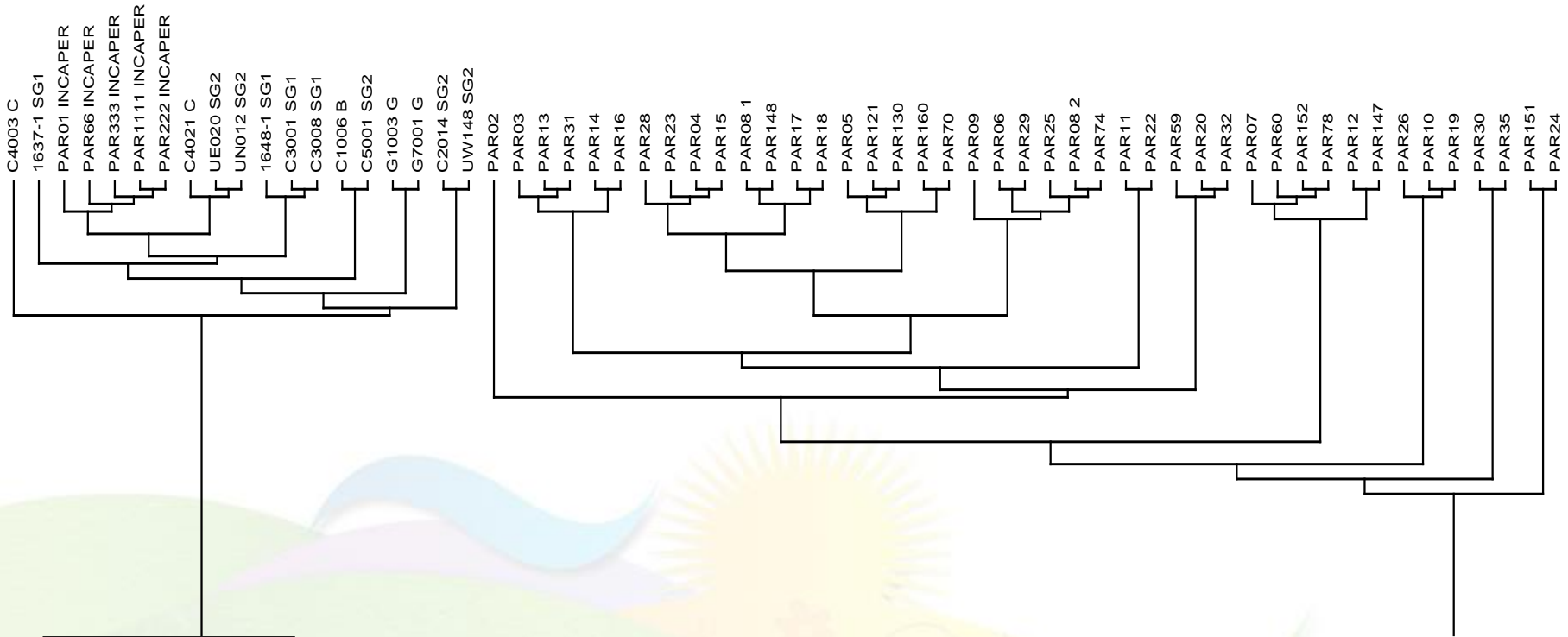
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Diversidade genética

- 11 SSRs
- 48 parentais do Incaper
- 250 indivíduos

Var. 8111
Var. 8121
Var. 8131
Var. 8141-Robustão
Var. 8142 C. Vitoria



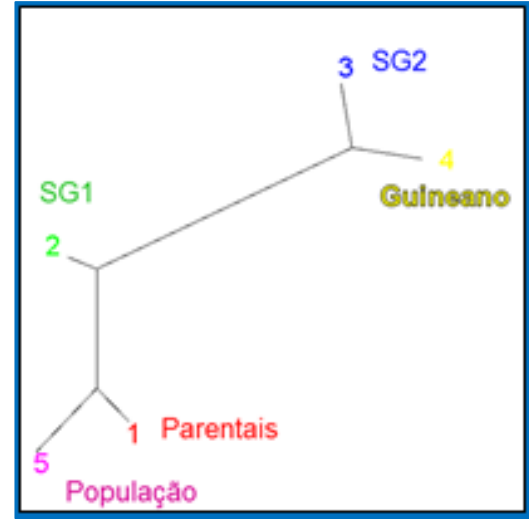
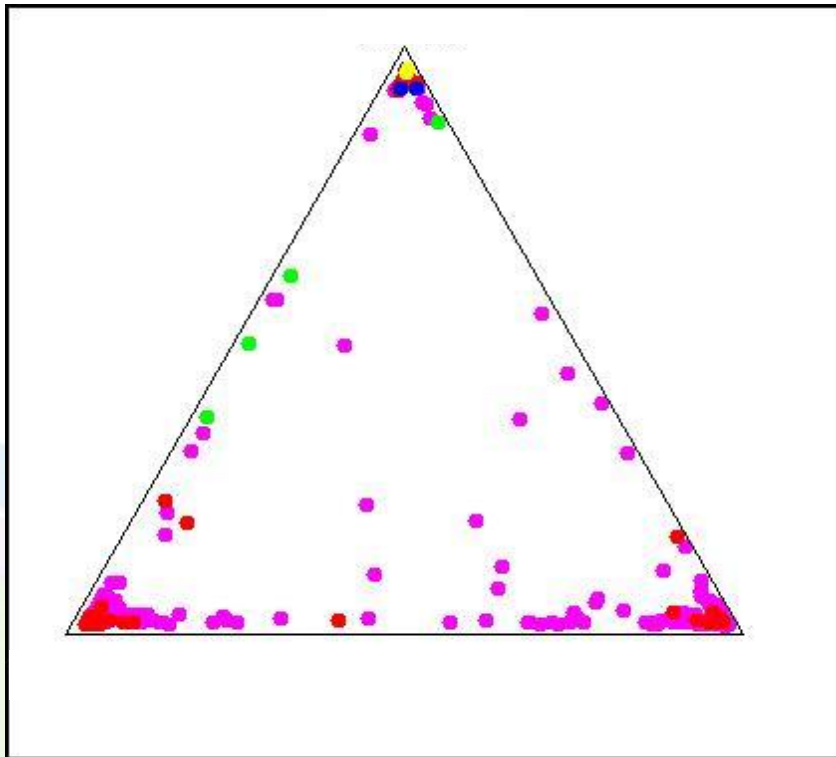
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Diversidade Genética

- Parentais INCAPER
- População EMBRAPA
- Grupo SG1
- Grupo SG2
- Grupo Guineano

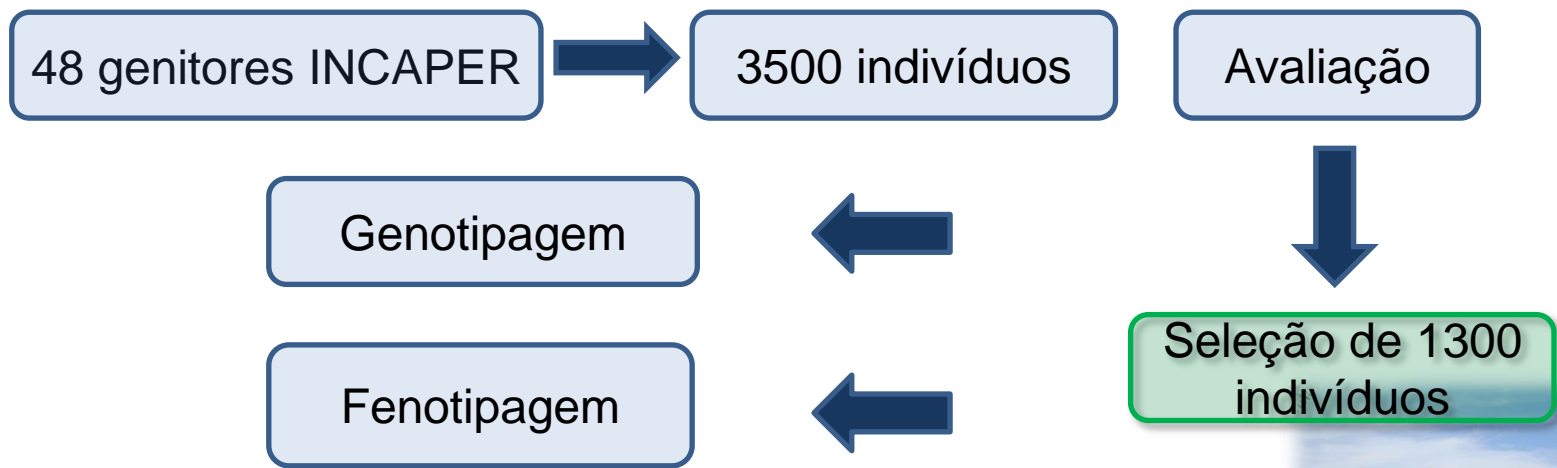


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MATERIAL VEGETAL



- SECA (Potencial hídrico de antemanhã- Ψ_{am})
- Produção (2012/2013)
- Qualidade Física
 - Classificação quanto aos defeitos e impurezas
 - Peneira
 - Peso de 100 grãos
- Medida e forma dos frutos



Embrapa



Avaliações fenotípicas

- Produção (2011/12; 2012/13)
- Vigor, Seca de Ponteiros, Ferrugem
- Morfologia de frutos
 - (Tamanho e formato dos frutos/grãos; Peneira)
- Potencial hídrico de antemanhã (Ψ_{am})
- Bioquímica (NIRs)



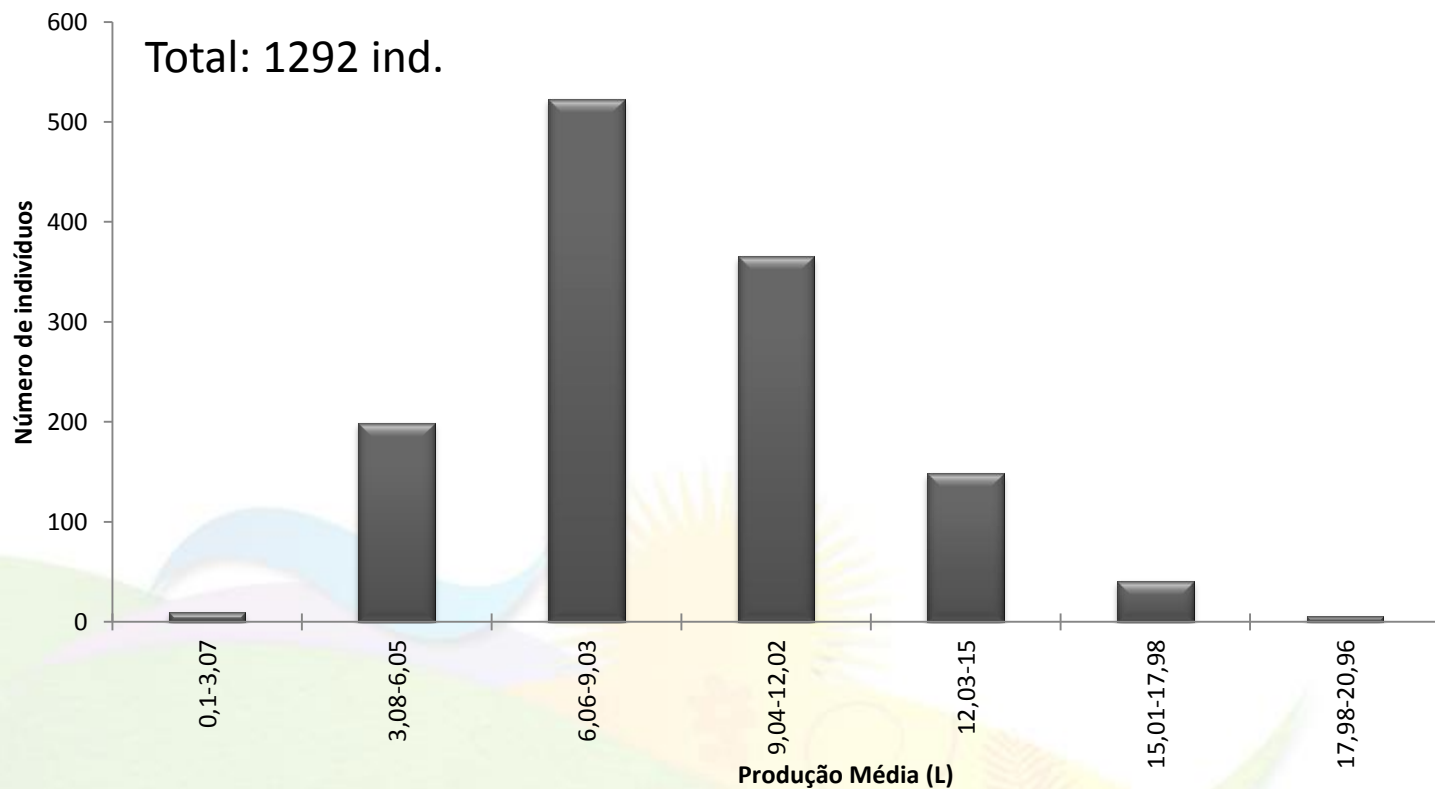
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Avaliações fenotípicas

Produção (Safras 2011/12; 2012/13).








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Avaliações fenotípicas

Morfologia de frutos

Total: 1292 ind.

Planta	Medidas (cm)	E1	E2	E3	E4	E5	Média	Foto
L1P15	Área	3,1942	3,0670	3,1608	2,0485	2,8882	2,8717	
	Perímetro	10,2846	10,0100	10,0965	8,3837	9,6723	9,6894	
	Menor eixo	0,8476	0,8548	0,8979	0,6393	0,8476	0,8174	
	Maior eixo	1,1996	1,1421	1,1206	1,0200	1,0846	1,1134	
L1P49	Área	2,7595	3,0292	2,9748	2,2486	2,4950	2,7014	
	Perímetro	9,5112	9,8257	9,7624	8,5690	9,0959	9,3528	
	Menor eixo	0,8045	0,9194	0,8907	0,7327	0,7470	0,8189	
	Maior eixo	1,0918	1,0487	1,0631	0,9769	1,0631	1,0487	
L1P65	Área	2,7543	2,7689	3,3083	2,4859	2,9070	2,8449	
	Perímetro	9,4084	9,4497	10,3036	8,9968	9,6568	9,5631	
	Menor eixo	0,8476	0,8404	0,9338	0,7758	0,8763	0,8548	
	Maior eixo	1,0344	1,0487	1,1277	1,0200	1,0559	1,0573	
L2P8	Área	3,5965	3,7512	3,4234	3,1398	3,5732	3,4968	
	Perímetro	10,8373	11,0463	10,5376	10,0058	10,7474	10,6349	
	Menor eixo	0,9266	0,9553	0,9194	0,9338	0,9482	0,9367	
	Maior eixo	1,2355	1,2499	1,1852	1,0703	1,1996	1,1881	
L4P51	Área	2,5131	2,3896	2,6324	2,6324	2,7232	2,5781	
	Perímetro	8,9727	8,7076	9,1905	9,1905	9,3274	9,0777	
	Menor eixo	0,8189	0,8404	0,8332	0,8332	0,8620	0,8375	
	Maior eixo	0,9769	0,9051	1,0056	1,0056	1,0056	0,9798	



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Avaliações fenotípicas

Peneira.

Total: 1292 ind.

Linha	Planta	Peso inicial	Peneiras (gramas)															Peso de 100 grãos
			19	13	18	12	17	11	16	10	15	9	14	13	8	12	Final	
1	15	98,978	0,427	5,12	10,888	13,455	31,255	7,1	23,169	3,445	2,987	0,692	0,222	0,093	0,047	0	0	18,964
2	10	98,977	1,208	2,951	2,368	22,948	15,352	14,382	22,983	6,684	8,271	0,8	0,971	0,077	0	0	0	13,85
5	60	98,97	8,304	23,666	21,004	14,626	22,828	2,18	4,112	1,406	0,384	0,101	0,196	0	0	0	0	19,642
8	42	100	0,371	3,461	0,957	12,412	12,929	11,323	25,453	10,941	11,446	6,584	2,101	1,501	1,52	0,167	0	17,045
12	68	99,679	0,828	3,253	0,836	11,368	15,698	13,821	30,097	9,441	10,897	1,642	1,276	0,286	0,069	0	0	16,042
18	100	97,61	0,181	0	0,148	10,38	14,125	10,359	27,088	7,622	18,036	3,024	2,482	0,967	0,222	0,128	0,027	14,301
21	36	98,836	3,963	5,57	22,041	17,924	25,757	5,953	11,88	1,769	2,643	0,962	0,353	0,076	0	0	0,111	16,218



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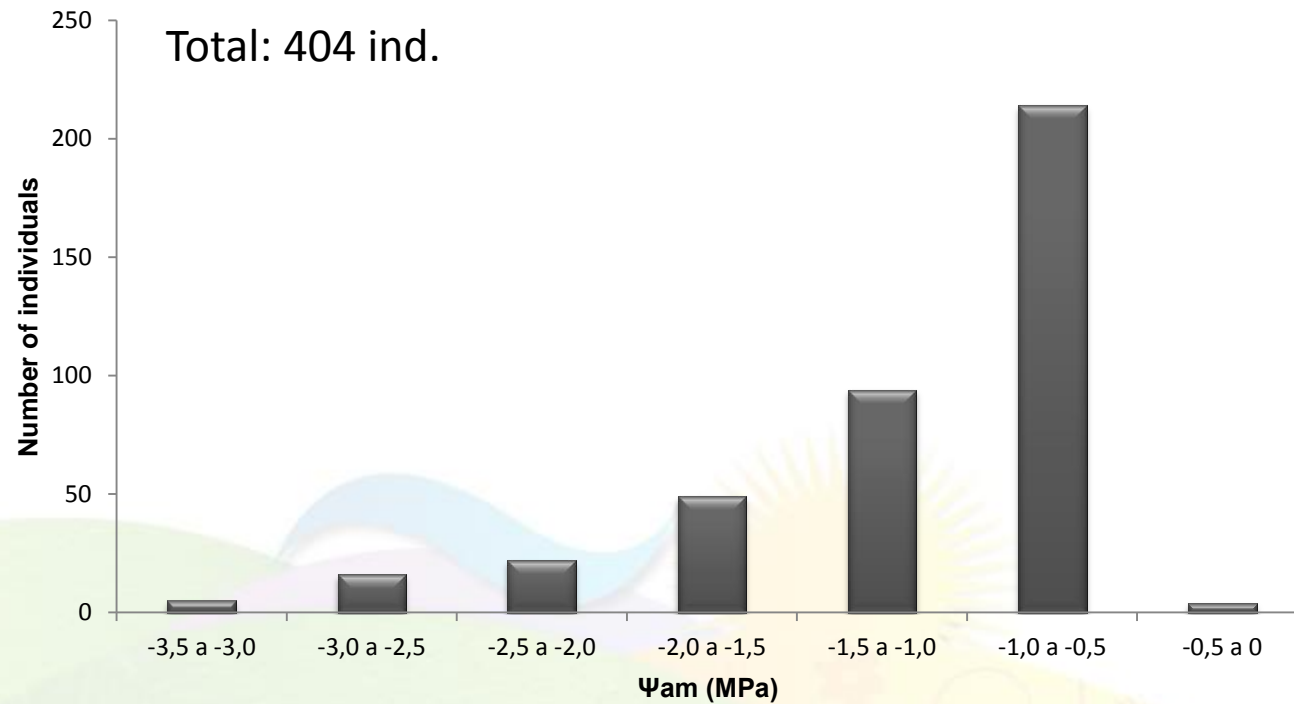




Avaliações fenotípicas

Ψ_{am} .

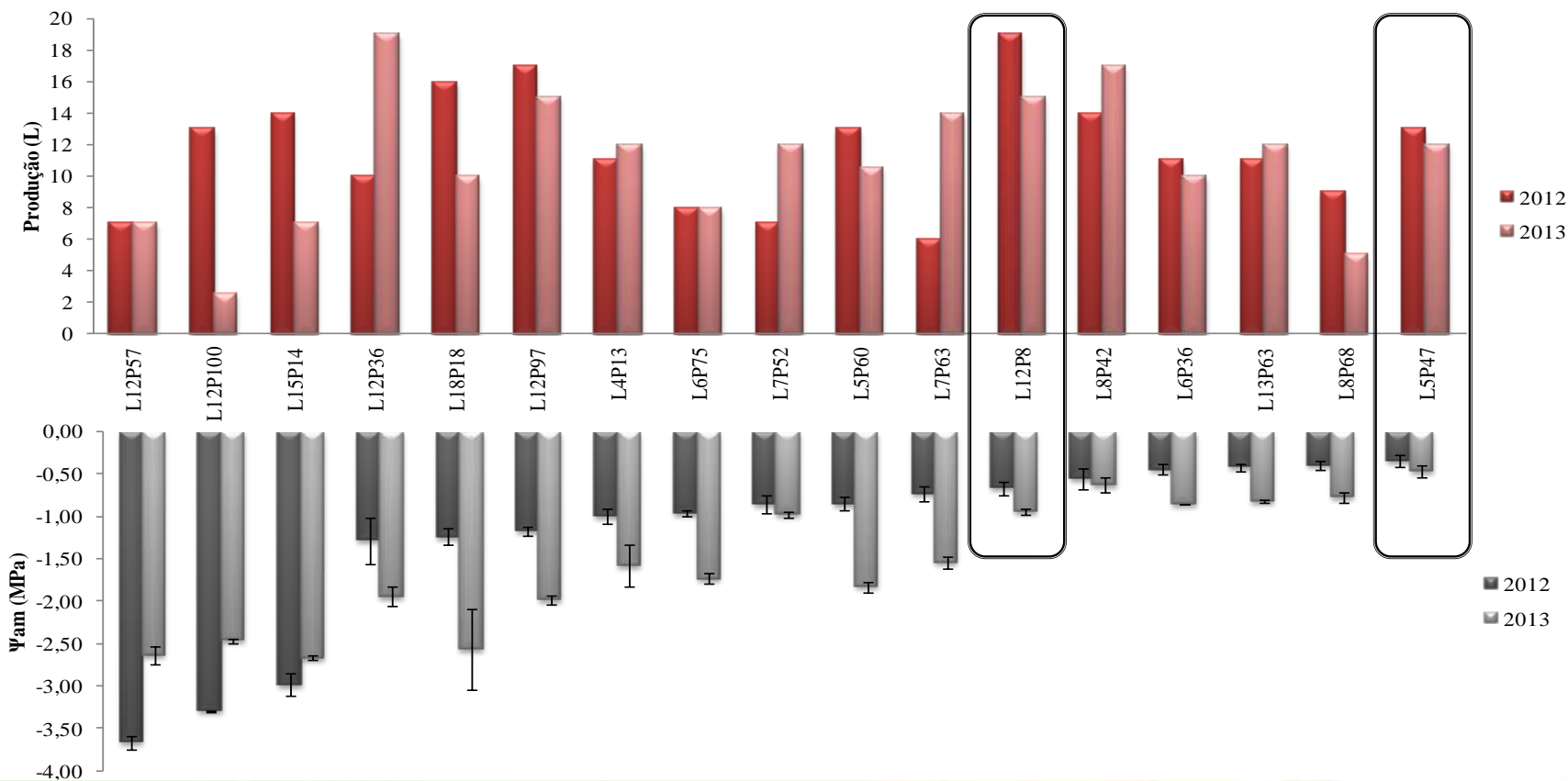
Total: 404 ind.



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Avaliações fenotípicas



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Ferramentas Genômicas



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Genômica Funcional

<https://alanine.cenargen.embrapa.br/>

<http://www.lge.ibi.unicamp.br/cafe/>



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<http://www.coffeegenome.org>)

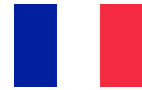


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Coffee Genomics (*C. Canephora*)



Patrick Wincker, Benoit Bertrand, Xavier Argout, Alexander de Kochko, Philippe Lashermes



Ray Ming, Victor A. Albert, Steve Rounsley, Chifumi Nagai



Alan C. Andrade



Giorgio Graziosi, Giovanni Giuliano



Robert Henry



David Sankoff



Jayarama

Artigo submetido !



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Coffee Genomics



	Seq. Length
• chr0	211,557,763
chr1	39,058,466
chr2	55,779,056
chr3	32,748,578
chr4	28,836,138
chr5	29,815,822
chr6	38,175,694
chr7	30,521,884
chr8	32,321,752
chr9	22,854,367
chr10	28,279,280
chr11	34,345,602



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Re-sequenciamento

- Paired end
- **Libraries**
 - 500 pb < fragment < 900pb 700pb
 - Kit *TruSeq DNA Sample Prep* da illumina®
 - Quantificada por PCR quantitativo em tempo real (qPCR)
 - Adaptadores
 - Read 1
AGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNATCTCGTATGCCGTCTTCTGCTTG
 - Read 2
AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCAT
- HiSeq2000
- Sequencing Kit SBS version 3.
- Fastq generated with Casava 1.8.2.



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Re-sequenciamento

Conilons

Bulk de indivíduos

Pool	Grupo	Nº de ind.
C1	Conilon	5
C2	Conilon	1
C3	Conilon	5
C4	Conilon	4
C5	Conilon	4
C6	Conilon	5
C7	Conilon	6
C8	Conilon	2
C9	Conilon	3
C10	Conilon	4
C11	Conilon	2
C12	Conilon	3
C13	Conilon	2
C14	Conilon	2



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Re-sequenciamento

Diversidade

Pool	Sub-Grupos	Grupos	Nº ind.
D1	Luki	SG1	10
D2	Niaouli	SG1	9
D3	Nana	C	10
D4	Libengue	B	9
D5	INEAC	SG2	4
D6	Erect	SG2	2
D7	Nganda	SG2	2
D8	Wild	SG2	3
D9	Pelezi	G	1
D10	Mouniandougou	G	1

Table 1 Characteristics and origins of the *C. canephora* diversity groups

Name	Origin	Diversity group	Size	Type
Nana	Central African Republic	C	92	Spontaneous
Pélézi	Ivory Coast	GP	35	Spontaneous
Guinean	Ivory Coast – Guinea	G	128	Cultivated & spontaneous
SG1 Bulk	Atlantic Coast (Gabon – Congo – Democratic Republic of Congo)	SG1	16	Cultivated & spontaneous
SG2 Bulk	Congo basin	SG2	85	Cultivated

Fonte: Cubry et al, 2013



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Re-sequenciamento

Bulk

Sequencias geradas

Conilon

704.388.334

Grupos de Diversidade

591.003.832

SEQUÊNCIAS MAPEADAS POR CROMOSSOMO NOS GRUPOS DE CONILON

GRUPO		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
SEQUÊNCIAS MAPEADAS POR CROMOSSOMO	Chr 0	22.847.673	23.266.498	26.251.519	22.920.224	26.500.581	27.815.681	17.130.483	19.537.273	22.522.141	17.383.775	17.976.528	24.389.429
	Chr 1	2.747.574	2.606.043	2.860.528	2.566.752	3.011.794	3.260.396	1.981.073	2.222.365	2.661.276	1.932.017	2.027.509	2.932.716
	Chr 2	3.745.735	3.461.916	3.694.033	3.362.678	3.978.005	4.306.896	2.622.638	2.981.014	3.600.217	2.572.280	2.672.530	3.958.483
	Chr 3	2.175.734	2.027.575	2.213.335	2.030.661	2.354.675	2.526.614	1.575.480	1.815.314	2.133.057	1.570.070	1.608.337	2.306.835
	Chr 4	1.980.196	1.878.082	2.023.810	1.828.973	2.166.485	2.310.580	1.423.346	1.602.356	1.945.111	1.406.433	1.448.269	2.111.932
	Chr 5	2.195.347	2.047.966	2.268.753	2.019.978	2.363.754	2.557.089	1.560.357	1.759.123	2.096.641	1.523.240	1.593.879	2.304.424
	Chr 6	2.584.643	2.522.983	2.728.238	2.452.092	2.891.910	3.097.469	1.897.330	2.146.752	2.586.767	1.870.663	1.949.751	2.813.507
	Chr 7	2.442.490	2.976.066	3.546.638	2.995.165	3.333.671	3.463.330	2.325.487	2.758.670	2.726.545	2.370.544	2.208.992	3.008.706
	Chr 8	2.261.443	2.186.163	2.402.402	2.162.838	2.522.483	2.802.880	1.675.104	1.884.538	2.228.929	1.610.152	1.682.928	2.536.347
	Chr 9	1.552.561	1.495.944	1.609.198	1.461.082	1.723.491	1.798.908	1.113.532	1.280.404	1.533.941	1.132.404	1.164.787	1.640.864
	Chr 10	1.915.275	1.851.555	1.990.848	1.831.830	2.122.887	2.305.051	1.396.694	1.604.950	1.915.852	1.384.972	1.441.490	2.117.205
Chr 11	2.565.490	2.386.719	2.607.106	2.376.587	2.763.460	3.008.855	1.834.382	2.060.489	2.461.882	1.800.893	1.833.626	2.751.430	
SEQUÊNCIAS MAPEADAS		49.014.161	48.707.510	54.196.408	48.008.860	55.733.196	59.253.749	36.535.906	41.653.248	48.412.359	36.557.443	37.608.626	52.871.878
SEQUÊNCIAS NÃO MAPEADAS		2.921.469	4.144.078	5.529.146	4.119.966	4.899.734	4.275.957	4.013.444	5.915.864	5.017.223	4.249.809	4.577.874	5.810.456
TOTAL DE SEQUÊNCIAS		51.935.630	52.851.588	59.725.554	52.128.826	60.632.930	63.529.706	40.549.350	47.569.112	53.429.582	40.807.252	42.186.500	58.682.334



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Re-sequenciamento

Bulk

Sequencias geradas

Conilon

704.388.334

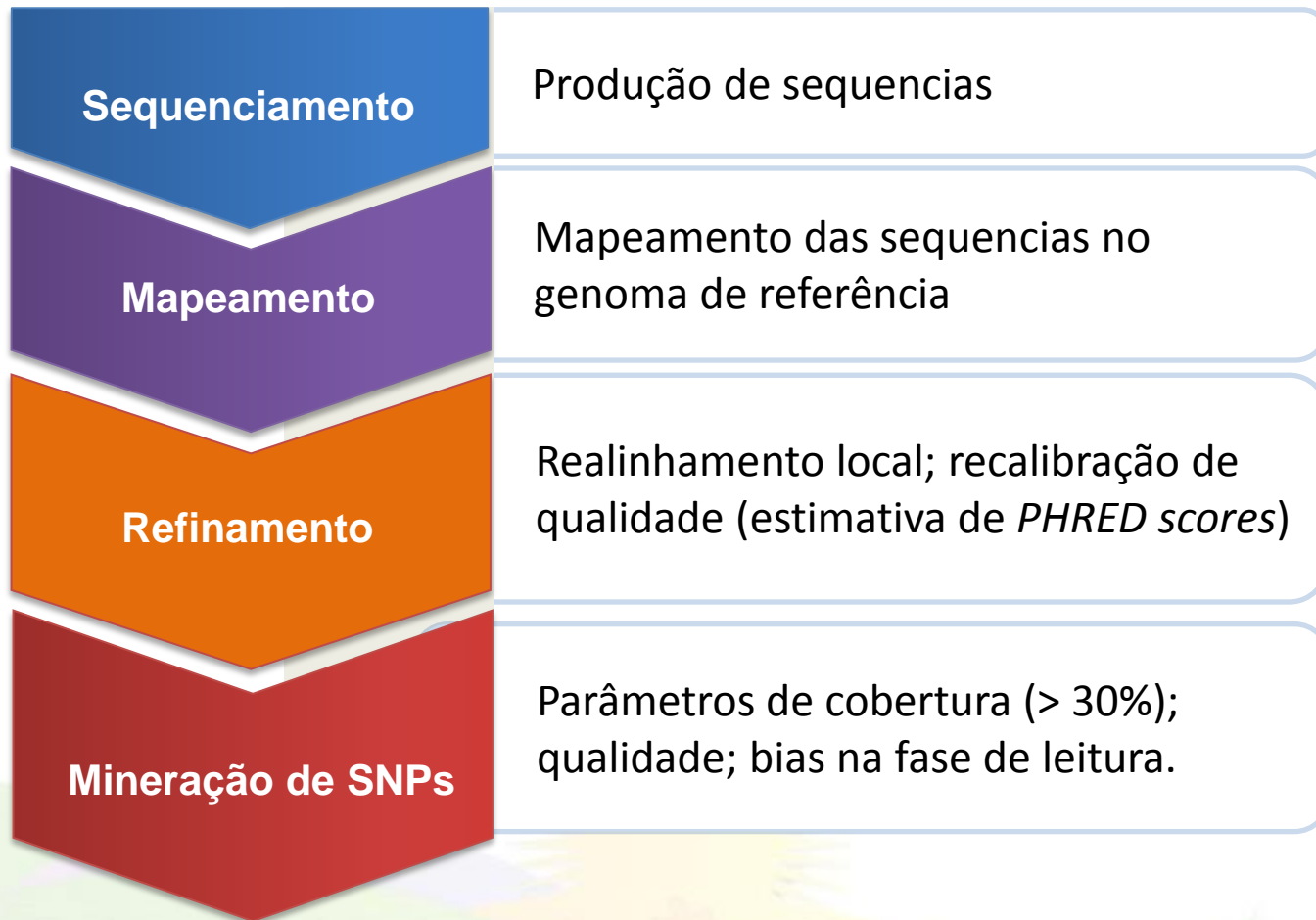
Grupos de Diversidade

591.003.832

SEQUÊNCIAS MAPEADAS POR CROMOSSOMO NOS GRUPOS DE DIVERSIDADE

GRUPO		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
SEQUÊNCIAS MAPEADAS POR CROMOSSOMO	Chr 0	27.695.876	27.101.635	16.271.875	23.141.784	23.074.050	28.571.894	26.753.857	29.538.728	18.418.614	18.892.558
	Chr 1	3.431.114	3.232.529	2.017.466	2.950.498	2.763.441	3.547.441	3.299.137	3.766.199	2.173.713	2.341.398
	Chr 2	4.603.996	4.309.656	2.676.556	4.054.325	3.743.728	4.791.640	4.540.919	5.078.303	2.970.096	3.265.888
	Chr 3	2.674.003	2.527.164	1.623.370	2.377.438	2.176.914	2.774.354	2.607.283	2.966.314	1.716.333	1.836.306
	Chr 4	2.481.739	2.322.979	1.493.817	2.170.156	2.023.848	2.539.815	2.432.384	2.746.025	1.598.272	1.717.224
	Chr 5	2.671.215	2.526.554	1.596.948	2.342.968	2.176.214	2.803.213	2.639.585	3.018.461	1.735.114	1.848.829
	Chr 6	3.258.288	3.078.079	1.901.349	2.804.512	2.569.606	3.271.167	3.106.704	3.502.127	2.088.515	2.262.538
	Chr 7	3.746.639	3.312.451	1.836.496	2.518.417	2.547.593	3.260.074	2.888.482	3.354.396	1.874.394	2.171.182
	Chr 8	2.838.105	2.760.295	1.608.770	2.471.645	2.267.623	2.922.573	2.775.392	3.076.853	1.835.139	1.968.567
	Chr 9	1.949.437	1.807.508	1.170.531	1.713.601	1.600.820	1.999.471	1.924.634	2.169.494	1.294.014	1.368.028
	Chr 10	2.437.177	2.313.103	1.390.730	2.084.703	1.906.496	2.426.099	2.304.655	2.616.276	1.543.520	1.688.319
	Chr 11	3.081.853	2.980.733	1.865.377	2.667.971	2.523.213	3.233.074	3.090.200	3.405.446	2.011.636	2.137.961
SEQUÊNCIAS MAPEADAS	60.869.442	58.272.686	35.453.285	51.298.018	49.373.546	62.140.815	58.363.232	65.238.622	39.259.360	41.498.798	
SEQUÊNCIAS NÃO MAPEADAS	3.988.502	11.152.922	8.002.597	4.918.534	10.100.290	5.181.175	3.473.498	5.587.170	11.452.480	5.378.860	
TOTAL DE SEQUÊNCIAS	64.857.944	69.425.608	43.455.882	56.216.552	59.473.836	67.321.990	61.836.730	70.825.792	50.711.840	46.877.658	

Bioinformática



DePristo MA, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet. 2011 May;43(5):491-8.

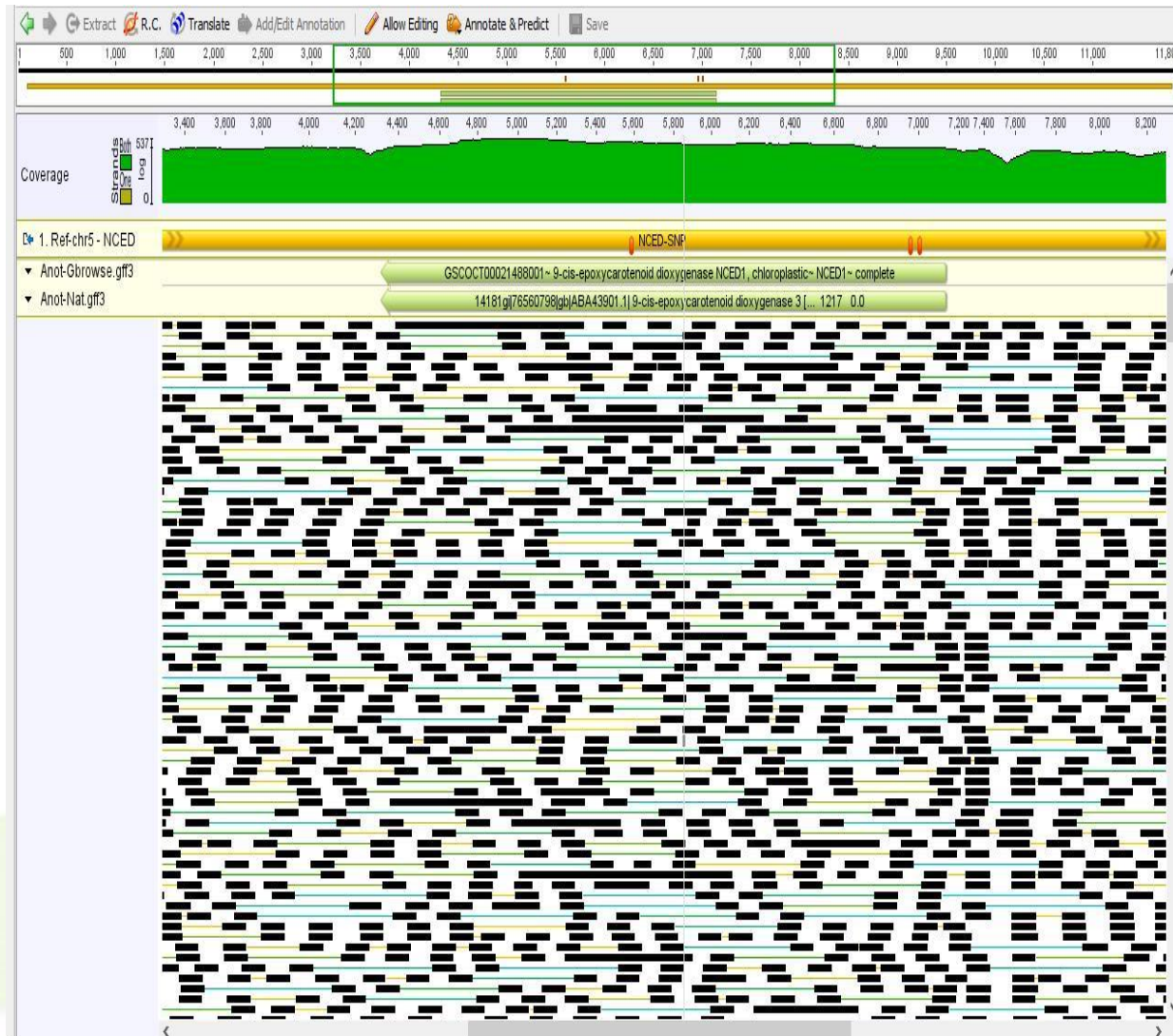
Nielsen R, Paul JS, Albrechtsen A, Song YS. Genotype and SNP calling from next-generation sequencing data. Nat Rev Genet. 2011 Jun;12(6):443-51.



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Realinhamento Local



Statistics

Stats include 1,242 hidden columns

Length: 11,890

Sequences: 16,068

Identical Sites: 3,308 (27.8%)

Pairwise % Identity: 97.2%

Confidence Mean: 33.8

Expected Errors: 51,614

At least Q20: 92.0%

At least Q30: 84.7%

At least Q40: 26.2%

Coverage of 11,890 bases:

Mean: 174.4 Std Dev: 90.9

Minimum: 1 Maximum: 537

Forward: ? Reverse: ?

Ref-Seq: 100% of 10,443 bp

Ungapped lengths of 16,067 reads:

Mean: 113.9 Std Dev: 74.6

Minimum: 22 Maximum: 1908

	Freq	% of non-gaps
A:	557,848	30.3%
C:	375,032	20.4%
G:	360,665	19.6%
T:	546,443	29.7%
N:	430	0.0%
GC:	735,697	35.3%
-:	245,222	11.8% (of any)

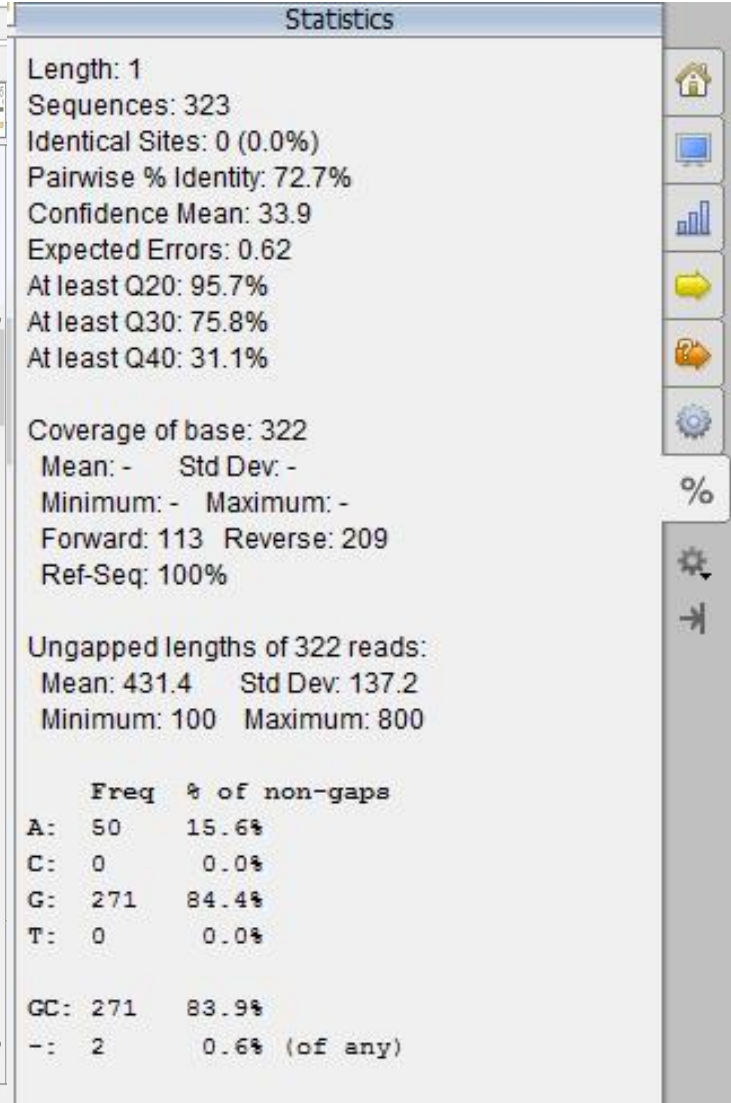
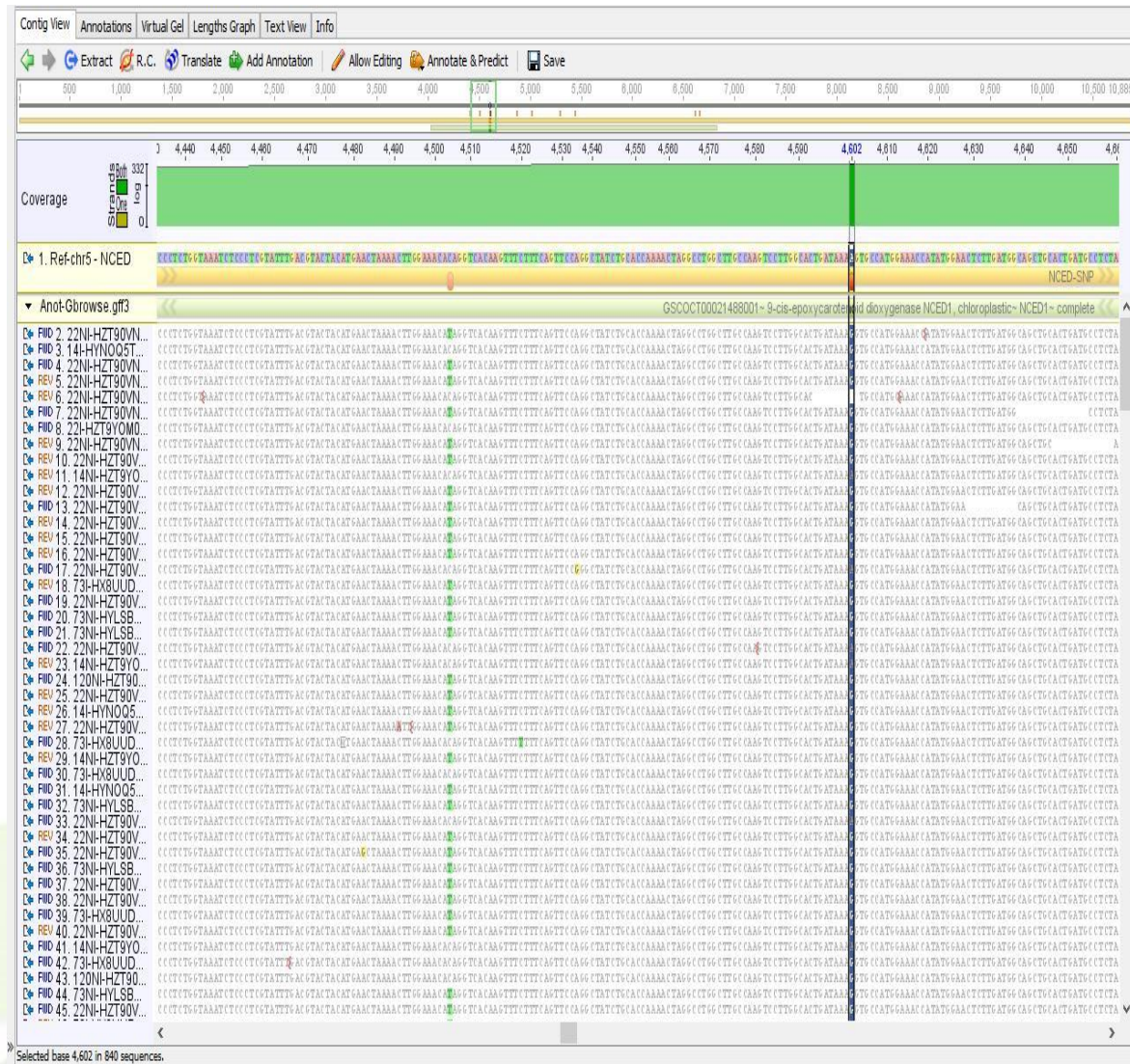
Cursor before base 11,860 (original base 24,538,763). Mouse over base 5,847 (-/Gap) in DBRHHJN1:339:C27LHACX:3:1211:18816:96340 1:N:0:GTCCGC (reversed)



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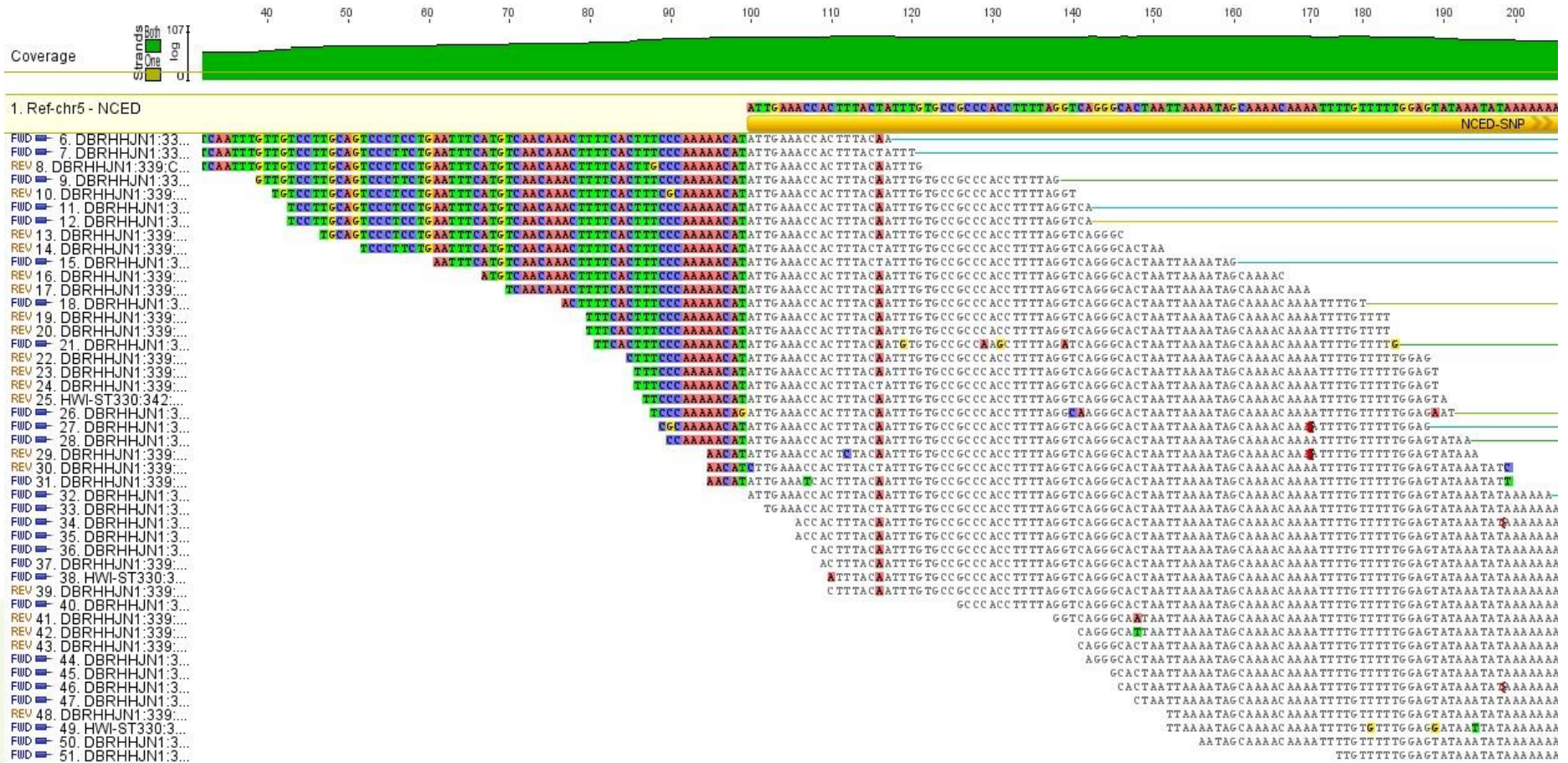
Identificação de SNPs



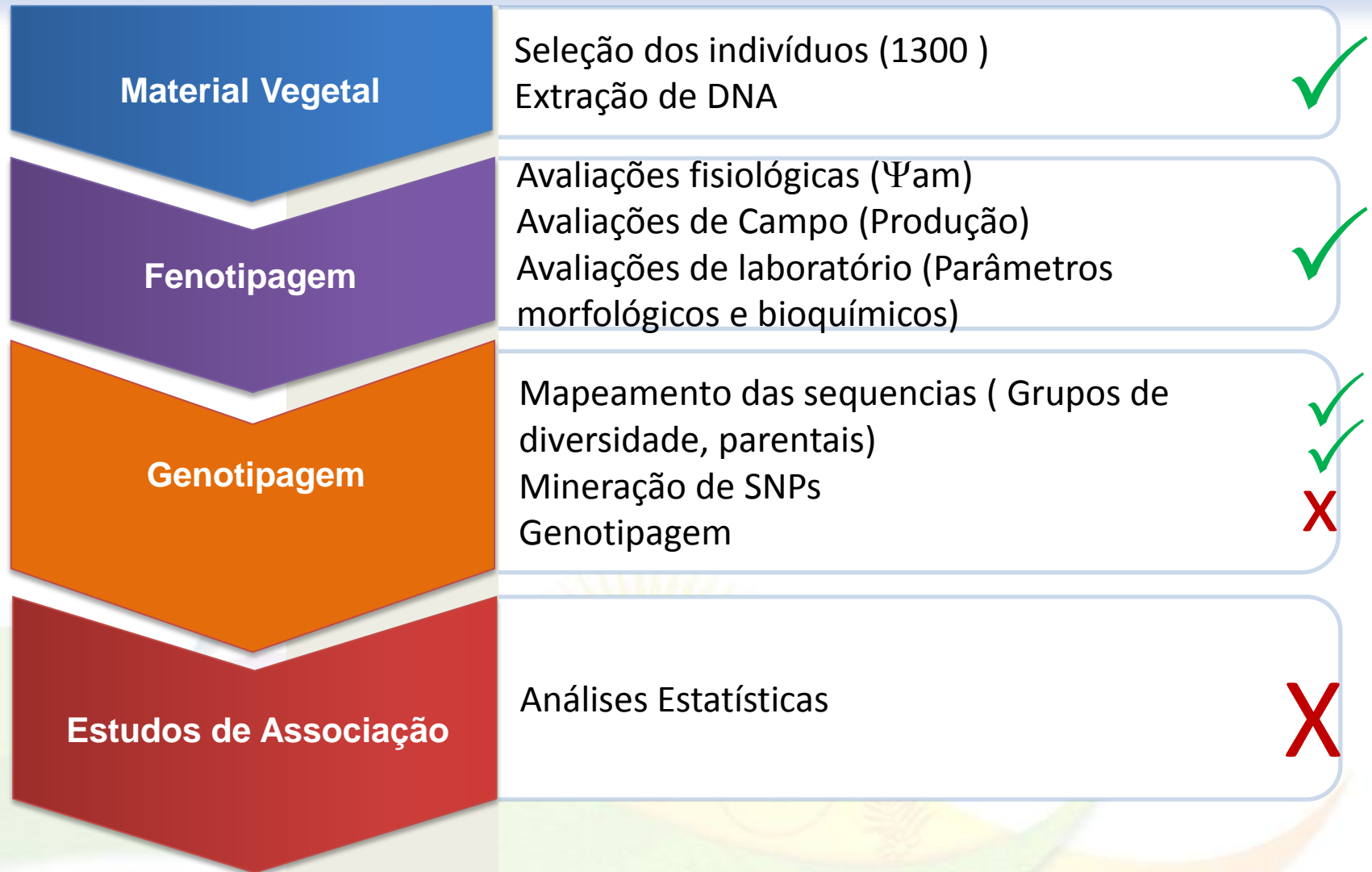
Selected base 4,602 in 840 sequences.



Alinhamento



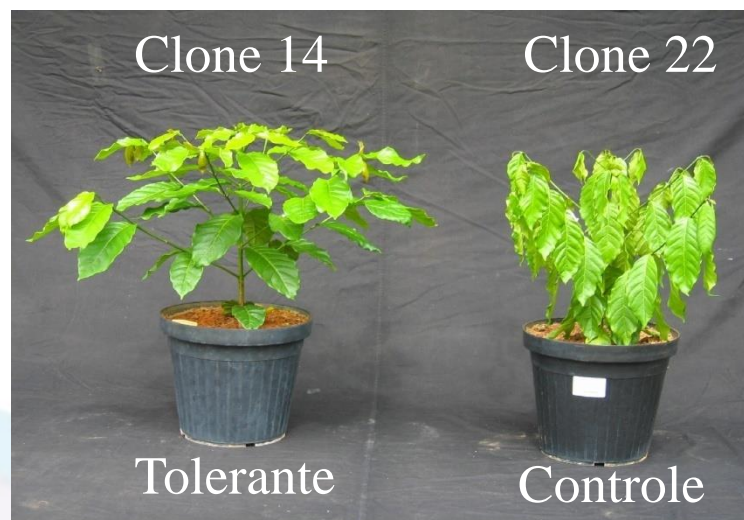
Estado da Arte





Re-sequenciamento

Tolerância à Seca e Nematóides



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Nematóides

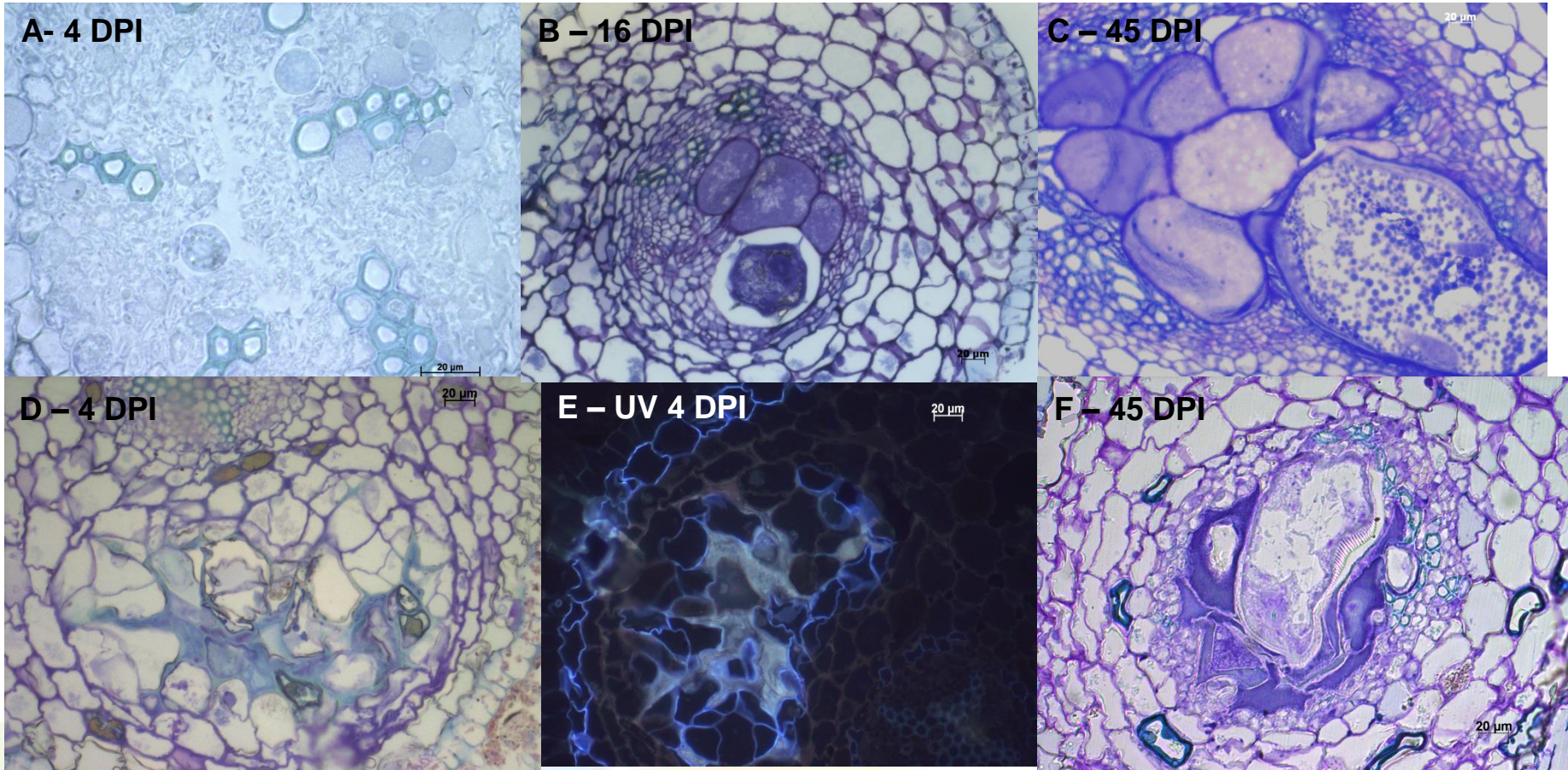


Fig. **A-C** clone 22, *Meloidogyne paranaensis* infection; **D-E** Incompatible reaction of *M. paranaensis* with clone 14 showing HR (D-E) e Cell-death (F) **Lima et al.2012**



Embrapa

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BRAZILIAN GOVERNMENT
BRASIL



Clones 22 e 14

Re-sequenciamento

Genome Sequencer FLX+



Whole genome sequencing

SID	Sample	Sample Type
23862	Clone 14	Genomic DNA
23863	Clone 22	Genomic DNA

6 runs

3 runs/library

Sequencing Summary

Sample Statistics

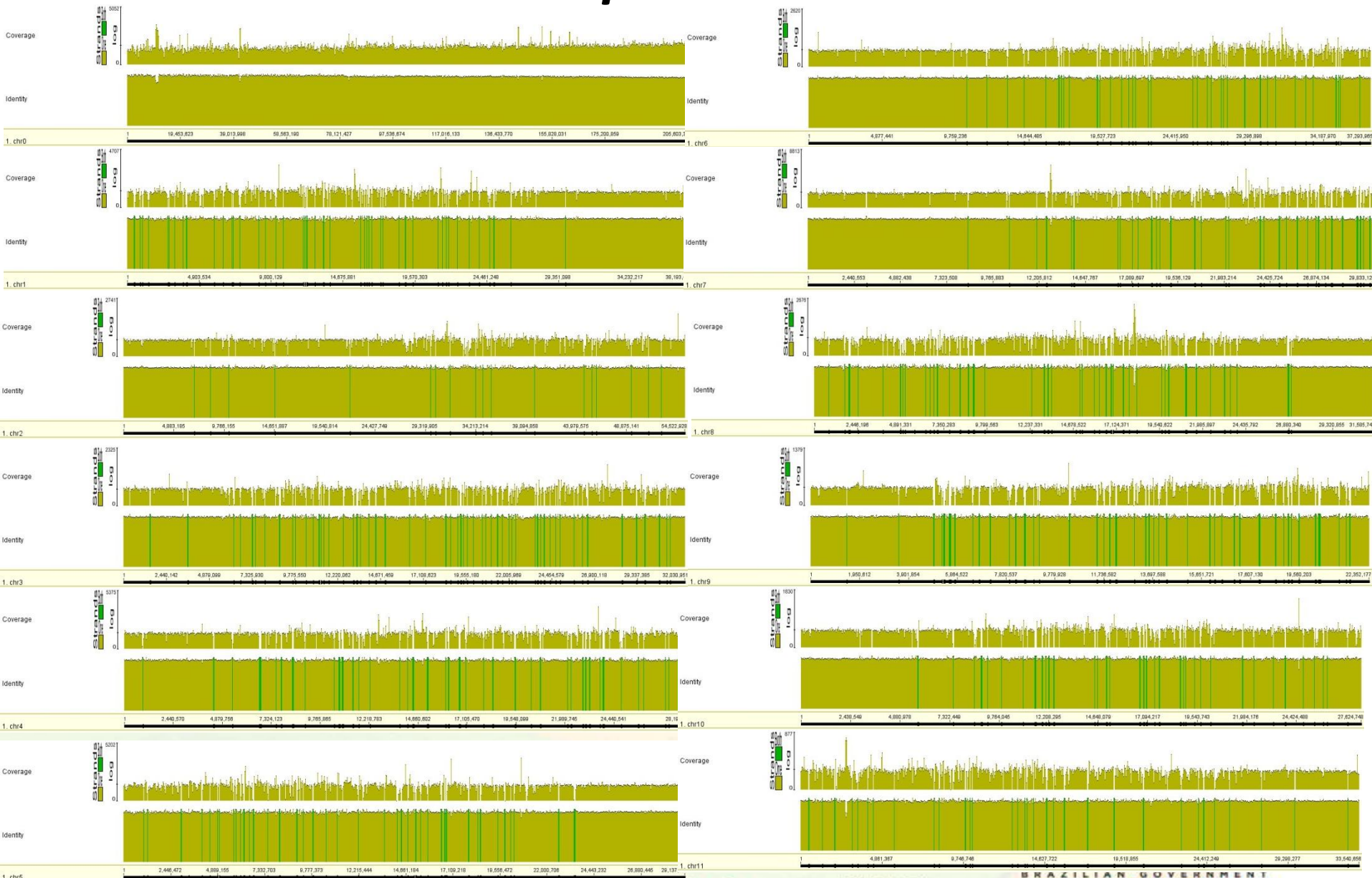
SID	Sample	HQ Reads	HQ Bases	Avg Read Length	%Mixed	%Dots
23862	Clone 14	3,963,286	1,908,581,953	481	10.63%	2.79%
23863	Clone 22	3,935,507	1,943,181,574	492	9.40%	2.47%



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Re-sequenciamento

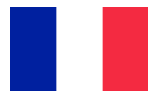


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Coffee Genomics (*C. arabica*)



Dominique Crouzilat (Nestlé), Alexandre de Kochko and Romain Guyot (IRD), Pierre Marraccini



Lukas Mueller and Susan Strickler (BTI), Ray Ming (UIUC)



Alan C. Andrade, Luis F. P. Pereira (Embrapa) and Douglas S. Domingues (IAPAR)

Decoding the genes in your beans:
Working towards a better coffee

<http://bti.cornell.edu/decoding-the-genes-in-your-beans-working-towards-a-better-coffee/>



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Resumo

- Existe potencial para cultivo de *C. canephora* em altitudes elevadas.
- Plantas perenes como o café serão as mais beneficiadas com programas de seleção genômica (Custo de testes de campo/seleção precoce!).
- A plataforma de genotipagem beneficiará outros programas de *C. canephora* (DNA Chip).
- Os dados obtidos, já nos permite desenvolver métodos moleculares eficientes e de baixo custo, para proteção de cultivares em *C. canephora*.



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HDR / AGAP-EGV



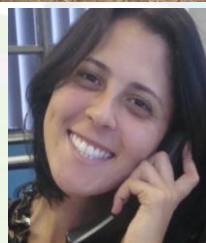
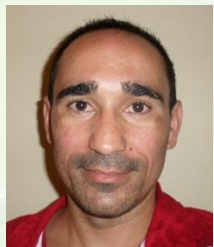
D. THIS
Montpellier Supagro



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Equipe do LGM



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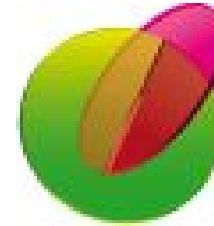




Parceiros:



*Consórcio
Pesquisa Café*



cirad

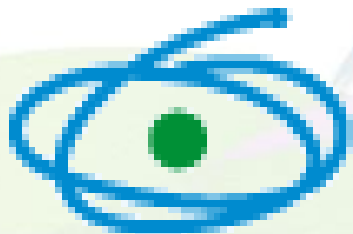


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CAPES



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Obrigada!



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