A Turfgrass Genome Project:
Integration of Cynodon Chromosomes with Detailed Molecular Maps of the Cereals

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<u>Project Description</u>: We are integrating new DNA probes for *Cynodon*, with tools that have been previously mapped in other Poaceae, to develop a primary molecular map of the *Cynodon* chromosomes. The map will be useful for investigating many aspects of turfgrass population biology and genetics, and a molecular conduit for turf improvement to benefit from the large body of genetic information now accumulated about cereals and other grasses. *Cynodon* is chosen as a focal point for turf genome analysis due to its importance across the southern USA, and abundance of phenotypic variation. Dr Wayne Hanna will assist in population development and maintenance.

<u>How Ours is Different</u>: To our knowledge, this project is the first effort to enable turf improvement to benefit from extensive genetic data for well-studied grains such as maize and rice. The "comparative approach" will reduce costs, and leverage much existing information and tools. Our experience in molecular analysis of complex populations such as sugarcane and buffelgrass, as well as grain crops such as rice, maize, and sorghum, together with our extensive repertoire of molecular tools, puts us in a strong position to efficiently develop a *Cynodon* molecular map useful for turf improvement.

<u>Progress to Date</u>: We have established techniques for bermudagrass DNA analysis, determined the genome size of C. dactylon and C. transvaalensis , demonstrated that there is ample DNA polymorphism between C. dactylon and C. transvaalensis, and begun to identify diagnostic DNA markers. We have identified most of the DNA differences needed, and are shifting our emphasis to preparation of DNA and blots to make the map of the segregating progenies. We have also sequenced the majority of Cynodon probes to be mapped, providing the largest amount of Cynodon DNA sequence available to date (which is beyond the goals of our proposal). The mapping cross to be used (T89 x T574) is sufficiently large (126 individuals) that we will conduct some preliminary searches for QTLs directly in this cross – realizing that we will only be able to detect those with large phenotypic effects – however these will come at no additional cost, so are unexpected fringe benefits.

<u>Plans for Continuation:</u> The focus of year 3 will be genetic linkage mapping, using about 150 Cynodon probes (prepared) together with a sampling of mapped probes from other taxa. By the end of year 3, we expect to be near the formal goals proposed for the full 5 years (some mapping and data analysis may continue into year 4). We will then proceed to applying the map to identification of QTLs, and DNA markers diagnostic of agriculturally-important traits, and also to development of a small BAC library for bermuda.

A detailed summary of progress and supplemental materials discussed during the site visit is attached.

US Golf Association
31 May 2000

A. Executive Summary – review basis for this project (copy attached).

B. Populations developed, and rationale for choice (table attached).

- DNA extarction of mapping population is just beginning, and will be a major activity this summer and fall.

C. DNA content of C. dactylon and C. transvaalensis (data attached) –

- Unexpected that it was so similar, as CD is tetraploid and CT is diploid – suggests that they may be quite divergent (consistent with RFLP data – below).
- Suggests that for longer-term goals (physical mapping and gene map assembly), there may be little gained by working with the diploid genome of CT, that it may be better to work with CD

D. Summary of results from surveys with various types of comparative clones (Table and Erica to bring films).

- high level of DNA polymorphism, suggesting that mapping will be routine.
- ca. 100 surveys generated, to date 138 probes have found at least 88 polymorphic loci (often multiple bands, but also some may not be single-dose)
- ongoing emphasis will be on Cynodon, rice and Pennisetum clones, in the latter two cases using clones that have been previously mapped on sorghum. Aim is to have a sufficient number of comparative probes that they represent all regions of all chromosomes of sorghum (and consequently other grasses)

E. Sequencing of pCD clones

- Not required by grant, but have done anyway (table illustrates some of the sequences).
- Creates new avenue to compare the bermuda map to Arabidopsis
- Sequence data creates new means to develop fingerprinting tools for bermuda (show DHPLC trace).

F. Timetable

- Expect to have sufficient survey data by mid-to-late 2000
- map should be well-advanced by late 2001 (about 2 years ahead of timetable)
- integrate with overall comparative genetic/physical map of grasses (figure attached).
- Probably will then explore for QTLs in mapping population – while it is a bit small, it may be adequate to find genes with largest effects.

G. Longer-term goal – 'comprehensive gene map' and 'physical map'

- BAC library (cost about $75,000 by taking advantage of our automation)
- Cynodon EST database – which is also warranted for other reasons – cost of a comprehensive EST database and data archival/retrieval system would be about $2-3 million

New contact information:

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Table . 1998 Crosses (grown in 1999) for molecular studies in turf bermudagrass (planted 1/22/99).

Number	Cross	Crosses made on		Plants
B1	98-T572 x T89	6/6	Hand crosses	2
B2	98-T89 x T572	6/6	Hand crosses	31
B3	98-T89 x T574	6/4 & 5	Hand crosses	4
B4	98-T574 x 93-157	6/6	Hand crosses	12
B5	98-T157 x T574	6/6	Hand crosses	67
B6	98-94-128 x T572	6/4 & 5	Hand crosses	8
B7	98-T574 x T89	6/4 & 5	Hand crosses	3
B8	98-T572 x 94-128	6/4 & 5	Hand crosses	1
B9	98-T574 x 94-128	6/6	Hand crosses	52
B10	98-T110 x T572	6/5	Hand crosses	12
B11	98-94-128 x T574	6/6	Hand crosses	53
B12	98-T574 x T110	6/7	Hand crosses	45
B13	98-T110 x T574	6/8	Hand crosses	2
B14	98-T572 x T110	6/5 & 6	Hand crosses	9
B15	98-T572 x T89	7/9	Pot crosses	1
B16	98-T572 x T110	7/9	Pot crosses	13
B17	98-T574 x T89	7/9	Pot crosses	123

From: karumuga@unlnotes.unl.edu
X-Lotus-FromDomain: UNIVERSITY OF NEBRASKA
To: "Andrew H. Paterson" <paterson@dogwood.botany.uga.edu>
Date: Fri, 13 Aug 1999 17:35:38 -0500
Subject: Re: C-value

Anderw,

tried

Here is the result. Let me know if you have any question.

with regards,

Aru.

Plant name		DNA content	St.Dev	Mbp/1C
		Pg/2C		
Kudzu-Local collection		2.06	0.05	994 mbp/1C
Opuntia nopalea TAM1308		1.82	0.01	878 mbp /1C
C. tactylon T89		1.64	0.02	791 mbp /1C
C. tranvaalensis T574		1.76	0.01	849 mbp/1C

tetraploid
diploid
!!

Category	# probes screened	#with interpretable pattern	# yield of polymorphisms	Avg. # of bands that differ:	
heterologous probes				**Eco RI**	**Hind III**
BCD (Barley cDNA)	4	4	4	1.5	1
UMC (Maize genomic DNA)	10	9	1	0.3	0.1
M (Millet genomic DNA)	7	6	1	0.2	0.3
pSB (Sorghum genomic DNA)	3	3	1	1.3	0.7
RZ (Rice cDNA)	11	11	9	2.4	2.1
C (Rice cDNA)	26	21	16	1.7	1.3
R (Rice cDNA)	22	15	14	1.9	2.2
S (Rice cDNA)	18	14	12	1.1	0.9
CDO (Oat cDNA)	6	1	1	5	2
pPAP (Pennisetum cDNA)	coming soon				
heterologous subtotal	**107**	**84**	**59**		
homologous probes					
PCD (Cynodon cDNA)	31	30	29	2	1.8
homologous subtotal	**31**	**30**	**29**		
Total probes	**138**	**114**	**88**		

These are heterologous grass probes that have been mapped ~~apo markers~~ on sorghum, suggesting that they may be sufficiently conserved to use as probes on other grasses. When investigating a new grass species, we suggest surveying perhaps 1-20 probes in each class, then choosing additional probes from the class(es) that is/are most useful.

Barley cDNA	Oat cDNA	Maize cDNA	Maize genomic	Millet genomic	Buffelgrass cDNA	Sorghum cDNA	Sorghum genomic	Rice genomic	Rice cDNA
BCD0098	CDO0017	CSU005	BNL05.09	M036	pPAP01A02	pRC121	pSB0041aA	RG123	C0147
BCD0147a	CDO0020a	CSU009a	BNL08.29	M044	pPAP01A08	pRC122	pSB0289cA	RG348	C0198
BCD0147b	CDO0020b	CSU009b	BNL09.11	M096	pPAP01A09	pRC125a	pSB1365cA	RG418	C0250
BCD01631	CDO0020c	CSU013a	BNL10.13a	M180	pPAP01A11	pRC125b	pSB1537bS	RG433a	C0356
BCD0178	CDO0036a	CSU013b	BNL10.13b	M180	pPAP01C03	pRC126	pSB1541A	RG433b	C0746
BCD0207	CDO0036b	CSU016	BNL10.13c	M190	pPAP01C05	pRC127	pSB1773dS	RG433c	RZ002
BCD0263	CDO0038	CSU027	BNL14.28	M265	pPAP01C09	pRC128		RG463a	RZ014
BCD0348	CDO0059	CSU028	UMC002a	M319	pPAP01D01	pRC130a		RG463b	RZ017
BCD0349	CDO0066	CSU029	UMC002b	M345	pPAP01D04	pRC130b		RG482a	RZ028
BCD0386	CDO0078a	CSU030	UMC002c	M371	pPAP01D10	pRC130c		RG482b	RZ053
BCD0450	CDO0078b	CSU031	UMC004	M386	pPAP01E05	pRC130d		RG561	RZ058
BCD0454	CDO0087	CSU033	UMC004	M428	pPAP01E10	pRC130e		RG598a	RZ069
BCD0738	CDO0109a	CSU034	UMC005	M443	pPAP01F01	pRC130f		RG598b	RZ087
BCD0828	CDO0109b	CSU036a	UMC006x	M443	pPAP01F12	pRC130g		RG653a	RZ141a
BCD0855	CDO0109c	CSU036b	UMC010	M458	pPAP01H02	pRC132		RG653b	RZ141b
BCD0926a	CDO0109d	CSU037	UMC012	M466	pPAP01H04	pRC136		RG716	RZ141c
BCD0926b	CDO0127	CSU038	UMC014a	M476	pPAP01H05	pRC137		RG944	RZ143a
BCD1072a	CDO0202	CSU039	UMC014b	M477	pPAP02A05	pRC139			RZ143b
BCD1072b	CDO0204	CSU040	UMC014c	M669	pPAP02A06	pRC140			RZ144a
BCD1381	CDO0226a	CSU048	UMC015	M696	pPAP02A08	pRC141			RZ144b
BCD1424	CDO0226b	CSU050	UMC016a	M696	pPAP02C02	pRC143			RZ161
	CDO0312a	CSU056	UMC016b	M713	pPAP02C03	pRC144			RZ166
	CDO0312b	CSU058	UMC019x	M716	pPAP02C06	pRC145			RZ228
	CDO0328	CSU059	UMC027x	M737	pPAP02D06	pRC147			RZ237
	CDO0337	CSU060	UMC032a	M738	pPAP02E06	pRC148			RZ244
	CDO0344a	CSU061	UMC032b	M738	pPAP02F07	pRC149			RZ260
	CDO0344b	CSU063a	UMC034	M815	pPAP03A04	pRC151a			RZ261
	CDO0344c	CSU063b	UMC035	M848	pPAP03A07	pRC151b			RZ273a
	CDO0345	CSU063c	UMC036	M858	pPAP03A08a	pRC151c			RZ273b
	CDO0348	CSU066a	UMC036	M866	pPAP03A08b	pRC152			RZ284
	CDO0365	CSU066b	UMC040	M866	pPAP03B10	pRC154a			RZ296
	CDO0385	CSU066c	UMC044	M869	pPAP03C01a	pRC154b			RZ323
	CDO0400	CSU068	UMC045a	M891	pPAP03C01b	pRC156a			RZ329
	CDO0405a	CSU070	UMC045b		pPAP03C08	pRC156b			RZ329b
	CDO0405b	CSU077	UMC047		pPAP03D01	pRC159			RZ387a
	CDO0407a	CSU081a	UMC051a		pPAP03D12	pRC160			RZ387b
	CDO0407b	CSU081b	UMC051b		pPAP03E01	pRC163a			RZ390
	CDO0412	CSU086	UMC054		pPAP03E07	pRC163b			RZ395
	CDO0455	CSU092	UMC055a		pPAP03E08	pRC164a			RZ400
	CDO0456	CSU095	UMC055b		pPAP03E09	pRC164b			RZ404
	CDO0457	CSU096a	UMC060a		pPAP03F08	pRC165			RZ413
	CDO0459	CSU096b	UMC060a		pPAP03F10	pRC166			RZ421
	CDO0470	CSU102a	UMC062a		pPAP03H01	pRC167			RZ421
	CDO0475	CSU102b	UMC062b		pPAP03H12	pRC168a			RZ444
	CDO0497	CSU102c	UMC063		pPAP04A06	pRC168b			RZ446
	CDO0507a	CSU102d	UMC064a		pPAP04A10	pRC169			RZ456a
	CDO0507b	CSU102e	UMC064b		pPAP04B06	pRC170			RZ456b
	CDO0516a	CSU103a	UMC065		pPAP04B07	pRC176			RZ467
	CDO0516b	CSU103b	UMC071		pPAP04E03	pRC179			RZ474
	CDO0520	CSU103c	UMC076		pPAP04F04	pRC181			RZ476a
	CDO0524	CSU108	UMC081		pPAP04F09	pRC182			RZ476b
	CDO0533	CSU109a	UMC083		pPAP04G01	pRC185			RZ500a

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Erica

pCD Clone Sequencing Summary

146 clones were sequenced
126 were of "good" quality (Phred/Phrap quality of 16 for over 300 contiguous base pairs)
Alignment of the sequences resulted in 5 contiguous blocks and 112 singlets
One microsatellite locus of 7 GTT repeat units was found

Summary of BLASTn Database Search Results

compares nucleotides

Clone	*Best Hit*	*E-Value*
1-A10	*Arabidopsis thaliana* DNA chromosome IV, BAC clone T30C3	1.00E-05
1-B04	*Oryza sativa* genomic DNA, chromosome I, clone:P0708G02	3.00E-04
1-B10	*Oryza sativa* mRNA for Ran	1.00E-36
1-C05	*Mesembryanthemum crystallinum* clone Mpc5 protein phosphatase-2C (PP2C) mRNA	2.00E-11
1-E09	*Oryza sativa* subsp. Japonica BAC nbxb0015J03, chromosome X	3.00E-34
1-G12	*Aegilops tauschii* starch synthase I gene	1.00E-05
1-H02	*Oryza sativa* BAC OSJNBa0049B20 genomic sequence	5.00E-27
2-C01	*Oryza sativa* genomic DNA, chromosome I, clone:P0424A08	4.00E-04
2-E03	*Arabidopsis thaliana* genomic DNA, chromosome V, P1 clone MGN6	4.00E-09

Summary of BLASTx Database Search Results

translates into proteins

Clone	*Best Hit*	*E-Value*
1-A01	*Arabidopsis thaliana* hypothetical protein T16H5.190	7.00E-13
1-A06	*Arabidopsis thaliana* glycerophosphodiester phosphodiesterase-like protein	5.00E-08
1-A12	*Arabidopsis thaliana* hypothetical protein F20D10.170	1.00E-14
1-B01	*Oryza sativa* gene for Pib. (AB013448)	1.00E-10
1-B04	*Zea mays* isoflavone reductase homolog IRL	3.00E-08
1-B08	*Arabidopsis thaliana* putative protein	4.00E-16
1-B10	*Zea mays* Ran related GTP binding protein	4.00E-31
1-C05	*Mesembryanthemum crystallinum* protein phosphotase-2C; PP2C	2.00E-52
1-C11	*Arabidopsis thaliana* hypothetical protein F24L7.13	1.00E-28
1-D07	*Arabidopsis thaliana* hypothetical protein F19.D11.7	2.00E-05
1-E09	*Arabidopsis thaliana* putative protein	5.00E-19
1-F02	*Arabidopsis thaliana* putative Cytochrome P450 protein	1.00E-14
1-G01	*Oryza sativa* reverse transcriptase (AC005315)	7.00E-23
1-G04	*Oryza sativa* long-staminate retrotransposon	7.00E-70
1-G06	*Arabidopsis thaliana* unknown protein	3.00E-05
1-G07	*Zea mays* rust resistance protein	2.00E-11
1-G11	*Arabidopsis thaliana* putative coatomer protein complex, subunit beta prime	3.00E-20
1-H02	*Oryza sativa* putative WRKY DNA binding protein	6.00E-08
1-H10	*Arabidopsis thaliana* F1K23.9	2.00E-29
1-H11	*Arabidopsis thaliana* T25K16.6	8.00E-07
2-A02	*Arabidopsis thaliana* lipid-like protein	2.00E-04
2-A05	*Arabidopsis thaliana* putative protein	7.00E-05
2-A12	*Oryza sativa* Pi-b protein	1.00E-07
2-B09	*Arabidopsis thaliana* hypothetical protein F7L13.80	2.00E-21
2-B10	*Arabidopsis thaliana* hypothetical protein F23A5.2	7.00E-25
2-B12	*Arabidopsis thaliana* putative laccase	5.00E-21
2-C04	*Arabidopsis thaliana* putative laccase	6.00E-34
2-C09	*Arabidopsis thaliana* unknown protein	2.00E-12
2-D03	*Arabidopsis thaliana* hypothetical protein F23A5.2	2.00E-26
2-D04	*Arabidopsis thaliana* hypothetical protein F23A5.2	1.00E-26
2-D07	*Arabidopsis thaliana* unknown protein	1.00E-09
2-E02	*Ipomoea nil* leaf protein	1.00E-49

pCD Sequence Assembly Summary

Contig	*Clone*
1	2-C04 2-B12
2	2-A07 2-A10
3	1-C10 1-D05
4	2-D03 2-D04 2-B10
5	2-C10 2-C11 2-C12 2-D09 2-D10

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Map Scale is 10.0 cM per cm
Kosambi Mapping Function
Segment Break Dist >= 999.9 cM
Segment Break Frac >= 50.0 %
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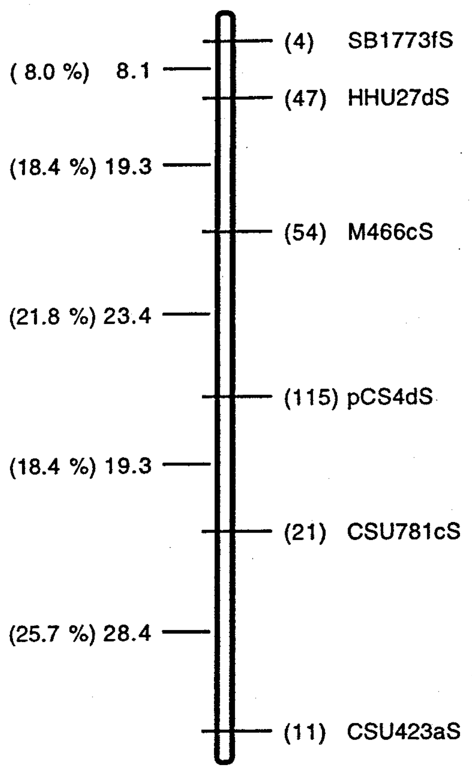
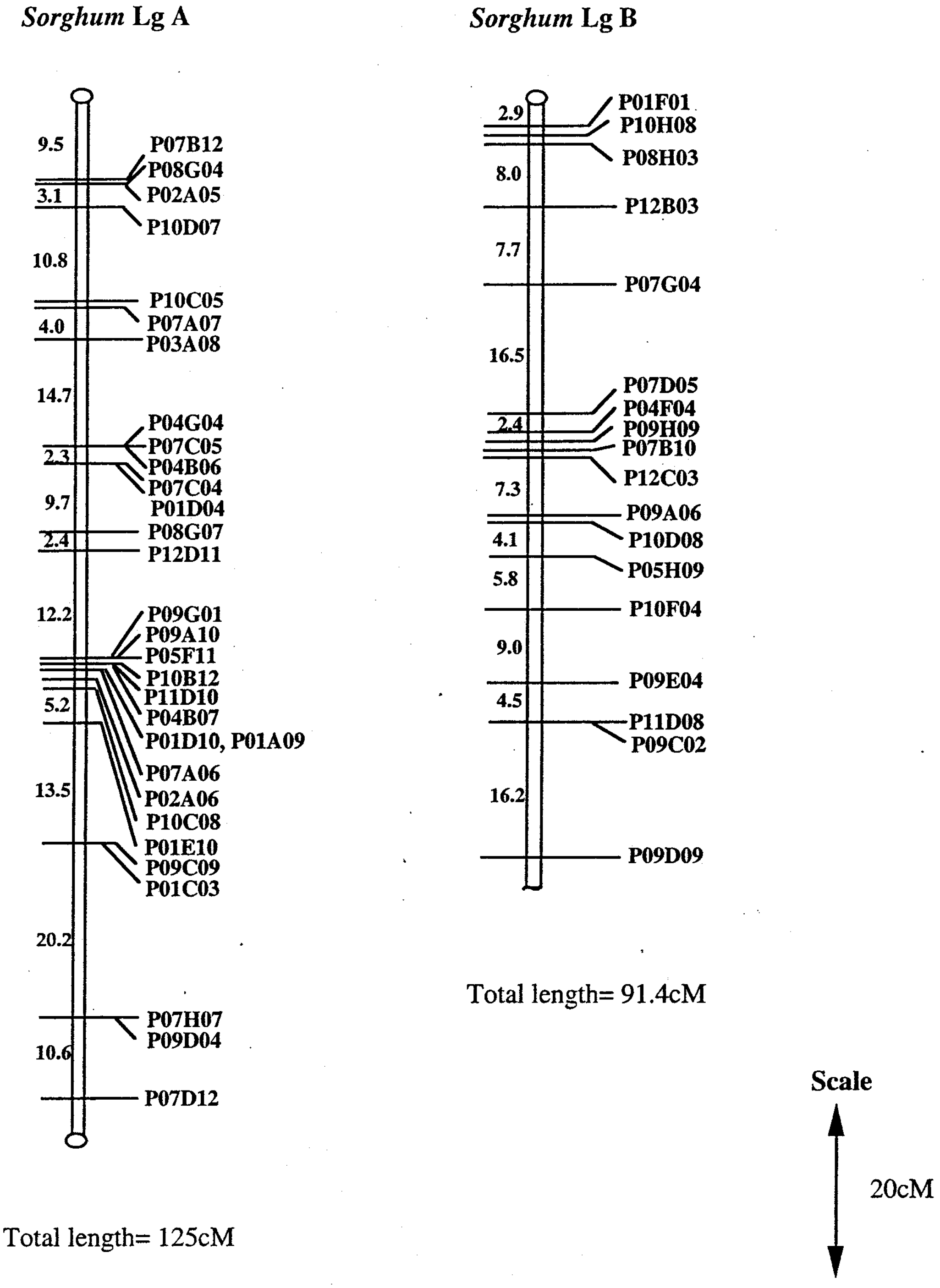
Iterations : 3
Longest Seg cM : 98.493
Loop Tolerance : 0.010
Inner Tolerance: 0.010

Rec Frac. Dist cM Marker Id Name





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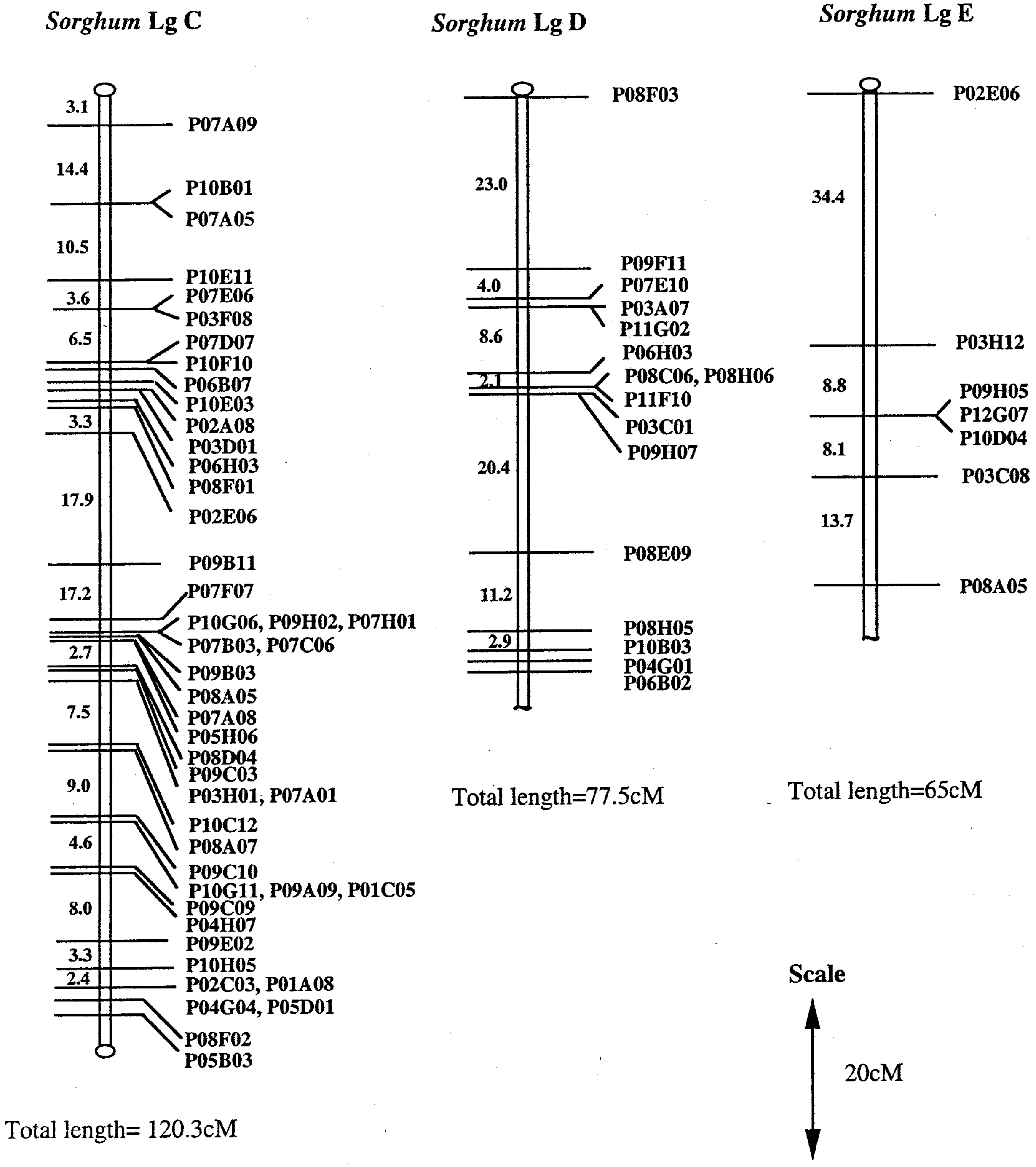Sorghum Lg C
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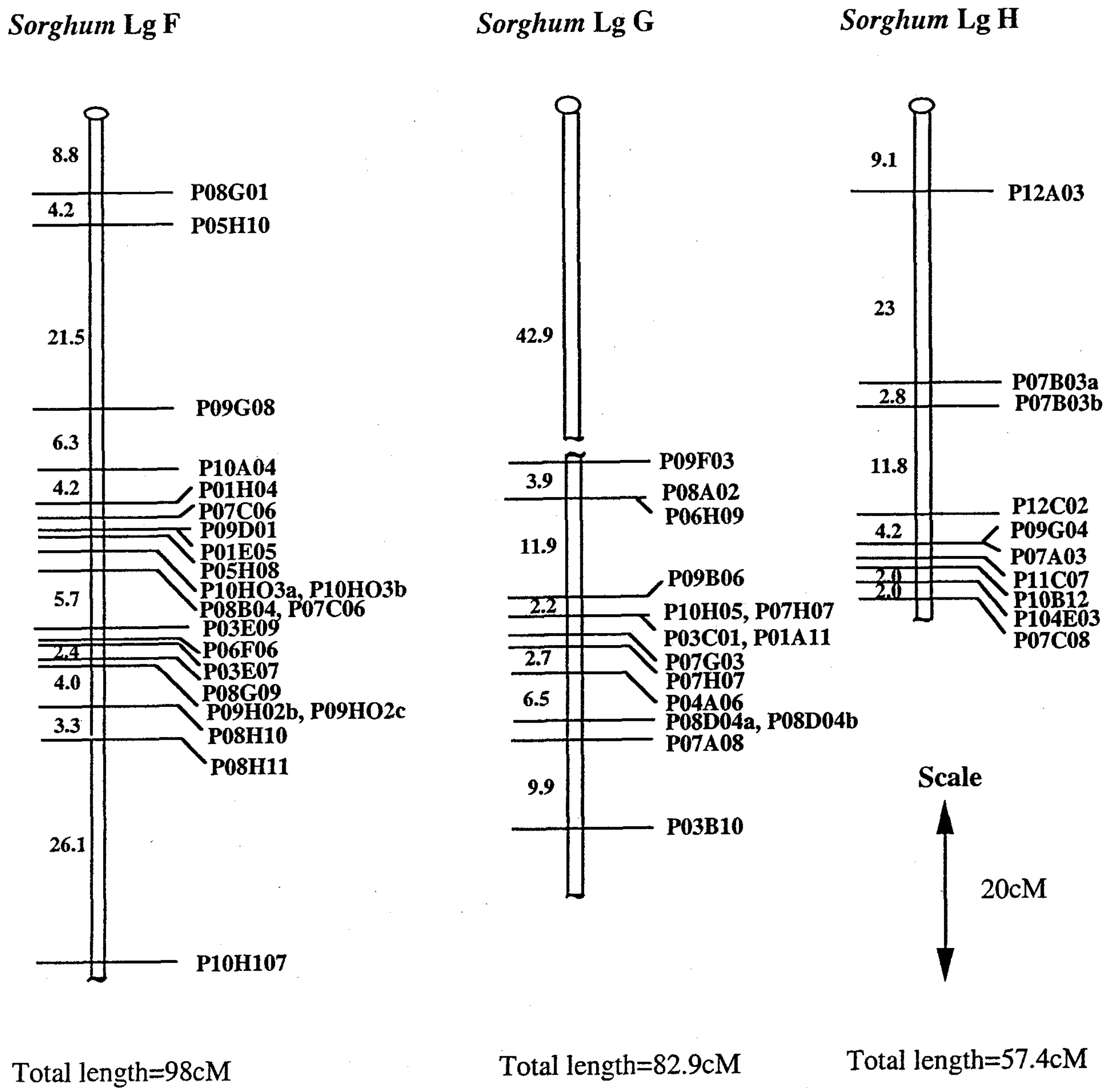
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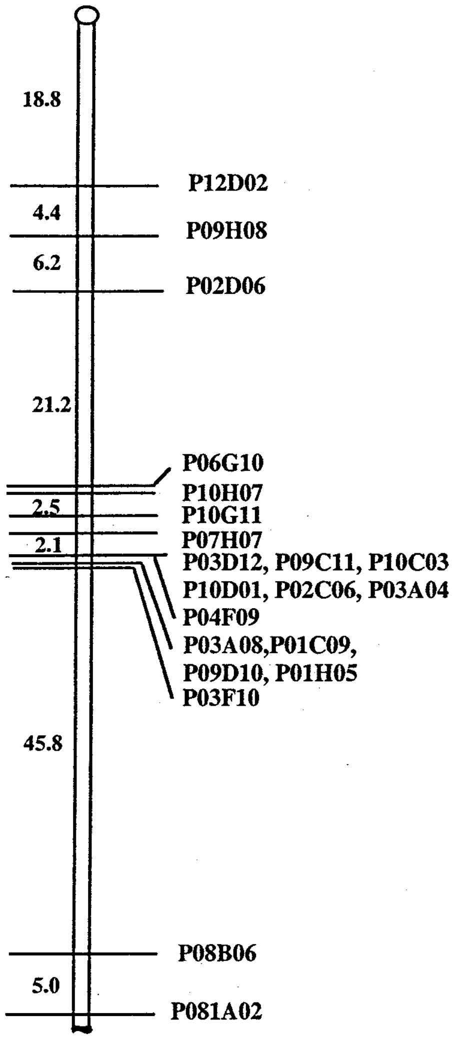
Sorghum Lg I



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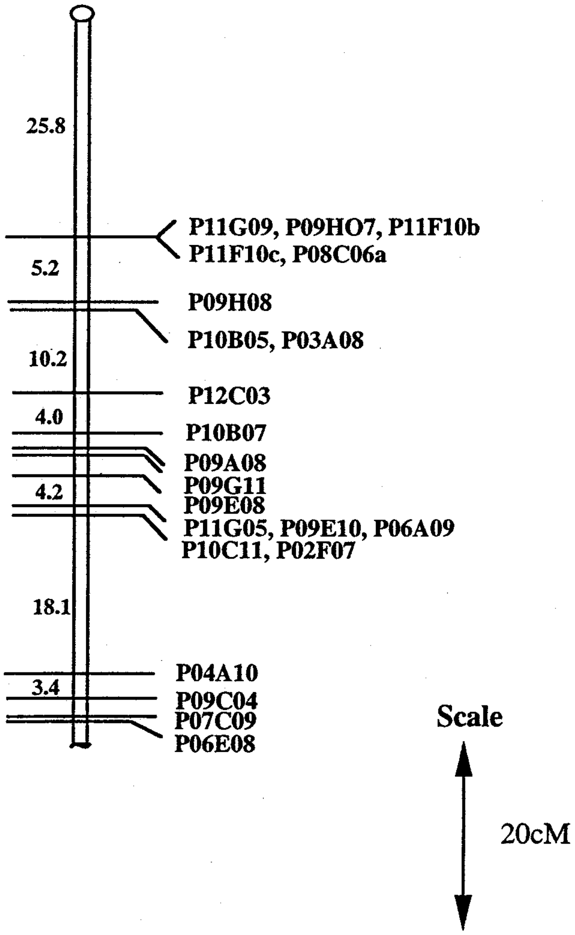
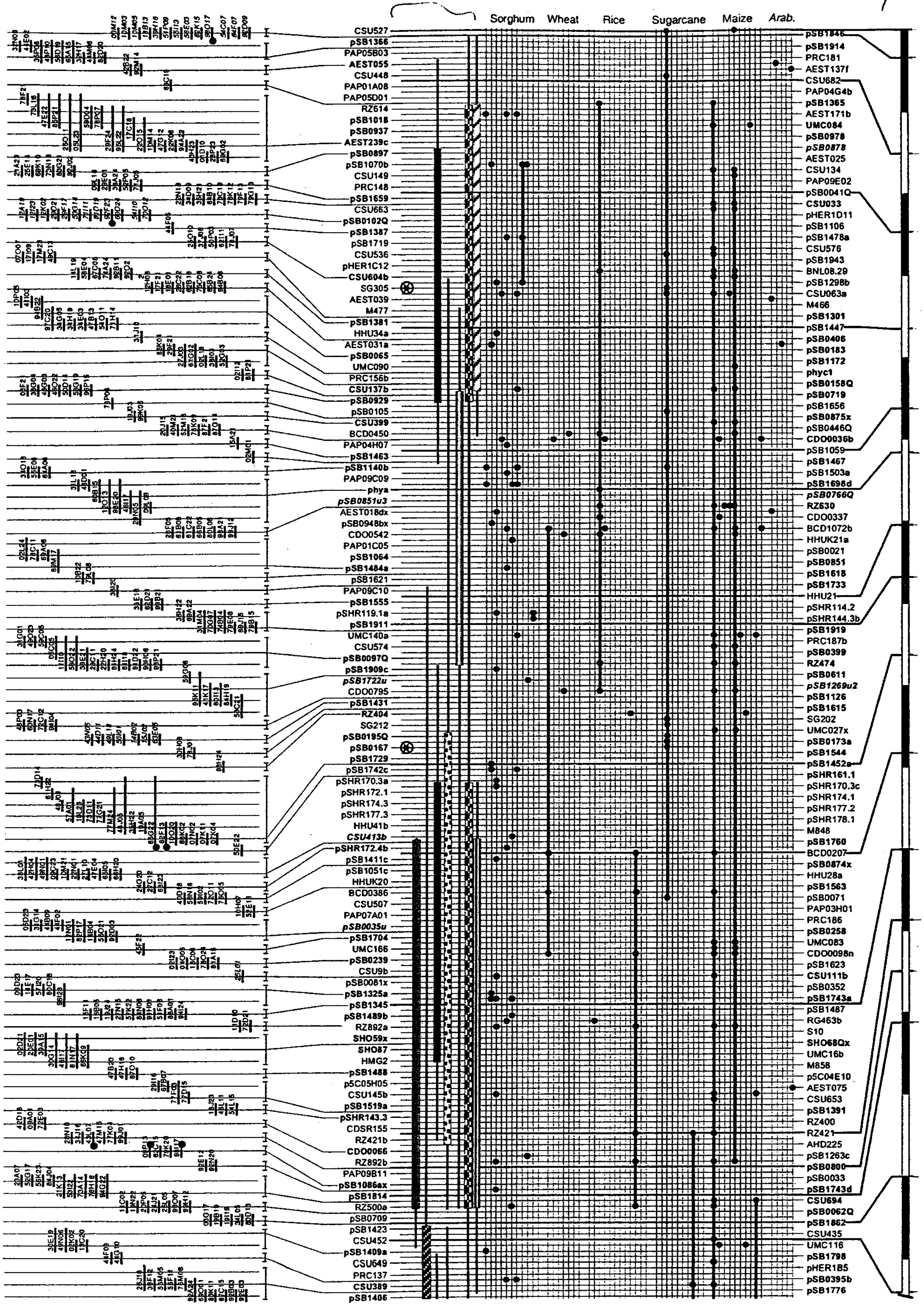
Sorghum Lg J



Total length=78.2cM


Sorghum
Wheat
Rice
Sugarcane
Maize
Arab.
CSU527
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PAP05B03
AEST055
CSU448
PAP01A08
PAP05D01
RZ614
pSB1018
pSB0937
AEST239c
pSB0897
pSB1070b
CSU149
PRC148
pSB1659
CSU663
pSB0102Q
pSB1387
pSB1719
CSU536
pHER1C12
CSU604b
SG305
AEST039
M477
pSB1381
HHU34a
AEST031a
pSB0065
UMC090
PRC156b
CSU137b
pSB0929
pSB0105
CSU399
BCD0450
PAP04H07
pSB1463
pSB1140b
PAP09C09
phya
pSB0851u3
AEST018dx
pSB0948bx
CDO0542
PAP01C05
pSB1064
pSB1484a
pSB1621
PAP09C10
pSB1555
pSHR119.1a
pSB1911
UMC140a
CSU574
pSB0097Q
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pSB1722u
CDO0795
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SG212
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UMC166
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phyc1
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pSHR144.3b
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pSB0399
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pSB1269u2
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SG202
UMC027x
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pSB1544
pSB1452a
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RG463b
S10
SHO68Qx
UMC16b
M858
p5C04E10
AEST075
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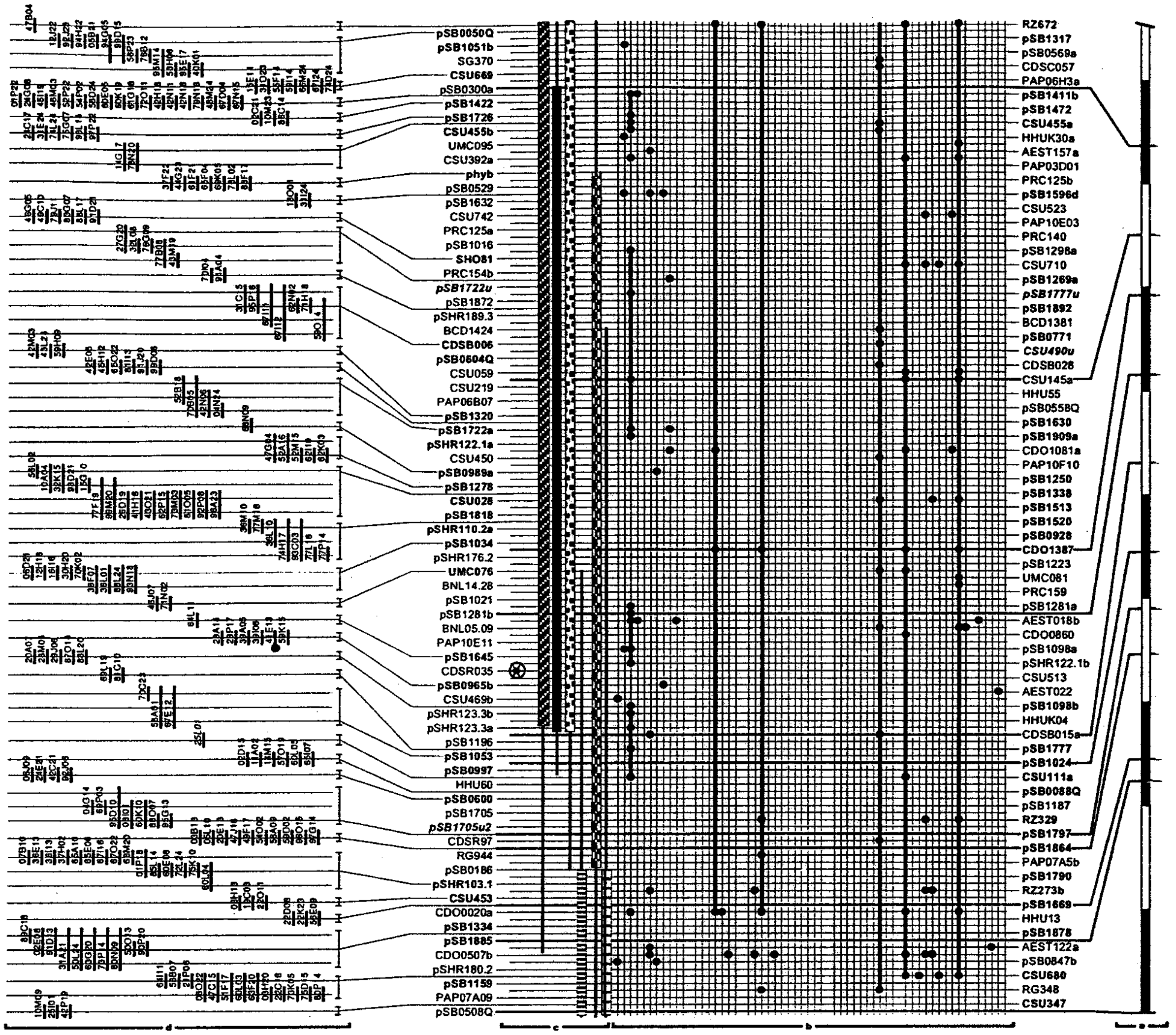

Sorghum QTLs: ▤ Average height of the main culm, tallest and shortest tillers; ◩ Log (non-zero rhoizome number); ■ Log (rhizome number); ▨ Rhizome distance; ◧ Log (subterranean rhizomatousness); ▩ Non-zero subterranean rhizomatousness; ▥ Seedling tillers; ■ Seed weight; ◨ Spikelet number; ⊞ Whorl number. **Sugarcane QTLs:** ⊛ Sugar content

From: Draye, et al, submitted
(provided by A. H. Paterson)