The Plant Genome
Original Research

An Open-Source First-Generation Molecular Genetic Map from a Sugarbeet × Table Beet Cross and its Extension to Physical Mapping

J. Mitchell McGrath*, Daniele Trebbi, Ann Fenwick, Lee Panella, Britta Schulz, Valerie Laurent, Steve Barnes, and Seth C. Murray

J.M. McGrath, USDA-ARS, Sugar Beet and Bean Research Unit, 494 PSSB, Michigan State Univ., East Lansing, MI 48824-1325; D. Trebbi, Keygene N.V., P.O. Box 216, 6700 AE Wageningen, The Netherlands; A. Fenwick and L. Panella, USDA-ARS, Crops Research Lab., 1701 Centre Ave., Fort Collins, CO, 80526; B. Schulz, KWS SAAT AG, Grimsehlstrasse 31, 37574 Einbeck, Germany; V. Laurent, UMR1281 Stress Abiotiques et Differencation des Vegetaux Cultives, Univ. des Sciences et Technologies de Lille, 59655 Villeneuve d'Ascq, France; and Florimond Desprez, 59242 Cappelle en Pévèle, France; S. Barnes, Sesvanderhave NV/SA, Industriepark, Soldatenplein Z2 nr 2, 3300, Tienen, Belgium; S.C. Murray; Institute for Genomic Diversity, Cornell Univ., Ithaca, NY 14853.
Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA or imply approval to the exclusion of other products that may also be suitable. Received 24 May 2006.
*Corresponding author (mitchmcg@msu.edu).

Abstract

In sugarbeet (*Beta vulgaris* subsp. *vulgaris*), many linkage maps have been constructed, but the availability of markers continues to limit utility of genetic maps in public domain programs. Here a framework genetic map is presented that is expandable and transferable to research programs interested in locating their markers on a consensus map. In its current framework, the primary markers used were amplified fragment length polymorphisms (AFLPs) that were anchored to Butterfass chromosome-nomenclature linkage groups using linkage group specific markers validated in other populations. Thus, a common framework has been established that anchors 331 markers, including 23 newly mapped simple sequence repeat (SSR) markers, having a combined total of 526.3 cM among the nine beet linkage groups. The source of the mapping population was a sugarbeet × table beet population, and this is the first report of a map constructed with a relatively wide cross in *B. vulgaris*. Segregation distortion was common (22% of loci), particularly extreme for Butterfass Chromosome 5, and predominantly favored the sugarbeet (seed parent) allele. Physical segments of the beet genome that carry mapped markers have been identified, demonstrating that physical and genetic mapping are facile and complementary applications for beet improvement.

Published in Crop Sci 47(S1) (2007). Published 11 Dec. 2006.
doi:10.2135/cropsci2006-05-0339tpg

677 S. Segoe Rd., Madison, WI 53711 USA

Genetic mapping seeks to determine the location of important genes in a framework of linkage groups that should equal the chromosome number of the species being mapped; here for beet, $n = x = 9$. Ideally, the framework should be constructed with a large number of neutral DNA markers that can be easily assayed and that are distributed uniformly across the genome. This ideal is rarely met, however, since most maps are generated to examine inheritance of particular traits with the most convenient marker system available, and genome coverage is rarely uniform without the availability of thousands of markers. Many mapped markers often do not segregate in a Mendelian fashion in the population of interest, limiting the utility of low-genome-coverage molecular maps. Developing reliable, robust, and informative markers is

Abbreviations: AFLP, amplified fragment length polymorphism; BAC, bacterial artificial chromosome; CMS, cytoplasmic male sterile; E/M, *Eco*RI/*Mse*I; EST, expressed sequence tag; M+3, *Mse*I primer with three selective nucleotides; PC, primer combination; PCR, polymerase chain reaction; P/M, *Pst*I/*Mse*I; RAPD, randomly amplified polymorphic DNA; RGA, resistance gene analog; RFLP, restriction fragment length polymorphism; SF, self fertility; SI, self-incompatibility; SSR, simple sequence repeat; STS, sequence tagged site; UTR, untranslated region.

an arduous undertaking and is prerequisite for modern genetic analyses, and a great deal of effort has been applied to sugarbeet genetic mapping to deduce the genetic control of agronomic and disease resistance traits. Unfortunately, few available public (e.g., nonrestricted use) markers has hindered wide adoption of genetic analyses of sugarbeet in public sector research programs, and the present work was performed to provide a public mapping resource whereby newly discovered genes and markers can be mapped in a common linkage group framework. It is hoped the community will add to this resource with discovery of additional molecular markers, particularly those residing in gene-rich regions of the beet genome.

A number of molecular marker genetic maps in sugarbeet have been constructed (Barzen et al., 1992, 1995; Halldén et al., 1996; Hansen et al., 1999; Nilsson et al., 1997; Pillen et al., 1992, 1993; Rae et al., 2000; Schondelmaier et al., 1996, 1997; Schumacher et al., 1997; Uphoff and Wricke 1992, 1995). Each has been constructed from sugarbeet, and other crop types [table and fodder beet, chard, wild beet (*B. vulgaris* subsp. *maritima*)] are not yet represented with genetic maps. Although their fundamental genetic basis is unlikely to be vastly different, allele frequencies will likely vary, and fixation of crop-type-specific alleles might be expected. Many marker systems have been used, most are anonymous, including restriction fragment length polymorphisms (RFLPs), randomly amplified DNA polymorphisms (RAPDs), AFLPs, and SSRs, as well as a few morphological (e.g., color, seed type) and isozyme markers. Some single nucleotide polymorphisms (SNPs) within protein-encoding genes are available for mapping in sugarbeet (Möhring et al., 2004; Schneider et al., 2001), and Pillen et al. (1996) determined linkage relationships among 12 nuclear genes encoding chloroplast thylakoid proteins. In these published maps, the number of markers used ranged from 85 to 413 markers, and the total genetic distance summed across nine linkage groups ranged from 621 cM to 1057 cM. Most maps showed strong clustering of markers in one or two regions of each linkage group, suggesting restricted genetic recombination, and perhaps influenced by the type of marker used (Nilsson et al., 1997). Genes linked in *Arabidopsis* were co-located to beet and other species chromosomes (Dominguez et al., 2003), demonstrating the blocks of conserved synteny extend among unrelated eudicot plant families. Importantly, Schondelmaier and Jung (1997) defined molecular, isozyme, and morphological linkage groups based on the Butterfass (1964) trisomic series, thus establishing a common nomenclature for beet linkage groups. Inconsistencies persist in the literature regarding chromosome assignments, although many maps contain a few morphological markers in common.

The work described here represents a step toward a public set of markers for genetic analyses in beet. A significant aspect of this work is that the nine linkage groups have been delineated, and named according to the Butterfass chromosome nomenclature. The DNA of this mapping population has been amplified using rolling circle amplification (Dean et al., 2001; Brukner et al., 2005). This mapping resource can be, and has been, shared among laboratories for efficient marker placement on a common genetic framework. Genetic maps rely on recombination to locate markers to linkage groups, thus a marker must be polymorphic in this population to be mapped. To circumvent this limitation, a physical map is being constructed using large insert clones from a bacterial artificial chromosome (BAC) library (McGrath et al., 2004). Physical maps only rely on the absolute distance between two loci measured in base pairs. The physical mapping resource is also amplified using rolling circle amplification and represented in pools of BAC clones that allow convenient screening via polymerase chain reaction (PCR). Typically, the BAC library is used to recover genes of interest in their native genomic state. These large genomic fragments are a rich source of potentially informative genetic markers that can be screened in any mapping population, and provide a means to link the nascent physical and genetic maps.

Materials and Methods

Plant Material

The mapping population utilized for this study was from an intraspecific cross between a single plant progenitor of the diploid sugarbeet release C869 (Lewellen, 2004) (i.e., 6869, McGrath et al., 1999) and a diploid table beet from W357B (Goldman, 1996). These parents were chosen because of their different genetic backgrounds and large phenotypic variability in anticipation of high molecular polymorphism levels for mapping. C869, used as the seed parent, carries a Mendelian dominant gene for self-fertility (*Sf*) and it is segregating for a Mendelian recessive gene conferring male sterility (*Aa*), and has medium-high root sucrose content (16%) with a white, conical root. W357B, used as the pollen parent, is homozygous dominant for both self-fertility and nuclear male fertility genes, and it is characterized by a lower root sucrose concentration (10%) and a dark-red, ball shaped root. Crosses were made by bagging an *aa* C869 progenitor with W357B. One-hundred and twenty-eight F_2 plants were obtained by self-pollinating a single fertile F_1 plant, and this population was genotyped for morphological and molecular genetic

markers. Plant DNA was extracted after grinding tissue in liquid N_2, removing lipids with one chloroform extraction, and isolating DNA using CsCl gradient centrifugation (McGrath et al., 1993).

AFLP Marker Mapping

Amplified fragment length polymorphism mapping followed the protocol of Vos et al. (1995) with modification (Myburg et al., 2001). Two different restriction enzyme pair combinations were used to generate the initial AFLP linkage map: the C-methylation-insensitive *Eco*RI/*Mse*I (E/M) combination and the C-methylation-sensitive *Pst*I/*Mse*I (P/M) combination. Preamplification was performed with one (A or C for *Eco*RI) or zero (for *Pst*I) selective nucleotide primers, and one selective nucleotide (A or C) for *Mse*I primers. A total of 16 and 20 different selective primer combinations (PCs) were analyzed for E/M and P/M restriction enzyme pair combinations, respectively. Fluorescence-labeled primers (IRD700 or IRD800; LI-COR Biosciences, Lincoln, NE) with three (*Eco*RI, E+3) or two (*Pst*I, P+2) selective nucleotides were used along with a single unlabeled *Mse*I primer also with three selective nucleotides (M+3) to generate the scored AFLP fingerprints. Expected Mendelian segregation ratios for 1:2:1 and 3:1 were tested by χ^2 analysis for codominant and dominant polymorphic amplified fragments, respectively, and marker clustering tendency was tested using the Poisson distribution.

Other Markers and Linkage Group Nomenclature Unification

A limited number of other marker types were used in the genetic map, including 25 RFLPs, 46 SSRs, 14 ESTs–UTRs (expressed sequence tags–untranslated regions), and three phenotypic markers (Table 1). The SSR sequences described but not mapped previously were tested (Cureton et al., 2002; Mörchen et al., 1996; Richards et al., 2004; Viard et al., 2002) and mapped if polymorphism was evident. Twenty-three SSR loci are newly described; those with the prefix FDSB were discovered and mapped in Florimond Desprez's population (Cappelle en Pévèle, France); SSRs prefixed USDA were identified and mapped in SESVANDERHAVE's population (Tienen, Belgium) from ESTs deposited in the NCBI database; and those named according to their GenBank numbers were mapped in this study from beet ESTs deposited in GenBank processed with SSR Primer software (Robinson et al., 2004). Fourteen SSR markers, owned by KWS SAAT AG (Einbeck, Germany), were run on the population used here to confirm and unify chromosome nomenclature according to Butterfass trisomics.

Integrated DNA Technologies (Coralville, IA) synthesized all primers (sequences are listed in Supplementary Table 1). The SSR and the sequence tagged site (STS) marker were detected using 1 × GoTaq Green master mix (Promega, Madison, WI), 0.375 μ*M* each forward and reverse SSR primer, and 50 ng DNA. The PCR conditions consisted of an initial denaturation at 94°C for 1.5 min, followed by 13 cycles of 94°C for 30 s, then 58°C for 30 s (touchdown using −0.8°C per cycle), 72°C for 60 s, and an additional 31 cycles of 94°C for 30 s, 47°C for 30 s, 72°C for 60 s, and final extension of 72°C for 10 min. The PCR fragments were analyzed using precast 4% agarose, 1 × TAE (Tris-acetate EDTA) with ethidium bromide gels (11.5 by 11.9 cm, No. GE-3577, Embitec, San Diego, CA), with six rows of 16 sample lanes and two lanes for DNA standards, loaded with 10 μL of PCR reaction, run at 100 V for 30 min.

Large-insert clones may help in discovering *cis*-linked polymorphisms.

The procedure for RFLP was as described by McGrath et al. (1993), using 5 μg of DNA and one of four restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, or *Xba*I). Probes were generated from randomly selected cDNA clones from a sugarbeet leaf library (courtesy of Dan Bush, Ft. Collins, CO) or germinating seedlings (de los Reyes et al., 2003), and were amplified from purified plasmids followed by excision from an agarose gel, labeled with ^{32}P-dCTP, and hybridized and detected as described.

The EST–UTR markers were generated from a single IRD700 labeled gene-specific primer in a pool of single enzyme digested genomic DNA ligated to unlabeled T7 adaptor sequences, detected as for AFLP. The EST–UTR markers were named with the GenBank Accession number followed by an E or D (for *Eco*RI or *Dra*I, respectively; if scored dominant in this case), or cd (if codominant) and a sequential number. All EST–UTR loci reported here were developed from the calmodulin-like EST BI543691 (sequence in Supplementary Table 1).

Linkage analysis used JoinMap 3.0 software (Van Ooijen and Voorrips, 2001) with LOD grouping threshold of 4.0. Marker order was calculated using pairwise data estimated with the REC threshold function set to 0.35 and LOD threshold > 3.0. Genetic distances were corrected for double crossover events using the Kosambi function.

Table 1. Segregation data for each locus mapped in the sugarbeet × table beet population.

Marker No.	Butterfass Linkage Group	Position cM	Locus name	Marker type†	Sugarbeet allele	Heterozygous	Table beet allele	χ^2	P	
1	1	0.0	BU089581	SSR	18	52	21	2.1	0.36	ns
2	1	12.5	BQ588629	SSR	31	–	61	3.7	0.05	*
3	1	15.5	PACMCAG058	AFLP	65	–	31	2.7	0.10	ns
4	1	16.9	521.6	SSR	30	38	16	5.4	0.07	ns
5	1	17.8	EACAMCTT750	AFLP	10	–	38	0.4	0.50	ns
6	1	18.0	PCAMCAT084	AFLP	11	–	37	0.1	0.74	ns
7	1	18.4	ECATMCCA152	AFLP	63	–	28	1.6	0.20	ns
8	1	18.4	PAGMACA220	AFLP	21	–	103	4.3	0.04	*
9	1	18.6	PAGMCTT184	AFLP	8	–	45	2.8	0.10	ns
10	1	19.1	EACAMCTT547	AFLP	9	–	39	1.0	0.32	ns
11	1	19.2	PCAMCAT177	AFLP	12	–	36	0.0	1.00	ns
12	1	19.2	EACAMCTT496	AFLP	9	24	15	1.5	0.47	ns
13	1	19.3	ECATMCAT140	AFLP	14	–	78	4.7	0.03	*
14	1	20.1	EACTMCTT090	AFLP	105	–	20	5.4	0.02	*
15	1	20.8	sbcD042	RFLP	12	28	19	1.8	0.40	ns
16	1	21.6	EAGCMCAG220	AFLP	62	–	30	2.8	0.09	ns
17	1	22.2	ECATMCAT170	AFLP	62	–	30	2.8	0.09	ns
18	1	23.5	BI543691E4	EST–UTR	54	–	34	8.7	0.00	***
19	1	23.7	PTCMCAT084	AFLP	35	–	23	6.6	0.01	**
20	1	24.7	PAGMAGC101	AFLP	22	–	102	3.5	0.06	ns
21	1	25.1	BI543691E7	EST–UTR	54	–	34	8.7	0.00	***
22	1	25.4	PCAMCAT083	AFLP	27	–	21	9.0	0.00	***
23	1	28.8	PACMCCA167	AFLP	18	–	73	1.3	0.25	ns
24	1	29.5	EACTMCTT059	AFLP	90	–	35	0.6	0.44	ns
25	1	31.1	A	Morphological	16	–	62	0.4	0.36	ns
26	1	31.4	ECTCMCAG148	AFLP	59	–	29	3.0	0.08	ns
27	1	35.8	PACMCCA415	AFLP	24	–	67	0.1	0.76	ns
28	1	36.5	PAGMCTT141	AFLP	39	–	13	0.0	1.00	ns
29	1	38.0	PCAMCTT162	AFLP	12	–	45	0.5	0.49	ns
30	1	42.8	EACAMCAT305	AFLP	68	–	28	0.9	0.35	ns
31	1	48.4	1KWS	SSR	16	50	24	2.5	0.28	ns
32	1	63.6	BQ583448	SSR	35	–	53	3	0.08	ns
33	2	0.0	PAGMCAT199	AFLP	19	–	74	1.0	0.31	ns
34	2	1.5	PTCMCCA083	AFLP	41	–	17	0.6	0.45	ns
35	2	3.6	PCAMAGC319	AFLP	26	–	98	1.1	0.30	ns
36	2	4.3	EACAMCTT340	AFLP	11	–	37	0.1	0.74	ns
37	2	5.8	PAGMCCA350	AFLP	71	–	24	0.0	0.95	ns
38	2	5.9	EACAMCCA276	AFLP	73	–	14	3.7	0.06	ns
39	2	8.5	BI543691cd1	EST–UTR	17	52	19	3.0	0.22	ns
40	2	9.6	PCAMACA207	AFLP	100	–	22	3.2	0.08	ns
41	2	10.1	EACAMCCA336	AFLP	32	–	10	0.0	0.86	ns
42	2	10.2	EAGCMCAG594	AFLP	18	–	72	1.2	0.27	ns
43	2	11.7	EACTMCTT228	AFLP	99	–	26	1.2	0.28	ns
44	2	14.6	sbcD66	RFLP	8	15	5	0.8	0.68	ns
45	2	15.5	BI543691E8	EST–UTR	15	–	73	3.0	0.08	ns
46	2	15.7	PTCMCAT275	AFLP	47	–	10	1.7	0.19	ns
47	2	16.3	2KWS	SSR	18	46	22	0.8	0.67	ns
48	2	16.3	PCAMCCA151	AFLP	21	–	75	0.5	0.48	ns
49	2	19.4	sbcD244	RFLP	14	32	14	0.3	0.88	ns
50	2	19.7	PCAMCTT073	AFLP	47	–	10	1.7	0.19	ns
51	2	21.8	BQ584037	SSR	21		71	0.2	0.63	ns
52	2	26.9	PAGMCAT270	AFLP	19	–	74	1.0	0.31	ns
53	2	29.1	PCAMCAG112	AFLP	13	–	50	0.6	0.42	ns
54	2	29.4	R	Morphological	27	–	46	5.7	0.02	*
55	2	31.5	PCAMCCA145	AFLP	72	–	24	0.0	1.00	ns
56	2	33.6	EACTMCTT114	AFLP	20	–	59	0.0	0.95	ns
57	2	39.4	sbcD143	RFLP	16	34	9	3.0	0.22	ns

Table 1 (continued).

Marker No.	Butterfass Linkage Group	Position cM	Locus name	Marker type†	Sugarbeet allele	Heterozygous	Table beet allele	χ^2	P	
58	2	45.3	ECATMCAT220	AFLP	12	–	80	7.0	0.01	**
59	3	0.0	ECTCMCAG130	AFLP	72	–	16	2.2	0.14	ns
60	3	1.6	PCAMCAG495	AFLP	16	–	47	0.0	0.94	ns
61	3	2.0	BI543628	SSR	23	–	66	0.1	0.85	ns
62	3	3.2	PCAMAGC068	AFLP	30	–	95	0.1	0.80	ns
63	3	6.6	PCAMCTT349	AFLP	46	–	11	1.0	0.32	ns
64	3	6.6	EACAMCTT688	AFLP	15	–	33	1.0	0.32	ns
65	3	7.3	EAGCMCAG506	AFLP	30	–	62	2.8	0.09	ns
66	3	8.2	PACMCGA104	AFLP	28	–	67	1.0	0.31	ns
67	3	9.5	EACAMCCA229	AFLP	65	–	19	0.3	0.61	ns
68	3	10.2	FDSB1027	SSR	22	43	19	0.3	0.88	ns
69	3	10.6	PCAMCAT169	AFLP	34	–	14	0.4	0.50	ns
70	3	11.3	PCAMACA468	AFLP	92	–	22	2.0	0.16	ns
71	3	11.6	EACAMCGG264	AFLP	26	–	69	0.3	0.59	ns
72	3	11.8	EACTMCTT121	AFLP	94	–	31	0.0	0.96	ns
73	3	12.1	BI543691E2	EST–UTR	64	–	24	0.2	0.62	ns
74	3	12.1	EACAMACA207	AFLP	25	45	21	0.4	0.83	ns
75	3	12.7	EACTMCTTcd1	AFLP	33	62	30	0.1	0.93	ns
76	3	12.7	ECATMCAT222	AFLP	71	–	21	0.2	0.63	ns
77	3	13.3	EACAMCCA303	AFLP	33	–	9	0.3	0.59	ns
78	3	15.0	Str1-C7	RFLP	19	22	21	5.3	0.07	ns
79	3	15.8	EACTMCAG311	AFLP	14	–	33	0.6	0.45	ns
80	3	17.0	PTCMCAT202	AFLP	16	–	34	1.3	0.25	ns
81	3	18.7	S1A-A9	RFLP	20	24	13	3.1	0.21	ns
82	3	20.3	PAGMCCA218	AFLP	24	–	71	0.0	0.95	ns
83	3	21.1	PCAMCAT338	AFLP	33	–	15	1.0	0.32	ns
84	3	22.0	PTCMCGA078	AFLP	69	–	25	0.1	0.72	ns
85	3	22.9	PCAMCAT156	AFLP	32	–	16	1.8	0.18	ns
86	3	23.7	PCAMCCAcd1	AFLP	24	47	25	0.1	0.97	ns
87	3	25.1	3bKWS	SSR	28	38	23	2.5	0.29	ns
88	3	25.9	PAGMCTT264	AFLP	13	–	40	0.0	0.94	ns
89	3	26.0	PAGMACA226	AFLP	26	–	89	0.3	0.55	ns
90	3	27.5	3aKWS	SSR	24	48	20	0.5	0.77	ns
91	3	28.9	EACAMCCA147	AFLP	20	–	73	0.6	0.44	ns
92	3	30.1	PACMCAG237	AFLP	24	–	71	0.0	0.95	ns
93	3	30.3	EAGCMCAG129	AFLP	63	–	29	2.1	0.15	ns
94	3	34.8	PAGMAGC068	AFLP	33	–	91	0.2	0.68	ns
95	3	35.2	PCAMCAT485	AFLP	14	–	34	0.4	0.50	ns
96	3	41.7	EAGCMCAG515	AFLP	32	–	59	5.0	0.03	*
97	3	43.0	PCAMCCA229	AFLP	24	–	72	0.0	1.00	ns
98	4	0.0	sbcD056	RFLP	16	30	14	0.1	0.94	ns
99	4	3.0	PTCMCGA459	AFLP	74	–	20	0.7	0.40	ns
100	4	4.6	BI543691E10	EST–UTR	20	–	68	0.2	0.62	ns
101	4	6.4	EAGCMCAT123	AFLP	16	–	76	2.8	0.09	ns
102	4	8.8	PCAMACA321	AFLP	21	–	102	4.1	0.04	*
103	4	12.0	PCAMCAT176	AFLP	38	–	10	0.4	0.50	ns
104	4	12.7	PCAMACA369	AFLP	91	–	30	0.0	0.96	ns
105	4	13.1	EAGCMCAG406	AFLP	22	–	70	0.1	0.81	ns
106	4	17.0	EACTMCTT111	AFLP	96	–	29	0.2	0.64	ns
107	4	18.3	ECATMCAT159	AFLP	21	46	25	0.3	0.84	ns
108	4	19.0	EACAMCTT110	AFLP	40	–	8	1.8	0.18	ns
109	4	20.1	ECATMCCA148	AFLP	21	–	71	0.2	0.63	ns
110	4	20.4	PCAMCCA088	AFLP	76	–	20	0.9	0.35	ns
111	4	20.9	PCAMCAG178	AFLP	17	–	46	0.1	0.72	ns
112	4	21.2	PTCMCAT250	AFLP	15	–	41	0.1	0.76	ns
113	4	21.9	FDSB1023	SSR	18	45	23	0.8	0.68	ns
114	4	23.1	PCAMCAG182	AFLP	71	–	16	2.0	0.15	ns

Table 1 (continued).

Marker No.	Butterfass Linkage Group	Position cM	Locus name	Marker type†	Sugarbeet allele	Heterozygous	Table beet allele	χ^2	P	
115	4	23.3	EAGCMCAG180	AFLP	16	–	76	2.8	0.09	ns
116	4	23.6	PAGMCTT150	AFLP	45	–	6	4.8	0.03	*
117	4	23.7	PCAMCAT343	AFLP	40	–	8	1.8	0.18	ns
118	4	23.8	PCAMCTT459	AFLP	11	–	39	0.2	0.62	ns
119	4	24.1	PCAMCTT107	AFLP	47	–	9	2.4	0.12	ns
120	4	24.3	EACTMCAG211	AFLP	39	–	8	1.6	0.21	ns
121	4	24.5	PCAMCAG180	AFLP	16	–	47	0.0	0.94	ns
122	4	24.5	PCAMCAT206	AFLP	9	–	39	1.0	0.32	ns
123	4	25.4	4bKWS	SSR	18	48	23	1.1	0.57	ns
124	4	25.6	FDSB1002-1	SSR	19	48	24	0.8	0.66	ns
125	4	27.1	FDSB1002-2	SSR	14	40	22	1.9	0.39	ns
126	4	27.4	4cKWS	SSR	17	47	25	1.7	0.42	ns
127	4	30.0	EACAMCCA242	AFLP	57	–	13	1.5	0.21	ns
128	4	31.0	BQ591109	SSR	19	–	73	0.9	0.34	ns
129	4	33.0	EACAMCCA133	AFLP	76	–	17	2.2	0.13	ns
130	4	35.3	PAGMAGC131	AFLP	24	–	100	2.1	0.15	ns
131	4	38.4	SB6-1	SSR	19	44	26	1.1	0.57	ns
132	4	39.7	PACMCCA103	AFLP	20	–	71	0.4	0.51	ns
133	4	41.3	SB6-2	SSR	16	39	20	0.6	0.76	ns
134	4	43.3	SB6-3	SSR	18	35	17	0.0	0.99	ns
135	4	45.3	4aKWS	SSR	24	46	22	0.1	0.96	ns
136	4	48.3	PAGMACA132	AFLP	18	–	44	0.5	0.46	ns
137	4	48.5	PCAMCAT126	AFLP	14	–	34	0.4	0.50	ns
138	4	50.4	M	Morphological	19	–	57	0.0	1.00	ns
139	4	53.1	BI643126	SSR	79	–	13	5.8	0.02	*
140	4	56.5	SB7	SSR	14	29	19	1.1	0.59	ns
141	4	58.6	EACTMCTT125	AFLP	89	–	36	1.0	0.33	ns
142	4	59.6	PCAMACA261	AFLP	89	–	33	0.3	0.60	ns
143	4	59.8	PAGMCAG207	AFLP	45	–	18	0.4	0.51	ns
144	4	74.3	BQ587612	SSR	36	–	56	9.8	0.01	**
145	5	0.0	BQ588947	SSR	32	–	60	4.7	0.03	*
146	5	2.3	PCAMCAT161	AFLP	16	–	32	1.8	0.18	ns
147	5	6.1	SB15-1	SSR	26	–	51	3.2	0.08	ns
148	5	8.2	SB15-2	SSR	40	31	3	39.0	0.00	***
149	5	11.4	BMB3	SSR	36	32	4	29.3	0.00	***
150	5	12.1	EACTMCTT375	AFLP	72	–	53	20.2	0.00	***
151	5	13.8	PTCMCGA396	AFLP	55	–	41	16.1	0.00	***
152	5	15.2	EAGCMCAG446	AFLP	45	–	47	28.1	0.00	***
153	5	16.0	EACAMACA253	AFLP	45	–	46	29.0	0.00	***
154	5	16.9	5KWS	SSR	44	37	5	37.1	0.00	***
155	5	17.7	EACAMCTT470	AFLP	44	–	4	7.1	0.01	**
156	5	18.2	sbcD119	RFLP	32	21	4	31.5	0.00	***
157	5	18.2	EACAMACG139	AFLP	86	–	6	16.8	0.00	***
158	5	18.6	PAGMCAT097	AFLP	86	–	7	15.1	0.00	***
159	5	19.4	PTCMCCA118	AFLP	22	–	62	0.1	0.80	ns
160	5	19.9	EACAMACG207	AFLP	82	–	10	9.8	0.00	***
161	5	21.6	PCAMACA426	AFLP	56	–	62	31.7	0.00	***
162	5	21.9	EACTMCTT220	AFLP	56	–	69	26.1	0.00	***
163	5	25.3	EAGCMCAG374	AFLP	41	–	51	18.8	0.00	***
164	5	27.0	PAGMAGC063	AFLP	108	–	16	9.7	0.00	***
165	5	27.1	PACMCGA221	AFLP	39	–	56	13.1	0.00	***
166	5	29.6	PAGMCAT115	AFLP	79	–	13	5.8	0.02	*
167	5	29.7	PTCMCAG170	AFLP	36	–	54	10.8	0.00	***
168	5	31.2	PCAMACA200	AFLP	38	–	26	8.3	0.00	***
169	5	32.9	SB04	SSR	40	–	20	2.2	0.14	ns
170	5	33.9	sbcD102	RFLP	31	21	5	27.7	0.00	***
171	5	35.9	PTCMCAT332	AFLP	44	–	13	0.1	0.70	ns

Table 1 (continued).

Marker No.	Butterfass Linkage Group	Position cM	Locus name	Marker type†	Sugarbeet allele	Heterozygous	Table beet allele	χ^2	P	
172	5	37.1	PAGMCTT086	AFLP	34	–	13	0.2	0.67	ns
173	5	37.4	PTCMCAG121	AFLP	28	–	61	2.0	0.16	ns
174	5	40.2	PCAMCCA127	AFLP	32	–	64	3.6	0.06	ns
175	5	43.4	PAGMCAT101	AFLP	34	–	56	7.8	0.01	**
176	5	48.6	PAGMCTT084	AFLP	14	–	33	0.6	0.45	ns
177	6	0.0	BQ591966	SSR	15	–	74	3.2	0.08	ns
178	6	6.4	6bKWS	SSR	22	50	18	1.5	0.48	ns
179	6	11.1	PAGMCAG264	AFLP	34	–	60	6.3	0.01	*
180	6	13.6	ECATMCAT210	AFLP	24	–	68	0.1	0.81	ns
181	6	14.4	PTCMCGA114	AFLP	73	–	20	0.6	0.44	ns
182	6	15.8	EACAMCGG424	AFLP	24	–	71	0.0	0.95	ns
183	6	17.4	sbcD150	RFLP	14	30	18	0.6	0.75	ns
184	6	17.8	EACAMACG145	AFLP	72	–	20	0.5	0.47	ns
185	6	20.4	ECTCMCAG646	AFLP	21	–	67	0.1	0.00	ns
186	6	21.1	ECATMCAT211	AFLP	65	–	27	0.9	0.34	ns
187	6	23.3	EACTMCTT165	AFLP	60	–	19	0.0	0.85	ns
188	6	23.4	ECTCMCAG279	AFLP	16	–	72	2.2	0.14	ns
189	6	23.8	PCAMCAT575	AFLP	12	–	36	0.0	1.00	ns
190	6	25.2	PCAMACA095	AFLP	93	–	31	0.0	1.00	ns
191	6	25.4	BI543691E11	EST–UTR	26	–	62	1.0	0.32	ns
192	6	27.6	PCAMAGC221	AFLP	27	–	97	0.7	0.41	ns
193	6	27.9	BI543691E16	EST–UTR	70	–	18	1.0	0.32	ns
194	6	28.1	EAGCMCAT208	AFLP	20	–	72	0.5	0.47	ns
195	6	28.6	EAGCMCAGcd1	AFLP	19	54	19	2.8	0.25	ns
196	6	28.7	ECATMCAT302	AFLP	73	–	19	0.9	0.34	ns
197	6	29.1	sbcD91	RFLP	11	26	17	1.4	0.49	ns
198	6	29.2	Str1-B8	RFLP	12	30	17	0.9	0.65	ns
199	6	29.6	PCAMCAG157	AFLP	49	–	14	0.3	0.61	ns
200	6	29.7	PCAMCAT337	AFLP	13	–	35	0.1	0.74	ns
201	6	29.9	EACAMCTT195	AFLP	36	–	12	0.0	1.00	ns
202	6	30.0	EACTMACA270	AFLP	70	–	21	0.2	0.67	ns
203	6	30.5	PAGMCAG070	AFLP	48	–	15	0.1	0.83	ns
204	6	30.9	ECATMCAT201	AFLP	19	–	73	0.9	0.34	ns
205	6	32.5	PAGMCTT217	AFLP	39	–	12	0.1	0.81	ns
206	6	33.1	6aKWS	SSR	20	46	21	0.3	0.86	ns
207	6	34.6	GTT1	SSR	19	47	18	1.2	0.54	ns
208	6	36.3	PAGMCAT217	AFLP	77	–	16	3.0	0.08	ns
209	6	39.8	EACAMCCA335	AFLP	33	–	9	0.3	0.59	ns
210	6	42.0	PAGMAGC183	AFLP	26	–	98	1.1	0.30	ns
211	6	46.9	BQ487642	SSR	25	–	67	0.2	0.63	ns
212	6	67.8	BQ591641	SSR	41	–	51	18.8	0.00	***
213	7	0.0	ECATMCAT290	AFLP	64	–	28	1.4	0.23	ns
214	7	9.9	sbcD010	RFLP	12	35	15	1.3	0.52	ns
215	7	10.6	BvGer165	STS	61†	–	63	44.0	0.00	***
216	7	12.1	7KWS	SSR	23	50	19	1.0	0.59	ns
217	7	18.1	PACMCAG188	AFLP	22	–	73	0.2	0.68	ns
218	7	22.0	USDA5	SSR	20	40	19	0.0	0.98	ns
219	7	22.1	EAGCMCAG479	AFLP	54	–	38	55.7	0.00	***
220	7	25.8	USDA3	SSR	27	38	24	2.1	0.35	ns
221	7	26.2	PCAMCAT128	AFLP	17	–	31	2.8	0.10	ns
222	7	29.4	sbcD203	RFLP	16	27	17	0.6	0.73	ns
223	7	30.3	EAGCMCAG579	AFLP	34	–	56	7.8	0.01	**
224	7	33.4	PAGMAGC253	AFLP	34	–	90	0.4	0.53	ns
225	7	36.4	FDSB1011	SSR	16	24	14	0.8	0.67	ns
226	7	38.8	EACAMCGG300	AFLP	71	–	24	0.0	0.95	ns
227	7	41.2	PAGMAGCcd1	AFLP	32	63	29	0.2	0.92	ns
228	7	42.6	BI543691E1	EST–UTR	20	–	68	0.2	0.62	ns

Table 1 (continued).

Marker No.	Butterfass Linkage Group	Position cM	Locus name	Marker type†	Sugarbeet allele	Heterozygous	Table beet allele	χ^2	P	
229	7	44.3	PAGMCAT134	AFLP	31	–	14	0.9	0.34	ns
230	7	45.1	Str1-B9	RFLP	16	24	19	2.4	0.31	ns
231	7	46.3	PACMCAT130	AFLP	17	–	39	0.9	0.35	ns
232	7	48.0	PACMCAG102	AFLP	27	–	69	0.5	0.48	ns
233	7	48.1	EACAMCAT069	AFLP	76	–	20	0.9	0.35	ns
234	7	49.5	Str1-A1	RFLP	18	22	20	4.4	0.11	ns
235	7	50.4	PAGMCAG243	AFLP	17	–	46	0.1	0.72	ns
236	7	51.1	PAGMCAT171	AFLP	72	–	21	0.3	0.59	ns
237	7	51.9	PACMCAGcd1	AFLP	28	45	22	1.0	0.60	ns
238	7	52.3	EACTMCTT184	AFLP	98	–	27	0.8	0.38	ns
239	7	54.5	PCAMCTT211	AFLP	47	–	10	1.7	0.19	ns
240	7	55.5	PTCMCAT080	AFLP	19	–	39	1.9	0.17	ns
241	7	57.5	EACAMCCA119	AFLP	72	–	21	0.3	0.59	ns
242	7	59.7	PCAMCTT067	AFLP	47	–	10	1.7	0.19	ns
243	7	63.1	EAGCMCAG291	AFLP	79	–	13	5.8	0.02	*
244	7	65.5	BI543691E3	EST–UTR	26	–	62	1.0	0.32	ns
245	7	68.2	BU089565	SSR	16	67	9	20.2	0.00	***
246	7	69.8	EACAMACG140	AFLP	8	–	84	13.0	0.00	***
247	7	70.5	EAGCMCAG628	AFLP	22	–	69	0.0	0.86	ns
248	7	70.7	EAGCMCAG574	AFLP	40	–	51	17.4	0.00	***
249	8	0.0	sbcD186	RFLP	36	–	25	8.3	0.00	***
250	8	9.0	PCAMCAG159	AFLP	17	–	77	2.4	0.12	ns
251	8	14.8	Str1-B6	RFLP	21	–	41	2.6	0.11	ns
252	8	16.9	EAGCMCAG141	AFLP	77	–	15	3.7	0.05	*
253	8	21.3	sbcD141	RFLP	18	29	13	0.9	0.64	ns
254	8	25.6	ECATMACC366	AFLP	24	–	67	0.1	0.76	ns
255	8	26.1	ECTCMCAG543	AFLP	27	–	62	1.4	0.24	ns
256	8	29.2	BI543691E9	EST–UTR	11	–	77	7.3	0.01	**
257	8	30.9	EACAMACG144	AFLP	77	–	15	3.7	0.05	*
258	8	31.3	PCAMCAT145	AFLP	42	–	6	4.0	0.05	*
259	8	32.1	Str1-A2	RFLP	20	–	42	1.7	0.19	ns
260	8	32.2	EACTMCTT167	AFLP	104	–	21	4.5	0.03	*
261	8	33.5	sbcD021	RFLP	14	34	9	3.0	0.22	ns
262	8	34.0	EACAMCCA458	AFLP	27	–	8	0.1	0.77	ns
263	8	34.2	EACAMCCA230	AFLP	23	–	61	0.3	0.61	ns
264	8	34.8	PCAMCAG381	AFLP	17	–	46	0.1	0.72	ns
265	8	35.2	PAGMACA405	AFLP	53	–	9	3.6	0.06	ns
266	8	35.5	PAGMAGC065	AFLP	103	–	21	4.3	0.04	*
267	8	35.9	EACTMCTT061	AFLP	14	–	33	0.6	0.45	ns
268	8	36.0	EACAMCTT263	AFLP	39	–	9	1.0	0.32	ns
269	8	36.6	EACAMCAT285	AFLP	82	–	14	5.6	0.02	*
270	8	37.0	EACTMACA143	AFLP	22	–	68	0.0	0.90	ns
271	8	37.4	EACTMCAG423	AFLP	39	–	8	1.6	0.21	ns
272	8	38.0	FDSB1007	SSR	23	54	15	4.2	0.12	ns
273	8	38.2	EACAMCAT212	AFLP	25	57	14	5.9	0.05	*
274	8	38.6	sbcD089	RFLP	19	35	6	7.3	0.03	*
275	8	39.4	PCAMCTT136	AFLP	14	–	43	0.0	0.94	ns
276	8	40.8	PACMCCAcd1	AFLP	23	53	15	3.9	0.14	ns
277	8	42.3	BI543691D1	EST–UTR	11	–	33	0.0	1.00	ns
278	8	42.9	8KWS	SSR	28	46	18	2.2	0.34	ns
279	8	43.6	EACTMCAG203	AFLP	13	–	34	0.2	0.67	ns
280	8	43.9	EACAMCAT126	AFLP	29	–	67	1.4	0.24	ns
281	8	44.7	EACAMCGG613	AFLP	27	–	65	0.9	0.34	ns
282	8	45.2	EACAMACA073	AFLP	28	–	64	1.4	0.23	ns
283	8	46.5	EACAMCTT132	AFLP	14	–	34	0.4	0.50	ns
284	8	47.2	EACAMACA345	AFLP	30	–	61	3.1	0.08	ns

Table 1 (continued).

Marker No.	Butterfass Linkage Group	Position cM	Locus name	Marker type†	Sugarbeet allele	Heterozygous	Table beet allele	χ^2	*P*	
285	8	48.7	PACMCAG120	AFLP	28	–	68	0.9	0.35	ns
286	8	50.1	EACAMACA195	AFLP	31	–	61	3.7	0.05	*
287	8	51.3	PAGMAGC127	AFLP	115	–	9	20.8	0.00	***
288	8	52.1	EACAMCAT343	AFLP	30	–	66	2.0	0.16	ns
289	8	52.2	ECTCMCAG484	AFLP	68	–	20	0.2	0.62	ns
290	8	55.2	PAGMCAG369	AFLP	16	–	47	0.0	0.94	ns
291	8	58.0	EACAMCAT174	AFLP	25	30	39	16.5	0.00	***
292	8	63.9	USDA29	SSR	8	34	21	5.8	0.06	ns
293	9	0.0	EACAMCCA204	AFLP	58	–	22	0.3	0.61	ns
294	9	10.0	PCAMAGC129	AFLP	96	–	28	0.4	0.53	ns
295	9	14.1	PCAMCAG053	AFLP	20	–	43	1.5	0.22	ns
296	9	21.2	PCAMAGC104	AFLP	93	–	32	0.0	0.88	ns
297	9	21.6	PAGMCAG200	AFLP	21	–	42	2.3	0.13	ns
298	9	24.1	PCAMACA081	AFLP	95	–	29	0.2	0.68	ns
299	9	24.9	EACAMCAT201	AFLP	27	–	69	0.5	0.48	ns
300	9	31.6	PAGMCAG522	AFLP	47	–	16	0.0	0.94	ns
301	9	31.8	BI543691E13	EST–UTR	31	–	57	4.9	0.03	*
302	9	33.3	EACAMACA490	AFLP	32	42	16	6.1	0.05	*
303	9	33.7	EACAMCCA231	AFLP	23	–	61	0.3	0.61	ns
304	9	35.6	EACTMACA120	AFLP	68	–	23	0.0	0.95	ns
305	9	35.9	PAGMAGC196	AFLP	42	–	82	5.2	0.02	*
306	9	36.5	ECATMCCA131	AFLP	37	–	55	11.4	0.00	***
307	9	36.8	PCAMCAG364	AFLP	47	–	16	0.0	0.94	ns
308	9	37.3	PTCMCAT075	AFLP	20	–	38	2.8	0.10	ns
309	9	37.8	EACAMACA282	AFLP	34	–	58	7.0	0.01	**
310	9	38.3	EACAMCTT065	AFLP	14	–	34	0.4	0.50	ns
311	9	38.5	EACAMCCA198	AFLP	68	–	23	0.0	0.95	ns
312	9	38.6	ECATMCAT286	AFLP	33	–	59	5.8	0.02	*
313	9	38.9	PCAMACA251	AFLP	40	–	83	3.7	0.05	*
314	9	39.4	EACAMACGcd1	AFLP	31	34	27	6.6	0.04	*
315	9	39.5	PCAMCTT181	AFLP	19	–	39	1.9	0.17	ns
316	9	39.8	EACAMCCA222	AFLP	67	–	23	0.0	0.90	ns
317	9	39.8	D12-F10 (G6PD)	RFLP	16	20	16	2.8	0.25	ns
318	9	39.9	EACAMCAT187	AFLP	72	–	24	0.0	1.00	ns
319	9	40.4	ECATMCAT305	AFLP	65	–	27	0.9	0.34	ns
320	9	40.6	PCAMCAT244	AFLP	16	–	32	1.8	0.18	ns
321	9	40.8	PCAMAGCcd1	AFLP	42	45	37	9.7	0.01	**
322	9	42.0	Pox1-A9	RFLP	21	22	18	5.0	0.08	ns
323	9	42.4	BI543691E12	EST–UTR	66	–	22	0.0	1.00	ns
324	9	43.1	PCAMCTT273	AFLP	43	–	14	0.0	0.94	ns
325	9	43.2	PCAMCCA244	AFLP	27	–	69	0.5	0.48	ns
326	9	44.3	9KWS	SSR	33	28	27	12.4	0.00	***
327	9	45.2	EACAMCTT214	AFLP	38	–	10	0.4	0.50	ns
328	9	45.7	P1P2C-A11	RFLP	21	23	16	4.1	0.13	ns
329	9	46.8	ECATMCAT320	AFLP	69	–	23	0.0	1.00	ns
330	9	48.1	PCAMCTT110	AFLP	23	–	33	7.7	0.01	**
331	9	49.2	FDSB1033	SSR	35	–	57	8.3	0.01	**
	Total cM	526.3	all 331 markers:		12 183	2643	12 238			
			69 co-dominant:		1 470	2643	1 259	17.9	0.00	***
			262 dominant (1:1):		10 713	–	10 979	3.3	0.07	ns

* Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

*** Significant at $P \leq 0.001$.

† In all cases but Marker #215, the dominant parental allele was the most frequent allele scored.

Mapping Population Replication and Immortalization

Genomiphi DNA Amplification (GE Healthcare Technologies, Waukesha, WI) was used exactly following manufacturer's directions, based on the methods of Dean et al. (2001) and Brukner et al. (2005). 100 ng of DNA (1 μL) was added to 9 μL of (proprietary) sample buffer containing random hexamer primers, and heated to 95°C for 3 min. To this was added 10 μL of *Phi*29 DNA polymerase and dNTP mix (proprietary concentrations consisting of 1 μL enzyme and 9 μL reaction buffer, based on Dean et al., 2001), incubated at 30°C for 20 h, followed by heat inactivation of *Phi*29 at 65°C for 10 min. The resulting DNA products were diluted with water to 50 ng μL^{-1}, and 1 μL of this diluted sample was used for traditional PCR.

Markers to BACs

The sugarbeet BAC library SBA (Amplicon Express, Pullman, WA), constructed from the hybrid US H20 sugarbeet genome (McGrath et al., 2004), was matrix pooled (Stormo et al., 2004). This allowed a specific clone to be identified in two rounds of PCR. Initially, a signal was identified within one of eight 4608 BAC clone superpools. Each superpool has a corresponding matrix pool consisting of 36 PCR reactions designed to resolve an individual plate, row, and column within each superpool. The second round of PCR identified the specific desired clone from among these 36 matrix pools, each with 1152 BAC clones, constructed from one superpool. These pools are available for research purposes. Mapped markers and genes were identified to individual BAC clones via the pooling strategy by PCR using 1 × GoTaq Green master mix (Promega, Madison, WI), 0.375 μ*M* each forward and reverse primer, and 50 ng DNA. The PCR conditions consisted of an initial denaturation at 94°C for 1.5 min, followed by 13 cycles of 94°C for 30 s, 58°C for 30 s (touchdown using −0.8 C per cycle), 72°C for 60 s, and an additional 31 cycles of 94°C for 30 s, 47°C for 30 s, 72°C for 60 s, and final extension of 72°C for 10 min.

Results

A genetic map was developed from a cross between sugarbeet and table beet using three morphological, 25 RFLP, 242 AFLP (115 with *Eco*RI and 127 with *Pst*I), 46 SSR, 14 EST–UTR, and one STS (total 331) markers mapped in a population of 128 F_2 generation plants derived from a single hybrid F_1 individual. The map spanned a total 526.3 cM among the nine beet linkage groups (Table 1).

The map framework was primarily based on the segregation of AFLP markers. Two enzyme systems were used, the more traditional *Eco*RI/*Mse*I combination and the *Pst*I/*Mse*I combination, whose details are elaborated here for beet. Similar numbers of fragments were scored for each combination between the parents (728 for *Eco*RI vs. 830 for *Pst*I). Overall, the 36 different restriction enzyme PCs generated 1558 amplified fragments (43.3 bands PC^{-1}), of which 316 (8.8 bands PC^{-1}; 20.3%) were polymorphic between parents. The 16 E/M PCs yielded 15 to 79 bands PC^{-1}, averaging 45.5 bands PC^{-1} (SD = 16.9), of which 10.4 bands PC^{-1} (SD = 7.1) showed polymorphism (22.9%). The 20 P/M PCs yielded 17 to 93 bands PC^{-1}, averaging 41.5 bands PC^{-1} (SD = 19.7), of which 7.5 bands PC^{-1} (SD = 4.8) were polymorphic (18.0%). Percentages of polymorphisms for each PC ranged from 3.7 to 41.4% for E/M and from 5 to 29.7% for P/M combinations. The percentage A/T nucleotide content of the selective nucleotides was not statistically correlated with the total number of amplified fragments, or with the percentage of polymorphisms.

Initially, AFLP markers were used to define unnamed linkage groups. In this first iteration, the number of AFLP markers and the length of individual linkage groups varied from 26 to 47, and from 36.8 to 69.7 cM, respectively (data not shown). The Poisson distribution of AFLP-derived markers indicated that, at a density of >5 markers per 5 cM, 51.7% (61/118) of E/M markers significantly clustered ($P < 0.001$) on six linkage groups (1, 3, 5, 6, 8, and 9), while 14.4% (19/132) of P/M markers clustered on just two (3 and 9). In the second map iteration, AFLP-identified linkage groups were named according to the Butterfass nomenclature using known chromosome assignments of morphological loci and chromosome-specific SSR markers (including those coded KWS, Table 1), and the combined map was integrated with other markers listed Table 1. In the final iteration reported here (Table 1), AFLP markers with LOD scores < 4.0 were discarded. The final map retained 115 E/M and 127 P/M AFLP markers.

All SSR markers were mapped with genomic DNA that had been amplified using *Phi*29 polymerase mediated rolling circle replication. Successful placement of these markers with respect to AFLP markers, in particular, demonstrated the utility of this method to amplify DNA of the mapping population, and thus can be used to provide adequate DNA amounts for continued discovery and mapping new SSRs. Genomiphi amplified DNA proved very reliable for PCR-based markers, but not RFLP or other hybridization-based detection approaches, where complex band patterns or smears were seen, perhaps the result of *strand switching* during the rolling circle replication process or the single stranded nature of the replicated products.

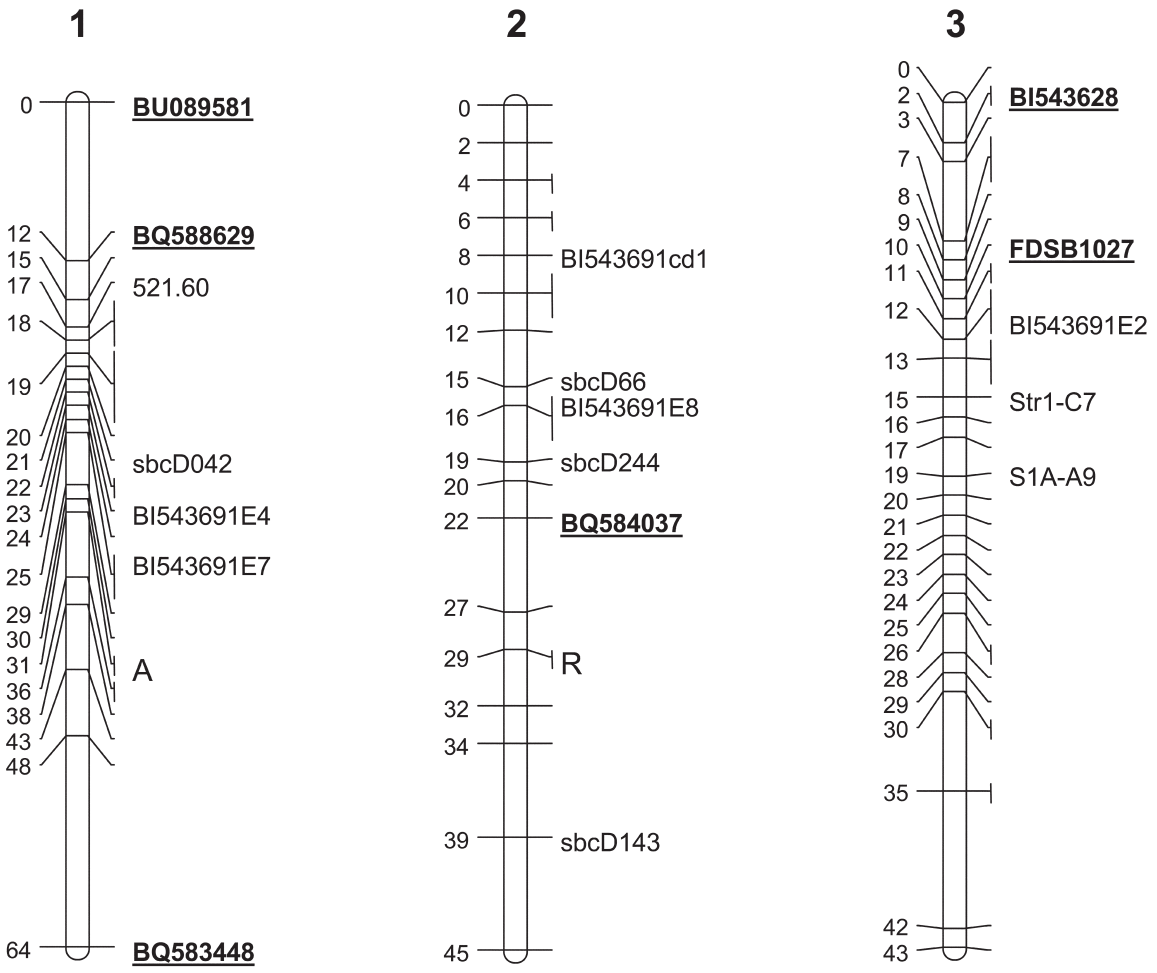

Fig. 1. Distribution of non-AFLP, nonproprietary markers on the genetic map. Bold, underlined labels indicate markers for which at least one BAC clone has been identified as a potential future physical map anchor point.

Morphological traits were scored and showed the expected results. The *R* locus, which governs production of betalain pigments typically used as a hypocotyl color marker for hybrid seed identification, was mapped to Butterfass Chromosome 2. The *M* locus, which conditions monogerm seed present in most modern hybrids and obviates the need for thinning stands, was located to Chromosome 4. Nuclear male sterility (locus *A*), often used in facilitating crosses, has been recently assigned to Butterfass Chromosome 1 (Friesen et al., 2006) and that assignment is confirmed here. The RFLP loci were scored using cDNA clones as probes; as most of these have been sequenced, their Genbank accession numbers are indicated in Supplementary Table 1. The EST–UTR genetic markers are described here for the first time, and could show unique utility for simultaneously mapping genes in families with conserved motifs, as demonstrated here for a calmodulin-containing motif where 14 separate loci with this motif were mapped to eight of the nine linkage groups (Fig. 1).

Of the 46 primer pairs targeted to amplify SSR loci, 9 had been previously reported but not mapped, 14 proprietary SSRs were applied for validating chromosome assignments, and 23 are newly reported here. An additional 35 newly described primer sequences have been shown to amplify genomic DNA, but were not polymorphic in this population, and 23 of these additional primers were used to map loci in one of two other populations (data not shown; primers and chromosome location are given in Supplementary Table 1). Excluding the proprietary SSRs, 37 SSR loci were disclosed in this population, with duplicate SSR loci all clustered on the same linkage group (e.g., Marker No. 124/125, 131/133/134, and 147/148; Table 1). Of the 32 newly mapped SSRs, 14 showed a presence–absence phenotype (e.g., dominant).

Overall, markers per chromosome ranged from 26 (Chromosome 2) to 47 (Chromosome 4). Average distance between markers ranged from 1.1 cM for Chromosome 3 to 2.0 cM on Chromosomes 1 and 7, with an average across all linkage groups of 1.61 cM between markers (SD = 0.31). Segregation

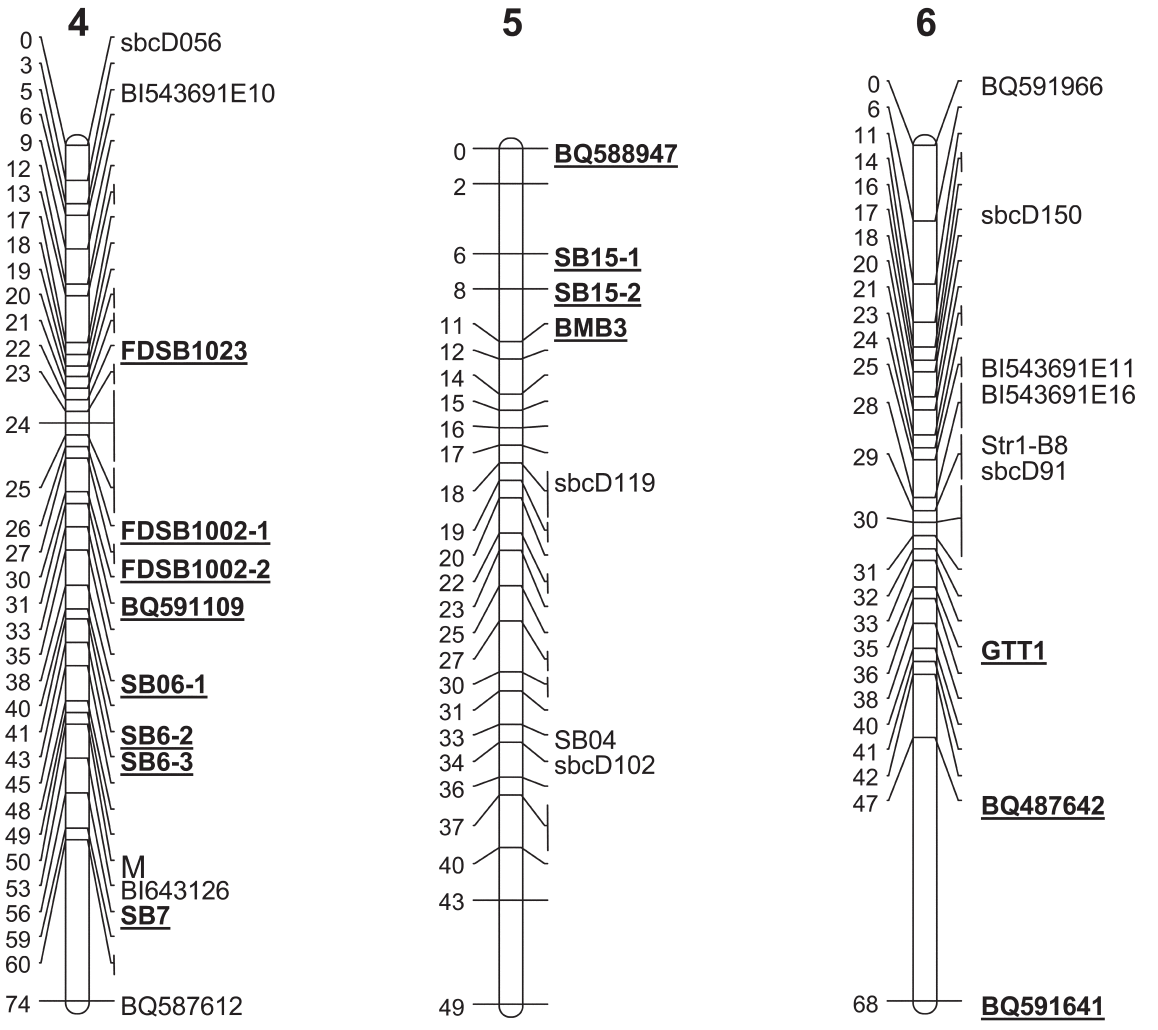

Fig. 1. Continued.

of individual markers was tested for consistency for expected Mendelian ratios using the Chi-square statistic, of which 72 of the 331 markers (21.8%) showed distorted segregation ratios. Markers were predominantly skewed in favor of the sugarbeet allele (53/72 = 74%), and 15 were skewed in favor of the table beet allele. Interestingly, three markers on Chromosome 9 and one of Chromosome 8 (Marker No. 291, 314, 321, 326; Table 1) showed an apparent heterozygote disadvantage, while one on Chromosome 7 (No. 245) showed an apparent heterozygote advantage.

Less than 10% of markers of Chromosomes 2, 3, 4, and 6 showed distorted segregation ratios. In contrast, the other chromosomes showed distorted segregations of nearly 20% or more of markers assigned to their respective chromosome [Chromosome 1 (25%); 5 (71.9)%; 7 (19.4%); 8 (29.5%); 9 (30.8%)]. The majority of skewed segregation on Chromosome 1 was toward the table beet allele (Marker No. 8, 13, 18, 19, 21, 22; Table 1) with two toward the sugarbeet parent (No. 2, 14). Interestingly, the *R* locus on Chromosome 2 (No. 54), which was scored here as a root trait and not hypocotyl color, showed a distorted ratio in favor of the recessive (*rr*), perhaps the result of unconscious selection due to our interest in sugarbeet improvement. The other Chromosome 2 marker (No. 58) with distortion was in favor of the table parent allele. All skewed segregation of markers on Chromosomes 3, 4, and 6 were in favor of the sugarbeet allele, with the exception of Marker No. 102. Similarly, all distortions on Chromosome 9 favored the sugarbeet allele (excepting the heterozygote disadvantages indicated above), as did all but two on Chromosome 8 (No. 249, 256, and also one heterozygote disadvantaged marker). Distortion on Chromosome 7 was evenly divided among sugarbeet and table beet alleles in excess (No. 223, 243, 248, and 215, 219, 246, respectively, not including No. 245 above). Interestingly, the sugarbeet gene-specific marker (No. 215) for a putative oxalate oxidase involved in enhanced germination (de los Reyes and McGrath, 2003) occurred less frequently than its

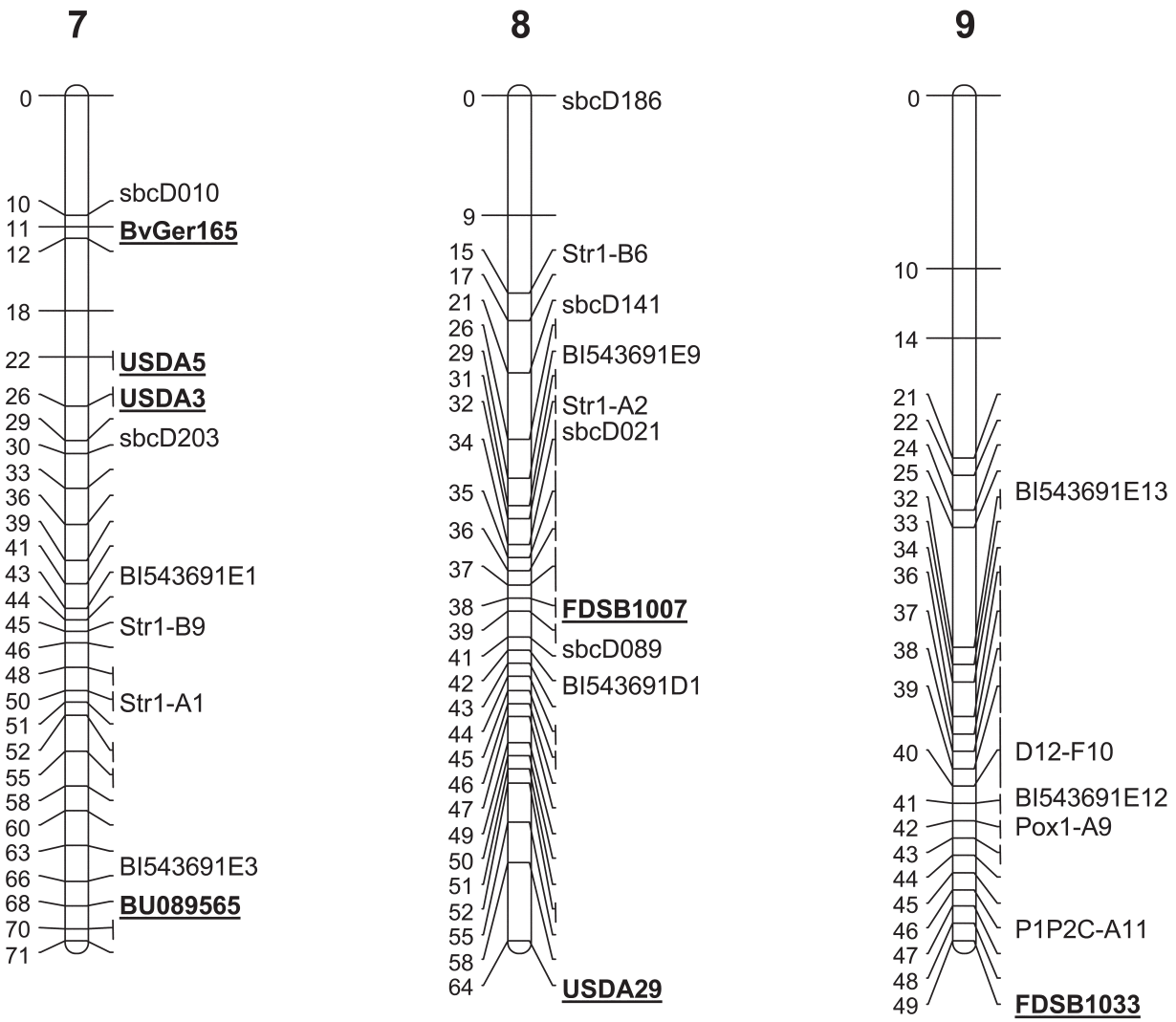

Fig. 1. Continued.

presumed table beet allele, suggesting an advantage of the table beet allele that could be exploited for germplasm improvement. Distortion was extreme for Chromosome 5, with 23 of 32 markers showing a distorted segregation ratio. All but three skewed markers were in excess from the sugarbeet allele (i.e., No. 150, 151, and 168; Table 1).

Genetic maps have utility for examining transmission of alleles through generations, and associating molecular markers with trait genes; however, all alleles will rarely be segregating in any one desired population of interest. The map presented here is a framework by which additional markers can be located on a common map. Not all alleles mapped in other populations will be segregating here, as was the case for additional published SSR primer sequences available for mapping in this population (Supplementary Table 1), and for this reason large-insert clones may help in discovering *cis*-linked polymorphisms. To evaluate whether such a strategy could be readily implemented, a BAC library was pooled, and the pools were used to identify BAC clones containing mapped markers from this map (Table 2). In all cases, at least one specific BAC clone was identified that carried a sequence similar to the mapped marker in as few as 44 PCR reactions. Such clones serve as genetic marker anchor points for physical mapping (Fig. 1).

Conversely, interesting candidate genes may become apparent for which nucleotide sequence is available but no marker has been developed. Discovering a series of *cis*-linked polymorphisms from large insert clones may allow mapping of the candidate gene in multiple populations. Resistance gene analogs (RGAs) are putatively involved in host plant disease resistance, and primers amplifying 47 sugarbeet RGAs were reported and many were mapped by Hunger et al. (2003). We recovered 31 of these from the BAC library. Two showed genetic polymorphism and were mapped in the sugarbeet × table beet population here (Marker

Table 2. Bacterial artificial chromosome clones containing mapped markers in the sugarbeet × table beet population, and RGA-containing BAC clones mapped by Hunger et al. (2003).

BAC clone ID	Mapped markers	Gene ID
SBA034F11	BI543628	unknown (*opaque-2*-like?)
SBA021K7	BMB3	anonymous SSR
SBA061E22	BQ487642	unknown (similar to cotton fiber protein E6)
SBA032G6	BQ583448	unknown (similar to ATPase in chromosome partitioning)
SBA004B12	BQ584037	phosphatidylglycerolphosphate synthase
SBA079G15	BQ588629	BSD domain-containing protein (Pfam PF03909)
SBA094K2	BQ588947	unknown
SBA015E22	BQ591109	unknown
SBA064F20	BQ591641	unknown
SBA083E16	BU089565	*4F03,* Transmembane-LRR-kinase
SBA029E13	BU089581	*AD-c-08c, Mi*-like
SBA009E14	BvGer165	germin-like protein (probable oxalate oxidase activity)
SBA025D20	FDSB1002	anonymous SSR
SBA069F10	FDSB1007	anonymous SSR
SBA078P16	FDSB1011	anonymous SSR
SBA079P16	FDSB1023	anonymous SSR
SBA068M15	FDSB1027	anonymous SSR
SBA025H18	FDSB1033	anonymous SSR
SBA073K18	GTT1	anonymous SSR
SBA036L23	SB06	anonymous SSR
SBA043C9	SB07	anonymous SSR
SBA013D19	SB15	anonymous SSR
SBA065N13	USDA29	BE590367: NHL-repeat containing protein (Pfam 01436.12)
SBA075B8	USDA3	BI544016: GDSL-motif lipase/ hydrolase family protein (Pfam PF00657)
SBA042I18	USDA5	BI543690: GDSL-motif lipase/ hydrolase family protein (Pfam PF00657)

BAC clone ID	Mapped markers	Gene ID
	RGA Genbank ID	Locus, Chromosome assignment, RGA-motif†
SBA054L3	BH897904	*E11,* Chr 7, NBS
SBA016E9	BU089548	*1D17,* Chr 5, *Pto*-like kinase
SBA004A19	BU089549	*1O14,* Chr 9, *Pto*-like kinase
SBA045I3	BU089550	*4L18,* Chr 1, *Pto*-like kinase
SBA034G20	BU089551	*5D23,* Chr 4, *Pto*-like kinase
SBA006G21	BU089552	*7A24,* Chr 3, *Pto*-like kinase
SBA005I8	BU089554	*9G24,* Chr 3, *Pto*-like kinase
SBA009F8	BU089555	*1F09,* Chr 9, Disease response protein
SBA024I8	BU089556	*4P09,* unassigned, *N*-like protein
SBA028A11	BU089558	*4M07,* unassigned, *Pto*-like kinase
SBA035E5	BU089559	*1L14,* Chr 7, *Pto*-like kinase
SBA059E21	BU089560	*9J14,* Chr 6, Transmembane-LRR-kinase
SBA035E5	BU089561	*7M20,* Chr 7, Transmembane-LRR-kinase
SBA019F3	BU089562	*7H14,* unassigned, Transmembane-LRR-kinase
SBA095E5	BU089563	*7B17,* Chr 8, Transmembane-LRR-kinase
SBA070O5	BU089564	*6L04,* Chr 7, Transmembane-LRR-kinase
SBA015B11	BU089566	*1D12,* Chr 7, Pto-like kinase
SBA007B17	BU089568	*8C05,* Chr 6, LRR
SBA012P16	BU089569	*5E07,* unassigned, LRR
SBA007K11	BU089570	*5C15,* unassigned, LRR
SBA019O4	BU089571	*2M02,* Chr 4, Transmembane-LRR
SBA015I3	BU089572	*8M01,* Chr 7, LRR
SBA070L17	BU089573	*8H04,* Chr 5, LRR
SBA035L7	BU089574	*2D06,* Chr 2, LRR
SBA029G18	BU089578	*AD-c-15c,* Chr 2, *Rp1*-like
SBA015B11	BU089579	*AD-c-01c,* Chr 7, *Mi*-like
SBA005O19	BU089580	*AD-c-11c,* unassigned, *Mi*-like
SBA008F23	BU089582	*AD-c-16c,* unassigned, *Mi*-like
SBA008F23	BU089583	*AD-c-12b,* Chr 6, *Mla*-like

† From Hunger et al., 2003.

No. 1 and 245; Table 1), where No. 1 was previously unassigned and the location of No. 245 was reported on Chromosome 5 but mapped to Chromosome 7 here, which is plausible because of common sequence motifs and predicted functions. These BAC clones are available for further characterization (Table 2). Further refinement of such a reciprocal genetic–physical mapping strategy will be required to quickly discover useful polymorphisms physically located on such BAC clones for which a nonpolymorphic PCR product has been used, either by sequencing outwards from the PCR primers or by implementing various other mutation scanning methods.

Discussion

The first demonstrated linkage in *B. vulgaris* was based on inheritance of the morphological markers for hypocotyl color (genes R and Y) and bolting behavior (B, annual vs. biennial), resulting in the widely known R–Y–B linkage association (Keller, 1936; Owen and Ryser, 1942), which is now known to reside on Chromosome 2 of the Butterfass chromosome series. The nomenclature adopted here is used as a standard. Various legacy marker types such as isozymes (Smed et al., 1989; Van Geyt et al., 1990; Wagner and Wricke, 1991; Wagner et al., 1992), RFLPs (Barzen et al., 1992; Boudry et al., 1994; Hallden et al., 1996, 1997; Heller et

al., 1996; Pillen et al., 1992, 1993), and RAPDs (Barzen et al., 1995; Laporte et al., 1998; Uphoff and Wricke 1992, 1995) have supported the R–Y–B association where examined. The AFLPs and SSRs have found wide use for molecular mapping in sugarbeet (Barnes et al., 1996; Rae et al., 2000; Schafer-Pregl et al., 1999; Schondelmaier et al., 1996; Schumacher et al., 1997); however, SSR primer sequences have remained trade secrets. To compare results of different sugarbeet genetic maps and define a common chromosome nomenclature, two sets of sugarbeet trisomic series were exploited; one was characterized by a heterogeneous genetic background (Butterfass, 1964) and the other set was established from inbred lines (Romagosa et al., 1986, 1987). Some of the Romagosa trisomic lines were lethal, and as proposed by Schondelmaier and Jung (1997), the sugarbeet standard chromosome nomenclature should be based on the Butterfass trisomic series.

In sugarbeet, clustering is generally observed with anonymous RFLP and RAPD markers. Information is limited on the clustering behavior of AFLP markers, and specific information generated with *Pst*I and *Mse*I restriction enzymes for sugarbeet genetic mapping is anecdotal. In this study, the average number of E/M-derived AFLP scorable bands (45.5 bands PC^{-1}) and the percentage polymorphism (22.9% = 10.4 polymorphic bands PC^{-1}) are in agreement with the results of Hansen et al. (1999) in an exhaustive evaluation of E/M-derived AFLP markers in the genus *Beta*. Those authors reported an average of 44.3 bands PC^{-1}, of which 27% were polymorphic (12 polymorphic bands PC^{-1}). Schondelmaier et al. (1996) found an average of 61 amplified bands PC^{-1}, of which 50% were polymorphic, only considering four selected E+3/M+3 PCs. Using 16 PCs of the *Hin*dIII/*Mse*I restriction enzyme pair, Schafer-Pregl et al. (1999) observed an average of 11 polymorphic bands PC^{-1}, where *Hin*dIII is similar to *Eco*RI in that it is also not senstive to 5-methyl-cytosine. In this study, similar numbers of amplified bands and polymorphisms were also obtained using the *Pst*I/*Mse*I pair using P+2/M+3 PCs. P+3/M+3 PCs were initially tested on the mapping population, but the lower number of amplified bands and polymorphism reduced the efficiency, and these combinations were discarded from further analysis. Overall, the sugarbeet × table beet cross appeared to show an average number of band amplification and polymorphism normally present between sugarbeet lines. Although we had not expected this, it may not be surprising considering table beet is a likely progenitor of sugarbeet (through fodder beet) and that heterozygosity has been maintained in the beet germplasm through out-crossing as open-pollinated populations. Sugarbeet and table beet likely diverged as recently as the 17th century (Biancardi et al., 2005; Draycott, 2006). The effect of selection in sugarbeet during the past 100 yr has not acted to reduce overall genetic diversity, but rather to partition diversity among breeding populations (McGrath et al., 1999). Plus in this case, our table beet parent is perhaps not as widely diverged as most since it carries the self-fertility and CMS (cytoplasmic male sterile) restorer genes introgressed from sugarbeet early after their discovery (Goldman, 1996).

Early sugarbeet genetic maps had large total map lengths with a relatively low number of markers, resulting in a very high intermarker distance average (Barzen et al., 1995; Pillen et al., 1992, 1993). The integration of AFLP and SSR markers increased marker density but did not significantly increase the length of the genetic maps (Rae et al., 2000; Schondelmaier et al., 1996; Schumacher et al., 1997). In this study, the uniformity and relatively high density of markers found on each chromosome and the expected number of linkage groups observed indicate a general confidence in this coverage of the *B. vulgaris* genome. The relatively high proportion of unlinked AFLP markers may suggest that a fraction of the genome is not represented by this genetic map, and that protein-encoding genes are vastly underrepresented, and current efforts are geared to improving the density of markers in gene-rich regions. Other good indicators of the general quality of this genetic map is that each chromosome-specific marker was correctly mapped to one of the nine chromosomes, including the three previously located morphological markers. Marker coverage of each chromosome was also relatively uniform (less than 2× variation), with the number of markers per chromosome roughly equivalent perhaps because of the similarity in size of *B. vulgaris* karyotyped chromosomes (Bosemark and Bormotov, 1972; de Jong et al., 1985; Nakamura et al., 1991).

Segregation distortion is common in sugarbeet. Wagner et al. (1992) and Pillen et al. (1992, 1993) found that approximately 15% of their markers showed distorted segregation ratios, which were attributed to lethal loci present on six linkage groups. Barzen et al. (1992, 1995) found that 19.3% of markers distributed on eight linkage groups showed segregation distortion, and attributed the causes for this high proportion to the presence of lethal loci, of structurally abnormal chromosomes, and to gametic self-incompatibility (SI), for which four loci have been described in sugarbeet (Larsen et al., 1977). These two maps were combined, extended, and correlated with the Butterfass chromosome nomenclature by Schumacher et al. (1997). Segregation distortion was not uniform between the maps; only Chromosome

3 showed no distortion, and our Chromosome 3 had only a single distorted marker (No. 96) distal on one end where, in general, distortions appear more frequently. For all other chromosomes, except in two cases, the proportion of distorted segregation ratios ranged from 0 to 21.1% in those mapping populations and generally occurred in clusters within a linkage map, but the clusters were not shared between maps, excepting perhaps one end of Chromosome 7 where all three maps show linkage of segregation distortions. Two exceptions showed entire linkage group segregation distortions; 77.3% of Chromosome 1 markers deviated from expectation in the Barzen-derived map and 96.2% of Chromosome 5 markers deviated in the Pillen-derived map (Schumacher et al., 1997). In our population, four chromosomes had distorted marker proportions > 25%, (i.e., Chromosomes 1, 5, 8, and 9).

Distribution of linkage distortion may be more instructive than an overall level of distorted segregation value. For instance, a modestly high frequency of marker segregation distortion (22%) was observed in this study, and overall, segregation distortion showed a basic trend to favor the sugarbeet (female) parent's alleles. Unfortunately, the phase is not reported in previous maps, so the direction of allelic selection is not yet comparable. Reciprocal crosses would be particularly instructive in the case that gross segregation distortions were a consequence of maternally inherited states (e.g., cytosine methylation, imprinting, nuclear-cytoplasmic incompatibility). Evidence suggests that Chromosome 5 is particularly vulnerable to distortion in sugarbeet × table beet crosses. Our Chromosome 5 showed a strong tendency for preferential transmission of the sugarbeet configuration (female), and another sugarbeet × table beet population showed the opposite with the male (table beet) Chromosome 5 configuration preferentially inherited (V. Laurent, 2006, unpublished data). The Pillen-derived sugarbeet map showing extreme distortion of Chromosome 5 was derived by selfing a single F_1 individual similar to these table beet maps (whereas the Barzen-derived mapping occurred in the F_1 between heterozygous parents), suggesting selfing may play an undefined role. Curiously, Abe et al. (1993) described inheritance of an as-yet-unmapped isozyme locus that showed distorted segregation in progeny from self-incompatible × self-compatible sugarbeet crosses, but not in progeny from self-incompatible crosses.

Segregation distortion in our population is unlikely due to segregation of lethal or sublethal alleles, but rather due to an undefined genetic discordance between sugarbeet and table beet. The cross here was homozygous for the dominant self fertility (SF, Owen 1942) allele, so SI per se is unlikely to explain the segregation distortion we observed in the F_2 generation. The table beet parent used here is homozygous at nearly 100% of its loci (data not shown) due to strong inbreeding during its development as a widely used CMS-maintainer line for table beet breeding, and the sugarbeet parent used here has reduced heterozygosity relative to most sugarbeets (McGrath et al., 1999). It should be noted that, due to SF, we have considered distortions as a favorable outcome of one parent or the other's allele, recognizing that it is equally probable for selection against a lethal allele, which may be more intuitive in many cases; however, such alleles likely have been purged from these parents. Positive selection for certain alleles in the homozygous state can be considered as a possibility, especially since four of five codominant loci showing distorted segregation appeared at a disadvantage for the heterozygote. Additional segregation analyses are needed for crosses involving each of the major crop types (chard, sugarbeet, fodder, table beet, wild beet) to test such hypotheses, and the availability of a common chromosome nomenclature and a set of common SSR markers will facilitate future comparisons.

The utility of this population, in conjunction with locating SSRs to BAC clones, will accelerate development of a physical map for beets, and will assist in developing SSR markers and other polymorphisms at physical locations in the genome. With these resources, a gene of interest needing confirmation via genetic co-segregation with a trait of interest, but lacking polymorphism in this population, can be mapped after recovering the gene-containing BAC clone, sequencing all or some of the clone until a putative SSR or SNP is identified, and testing that marker for segregation. End sequencing of BAC clones in this library is underway with the expectation that many of these sequences will carry useful SSR markers, and thus contribute to improving the coverage and resolution of this genetic mapping resource.

Acknowledgments

We thank Dr. Benildo de los Reyes, R. Scott Shaw, Pat Reeves, Cathy Derrico, Chad Moore, and Susan Myers for technical assistance. We thank the member companies of the Beet Sugar Development Foundation for partial funding for this research.

References

Abe, J., G.-P. Guan, and Y. Shimamoto. 1993. Linkage maps for nine isozyme and four marker loci in sugarbeet (*Beta vulgaris* L.). Euphytica 66:117–126.

Barnes, S., G. Massaro, M. Lafebvre, M. Kuiper, and E. Verstege. 1996. A combined RFLP and AFLP genetic map for sugar beet. p. 555–560. *In* Proc. 59th Congr. of the IIRB, Brussels. Int. Inst. for Beet Res., Brussels.

Barzen, E., W. Mechelke, E. Ritter, E. Schulte-Kappert, and F. Salamini. 1995. An extended map of the sugar beet genome containing

RFLP and RAPD loci. Theor. Appl. Genet. 90:189–193.
Barzen, E., W. Mechelke, E. Ritter, J.F. Seitzer, and F. Salamini. 1992. RFLP markers for sugar beet breeding: Chromosomal linkage maps and location of major genes for rhizomania resistance, monogermy and hypocotyl colour. Plant J. 2:601–611.
Biancardi, E., L.G. Campbell, G.N. Skaracis, and M. de Biaggi (ed.). 2005. Genetics and breeding of sugar beet. Science Publ., Enfield, NH.
Bosemark, N.O., and V.E. Bormotov. 1971. Chromosome morphology in a homozygous line of sugar beet. Hereditas 69:205–212.
Boudry, P., R. Wieber, P. Saumitou-Laprade, K. Pillen, H. van Dijk, and C. Jung. 1994. Identification of RFLP markers closely linked to the bolting gene B and their significance for the study of the annual habit in beets (*Beta vulgaris* L.). Theor. Appl. Genet. 88:852–858.
Brukner, I., B. Paquin, M. Belouchi, D. Labuda, and M. Krajinovic. 2005. Self-priming arrest by modified random oligonucleotides facilitates the quality control of whole genome amplification. Anal. Biochem. 339:345–347.
Butterfass, T. 1964. Die chloroplastenzahlen in verschiedenartigen zellen trisomer zuckerruben (*Beta vulgaris* L.). Z. Bot. 52:46–77.
Cureton, A.N., M.N. Barnes, B.V. Ford-Lloyd, and H.J. Newbury. 2002. Development of simple sequence repeat (SSR) markers for the assessment of gene flow between sea beet (*Beta vulgaris* ssp. *maritima*) populations. Mol. Ecol. Notes 2:402–403.
de Jong, J.H., G.J. Speckmann, T.S.M. De Bock, and A. Van Voorst. 1985. Monosomic additions with resistance to beet cyst nematode obtained from hybrids of *Beta vulgaris* and wild *Beta* species of the selction Patellares. II. Comparative analysis of the alien chromosomes. Z. Pflanzenzuecht. 95:84–94.
De los Reyes, B.G., and J.M. McGrath. 2003. Cultivar-specific seedling vigor and expression of a putative oxalate oxidase germin-like protein in sugar beet (*Beta vulgaris* L.). Theor. Appl. Genet. 107:54–61.
De los Reyes, B.G., S.J. Myers, and J.M. McGrath. 2003. Differential stress-induction of glyoxylate cycle enzymes as a marker for seedling vigor in sugar beet (*Beta vulgaris*). Mol. Genet. Genom. 269:692–698.
Dean, F.B., J.R. Nelson, T.L. Giesler, and R.S. Lasken. 2001. Rapid amplification of plasmid and phage DNA using Phi29 DNA polymerase and multiply-primed rolling circle amplification. Genome Res. 11:1095–1099.
Dominguez, I., E. Graziano, C. Gebhardt, A. Barakat, S. Berry, P. Arus, M. Delseny, and S. Barnes. 2003. Plant genome archaeology: Evidence for conserved ancestral chromosome segments in dicotyledonous plant species. Plant Biotechnol. J. 1:91–99.
Draycott, A.P. (ed.). 2006. Sugar beet. Blackwell Publ., Oxford, UK.
Friesen, T.L., J.J. Weiland, M.L. Aasheim, S. Hunger, D.C. Borchardt, and R.T. Lewellen. 2006. Identification of a SCAR marker associated with *Bm*, the beet mosaic virus resistance gene, on chromosome 1 of sugar beet. Plant Breed. 125:167–172.
Goldman, I.L. 1996. A list of germplasm releases from the University of Wisconsin Table Beet Breeding Program, 1964–1992. HortScience 31:880–881.
Halldén, C., A. Hjerdin, I.M. Rading, T. Sall, B. Fridlundh, G. Johannisdottir, S. Tuvesson, C. Akesson, and N.O. Nilsson. 1996. A high density RFLP linkage map of sugar beet. Genome 39:634–645.
Halldén, C., T. Sall, K. Olsson, N.O. Nilsson, and A. Hjerdin. 1997. The use of bulk segregant analysis to accumulate RAPD markers near a locus for beet cyst nematode resistance in *Beta vulgaris*. Plant Breed. 116:18–22.
Hansen, H., T. Kraft, M. Christiansson, and N.O. Nilsson. 1999. Evaluation of AFLP in Beta. Theor. Appl. Genet. 98:845–852.
Heller, R., J. Schondelmaier, G. Steinrucken, and C. Jung. 1996. Genetic localization of four genes for nematode (*Heterodera schachtii* Schm.) resistance in sugar beet (*Beta vulgaris* L.). Theor. Appl. Genet. 92:991–997.
Hunger, S., G. Di Gaspero, S. Möhring, D. Bellin, R. Schäfer-Pregl, D.C. Borchardt, C.E. Durel, M. Werber, B. Weisshaar, F. Salamini, and K. Schneider. 2003. Isolation and linkage analysis of expressed disease-resistance gene analogues of sugar beet (*Beta vulgaris* L.). Genome 46:70–82.
Keller, W. 1936. Inheritance of some major colour types in beets. J. Agric. Res. (Washington, DC) 52:27–38.
Laporte, V., D. Merdinoglu, P. Saumintou-Laprade, G. Butterlin, P. Vernet, and J. Cuguen. 1998. Identification and mapping of RAPD and RFLP markers linked to a fertility restorer gene for a new source of cytoplasmatic male sterility in *Beta vulgaris* ssp. *maritima*. Theor. Appl. Genet. 96:989–996.
Larsen, K. 1977. Self-incompatibility in *B. vulgaris* L: I. Four gametophytic, complementary S-loci in sugar beet. Hereditas 85:227–248.
Lewellen, R.T. 2004. Registration of rhizomania resistant, monogerm populations C869 and C869CMS sugarbeet. Crop Sci. 44:357–358.
Mörchen, M., J. Cuguen, G. Michaelis, C. Hänni, and P. Saumitou-Laprade. 1996. Abundance and length polymorphism of microsatellite repeats in *Beta vulgaris* L. Theor. Appl. Genet. 92:326–333.
McGrath, J.M., C.A. Derrico, and Y. Yu. 1999. Genetic diversity in selected, historical USDA sugarbeet germplasm releases and *Beta vulgaris* ssp. *maritima*. Theor. Appl. Genet. 98:968–976.
McGrath, J.M., M.M. Jancso, and E. Pichersky. 1993. Duplicate sequences with similarity to expressed genes in the genome of *Arabidopsis thaliana*. Theor. Appl. Genet. 86:880–888.
McGrath, J.M., R.S. Shaw, B.G. de los Reyes, and J.J. Weiland. 2004. Construction of a sugar beet BAC library from a hybrid that combines diverse traits. Plant Mol. Biol. Rep. 22:23–28.
Möhring, S., F. Salamini, and K. Schneider. 2004. Multiplexed, linkage group-specific SNP marker sets for rapid genetic mapping and fingerprinting of sugar beet (*Beta vulgaris* L.). Mol. Breed. 14:475–488.
Myburg, A.A., D.L. Remington, D.M. O'Malley, R.R. Sederoff, and R.W. Whetten. 2001. High-throughput AFLP analysis using infrared dye-labeled primers and an automated DNA sequencer. Biotechniques 30:348–357.
Nakamura, C., G.N. Skaracis, and I. Romagosa. 1991. Cytogenetics and breeding in sugar beet. p. 295–313. *In* T.Tsuchiya and P.K. Gupta (ed.) Chromosome engineering in plants: Genetics, breeding, evolution. Elsevier, Amsterdam.
Nilsson, N.O., C. Hallden, M. Hansen, A. Hjerdin, and T. Sall. 1997. Comparing the distribution of RAPD and RFLP markers in a high density linkage map of sugar beet. Genome 40:644–651.
Owen, F.V. 1942. Inheritance of cross- and self-sterility and self-fertility in *Beta vulgaris*. J. Agric. Res. (Washington, DC) 64:679–698.
Owen, F.V., and G.K. Ryser. 1942. Some Mendelian characters in *Beta vulgaris* L. and linkages observed in the Y-R-B group. J. Agric. Res. (Washington, DC) 65:153–171.
Pillen, K., G. Steinrucken, R.G. Herrmann, and C. Jung. 1993. An extended linkage map of sugar beet (*Beta vulgaris* L.) including nine putative lethal genes and the restorer gene *X*. Plant Breed. 111:265–272.
Pillen, K., G. Steinrucken, G. Wricke, R.G. Herrmann, and C. Jung. 1992. A linkage map of sugar beet (*Beta vulgaris* L.). Theor. Appl. Genet. 84:129–135.
Pillen, K., J. Schondelmaier, C. Jung, and R.G. Herrmann. 1996. Genetic mapping of genes for twelve nuclear-encoded polypeptides associated with the thylakoid membranes in *Beta vulgaris* L. FEBS Lett. 395:58–62.
Rae, S.J., C. Aldam, I. Dominguez, M. Hoebrechts, S.R. Barnes, and K.J. Edwards. 2000. Development and incorporation of microsatellite markers into the linkage map of sugar beet (*Beta vul-*

garis spp.). Theor. Appl. Genet. 100:1240–1248.
Richards, C.M., M. Brownson, S.E. Mitchell, S. Kresovich, and L. Panella. 2004. Polymorphic microsatellite markers for inferring diversity in wild and domesticated sugar beet (*Beta vulgaris*). Mol. Ecol. Notes 4:243–245.
Robinson, A.J., C.G. Love, J. Batley, G. Barker, and D. Edwards. 2004. Simple sequence repeat marker loci discovery using SSR Primer. Bioinformatics 9:1475–1476.
Romagosa, I., L. Cistue, T. Tsuchiya, J.M. Lasa, and R.J. Hecker. 1987. Primary trisomics in sugar beet. II. Cytological identification. Crop Sci. 27:435–439.
Romagosa, I., R.J. Haecker, T. Tsuchiya, and J.M. Lasa. 1986. Primary trisomics in sugar beet. I. Isolation and morphological characterization. Crop Sci. 26:243–249.
Schafer-Pregl, R., D.C. Borchardt, E. Barzen, C. Glass, W. Mechelke, J.F. Seitzer, and F. Salamini. 1999. Localization of QTLs for resistance to *Cercospora beticola* on sugar beet linkage groups. Theor. Appl. Genet. 99:829–836.
Schneider, K., B. Weisshaar, D.C. Borchardt, and F. Salamini. 2001. SNP frequency and allelic haplotype structure of *Beta vulgaris* expressed genes. Mol. Breed. 8:63–74.
Schondelmaier, J., G. Steinrucken, and C. Jung. 1996. Integration of AFLP markers into a linkage map of sugar beet (*Beta vulgaris* L.). Plant Breed. 115:231–237.
Schondelmaier, J., and C. Jung. 1997. Chromosomal assignment of the nine linkage groups of sugar beet (*Beta vulgaris* L.). Theor. Appl. Genet. 95:590–596.
Schumacher, K., J. Schondelmaier, E. Barzen, G. Steinrucken, D.C. Borchardt, F. Salamini, C. Jung, and W.E. Weber. 1997. Combining different linkage maps in sugar beet (*Beta vulgaris* L.) to make one map. Plant Breed. 116:23–38.
Smed, E., J.P.C. Van Geyt, and M. Oleo. 1989. Genetical control and linkage relationships of isozyme markers in sugar beet (*B. vulgaris* L.). Theor. Appl. Genet. 78:97–104.
Stormo, K.E., Q.Z. Tao, and R. Bogden. 2004. Pool and superpool matrix coding and decoding designs and methods. United States Patent Application No. 20040224346, filed 5 May 2004.
Uphoff, H., and G. Wricke. 1992. Random amplified polymorphic DNA (RAPD) markers in sugar beet (*Beta vulgaris* L.): Mapping the genes for nematode resistance and hypocotyl colour. Plant Breed. 109:168–171.
Uphoff, H., and G. Wricke. 1995. A genetic map of sugar beet (*Beta vulgaris* L.) based on RAPD markers. Plant Breed. 114:355–357.
Van Geyt, J.P.C., E. Smed, and M. Oleo. 1990. Genetical control and linkage relationships of isozyme markers in sugar beet (*Beta vulgaris* L.). Theor. Appl. Genet. 80:593–601.
Van Ooijen, J.W., and R.E. Voorrips. 2001. JoinMap 3.0, Software for the calculation of genetic linkage maps. Plant Research Int., Wageningen, the Netherlands.
Viard, F., J. Bernard, and B. Desplanque. 2002. Crop–weed interactions in the *Beta vulgaris* complex at a local scale: Allelic diversity and gene flow with sugar beet fields. Theor. Appl. Genet. 104:688–697.
Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407–4414.
Wagner, H., W.E. Weber, and G. Wricke. 1992. Estimating linkage relationship of isozyme markers and morphological markers in sugar beet (*Beta vulgaris* L.) including families with distorted segregations. Plant Breed. 108:89–96.
Wagner, H., and G. Wricke. 1991. Genetical control of five isozyme systems of sugar beet (*Beta vulgaris* L.). Plant Breed. 107:124–130.