Theor Appl Genet (1999) 98: 968–976

J. M. McGrath · C. A. Derrico · Y. Yu

Genetic diversity in selected, historical US sugarbeet germplasm and *Beta vulgaris* ssp. *maritima*

Received: 20 October 1998 / Accepted: 28 October 1998

Abstract Diversity among sugar beet accessions released over the first 50 years of public breeding in the United States was examined to ascertain a baseline of genetic diversity and to gauge the effect of breeding on the loss or gain of diversity over this time period. Accessions were chosen as released germplasm from the major breeding stations contributing to the US germplasm pool and their presumed ancestors from Europe, including representatives for the wild forms *Beta vulgaris* ssp. *maritima*. Sixty nine polymorphic RAPD fragments were used for gene frequency analysis, and heterozygosity was determined within and among groups of accessions related either by breeding station or simply inherited agronomic characters for monogerm seed and restoration of fertility in a cytoplasmic male-sterile background. In general, heterozygosity within releases declined with time, but total genetic diversity in the US germplasm pool remained constant. Breeding for the agronomic characters had a marked influence in reducing diversity.

Key words RAPDs · Disease resistance · Monogerm · Self fertility

Communicated by H. F. Linskens

J. M. McGrath (✉) · C. A. Derrico
USDA-ARS Sugarbeet and Bean Research Unit,
494D Plant and Soil Science Bldg, Michigan State University,
East Lansing, MI 48824-1325, USA
Fax: + 1-517-337-6782
E-mail: mitchmcg@pilot.msu.edu

Y. Yu
Department of Crop and Soil Sciences, 494D Plant and Soil Sciences Bldg, Michigan State University, East Lansing,
MI 48824-1325, USA

Introduction

Sugarbeet (*Beta vulgaris* L.) has been specifically selected and bred for sucrose production over the past two centuries (de Bock 1986; Winner 1993). During the first 100 years of selection, sucrose levels in *B. vulgaris* dramatically increased from relatively low levels of sucrose (e.g. from less than 6% to more than 12%), beginning from open-pollinated selections of fodder beets grown in a small region of Europe (Fischer 1989). The bulk of the United States germplasm is derived from this European material, probably from a limited number of introductions selected for curly top virus resistance and *Cercospora* leaf spot resistance beginning in the late 1920s (Coons 1936; Lewellen 1992). Breeding for additional and higher levels of disease resistances, e.g. *Cercospora* leaf spot, *Rhizoctonia* root rot, rhizomania, *Aphanomyces* seedling disease and a host of viral and storage rot organisms, continues to be important (Bosemark 1993) as does understanding the genetic basis of these and other characters (Smith 1980).

The relatively recent inception of sugarbeet compared with other major crops, as well as its migration from Europe to America, have caused concern that the extent of genetic diversity within the United States sugarbeet germplasm pool, and the sugarbeet germplasm as a whole, may be narrow (Coons 1936, Bosemark 1979, Panella 1996). For much of its cropping history sugarbeet varieties have been maintained as open-pollinated populations with large numbers of effective parents. Breeding has relied to a great extent on mass selection, with a presumption that allelic diversity has been maintained in these populations. Only within the last 25 years have hybrids assumed importance (Hecker and Helmerick 1985). The introduction of hybrid breeding and the near ubiquitous deployment of three Mendelian recessive characters, two involved in the restoration of male fertility (Owen 1945) and one for monogerm seed (Savitsky 1952), has likely

narrowed the germplasm base of sugarbeet further from initial (unknown) levels.

Genetic diversity in sugarbeets, related species and subspecies has been examined at a number of levels. For instance, Shen et al. (1996) examined 26 accessions of wild beets closely related to cultivated *Beta vulgaris* and, using random amplified polymorphic DNAs (RAPDs), were able to finely discriminate relationships among the taxonomically difficult subspecies within the section *Beta*. Mita et al. (1991) examined accessions within sections *Beta* and *Patellares* with restriction fragment length polymorphisms (RFLPs) and concurred with taxonomic treatments (Ford-Lloyd and Williams 1975, Letschert et al. 1993) that the two sections are widely divergent. These and additional sections have been examined at the level of nuclear ribosomal gene structure (Santoni and Bervillé 1992) and repetitive DNA sequences (Jung et al. 1993, Schmidt and Heslop-Harrison 1994; Kubis et al. 1997), each supportive of the current taxonomic view of the genus *Beta*. Recently, Kraft et al. (1997) examined a wide range of working sugarbeet breeding lines and wild beet (*Beta vulgaris* ssp. *maritima*) accessions with RFLPs and a pooled sampling strategy and showed that wild beets had the greatest number of alleles per locus, followed by multigerm and then monogerm sugarbeet accessions. In large part these studies examined diversity from one or a few plants from each accession or from bulked samples from a larger number of individuals. While useful in detecting accession-specific differences, the effect of changing allele frequencies during breeding appears to be an overlooked component of genetic diversity within sugarbeet.

We report on a number of varieties and germplasm releases which have likely played a significant role in the development of the United States germplasm base from its beginnings from European germplasm in the early 1920s (reviewed in Lewellen 1992). These varieties span the history of germplasm releases of the USDA (comprehensively listed in Doney 1995) to about 1970. A few representatives of *B. vulgaris* ssp. *maritima* were also included to better assess the relative differentiation of sugarbeet with its putative wild ancestors. While not designed to be comprehensive in scope, these results provide an assessment of population structure in a selected, diverse set of sugarbeet germplasm used in United States varietal development as well as comparative information on wild germplasm populations which may be important sources of genetic variability for the future (Dale et al. 1985, Van Geyt et al. 1990).

Materials and methods

Plant material

Plant material from 15 USDA sugarbeet parent and germplasm releases (Doney 1995), four sugarbeet lines deposited in the USDA National Germplasm System by the (then) Great Western Sugar, Co (Longmont, Colo.), 4 accessions classified as *Beta vulgaris* ssp. *maritima* (L.) and one breeding line generously supplied by Dr. Robert Lewellen (Salinas, Calif.). Salient features of these accessions are given in Table 1. All accessions except EL48, SP6822 and C6869 areavailable through the National Plant Germplasm System (http://www.ars-grin.gov/npgs/). The exceptions may be obtained from the first author. It should be mentioned that SP6822, obtained in this case from a generous donation by American Crystal Seed (Moorhead, Minn.), is a seed increase of SP6322 listed by NPGS.

DNA extraction and polymerase chain reaction (PCR)

DNA was isolated and PCR reactions done as per methods described in McGrath et al. (1994). Primers were obtained from Operon Technologies (Alameda, Calif.). DNA was amplified with a Thermolyne Amplitron II thermocycler. Digital images of each gel obtained with the same primer were assembled into a single file using Photoshop (Adobe) and scored.

Data Analysis

Seven primers were used in this study with a total of 69 markers scored as either present or absent (Table 2). Gene frequencies were tabulated and input into genetic distance and phylogenetic analysis package DISPAN (Ota 1993, based on Nei 1987).

Results

Twenty-three accessions of *Beta vulgaris* (Table 1) were examined with seven decamer primers. These seven primers were chosen to amplify the fewest and best resolved number of fragments from 31 tested, as reported in Eagen and Goldman (1996). From the seven primers used, 69 polymorphic amplified fragments (RAPDs) were scored. Many other fragments were evident, however they were not considered informative since they were either weak in their expression, monomorphic among all accessions or not directly comparable between accessions due to closely migrating fragments.

The accessions tested were chosen as a representative sample of the sugarbeet germplasm base which has contributed significantly to sugarbeet varieties and hybrids developed in the United States from about 1920 through the 1970s. These accessions are publicly available, and their derivatives still contribute significantly to current varieties grown today, particularly with regard to disease resistance. Additionally, 5 accessions were examined that may have utility for future USDA-ARS breeding efforts at East Lansing; C6869 and 4 wild accessions. Generally, 12 plants of each accession were analyzed individually, though some accessions had fewer plants (indicated in Table 2).

For the amplified fragments scored, gene frequencies were determined from the presence or absence of a particular amplified fragment across all accessions (Table 2). Fragments of identical size were assumed to

Table 1 Key to the accessions used in this study, their primary selection pressure, approximate date of release and special breeding considerations

D	Cultivar	Accession no.	Trait selected	Date	Unique features[a]
1	R&G Old Type	NSSL 142033	German progenitor	Pre-1920	
2	Klein E	PI590588	German progenitor	Pre-1920	
3	US33	PI590580	Curly top	1936	
4	US22/3	PI590708	Curly top	1948	
5	SLC101	NSSL29900	Monogerm progenitor	1948	mm, SF
6	US75	PI590586	Curly top	1952	
7	NB1 (S20)	PI590676	Non-bolting	1954	SF, O-type
8	SLC 133 (7401)	Ames2663	Curly top	1960	mm, O-type
9	SLC 129 cms	PI590862	Curly top	1968	mm, cms
10	GW85-46R	NSSL28036	Cercospora	ca. 1940	
11	GW59-47A	NSSL28033	Cercospora	ca. 1940	
12	GW359-56A	NSSL28059	Cercospora	ca. 1950	
13	GW304-50A	NSSL28044	Cercospora	ca. 1950	
14	US401	W617102	Aphanomyces	1960	
15	EL48	E. Lansing, Mich.	Multiple disease	1984	mm, O-type
16	SP6822	ACH	Aphanomyces	ca. 1958	
17	FC701	PI590661	Rhizoctonia	1968	
18	C01	PI590679	Virus yellows	1977	
19	C6869	Salins, Calif.	Rhizomania	NR	mm, SF
20	Wild beet WB879	PI540625	*B. vulgaris* spp. *maritima*	Wild-France	
21	Wild beet WB185	PI546409	*B. vulgaris* spp. *maritima*	Wild-UK	
22	Wild beet 002/79	PI546523	*B. vulgaris* spp. *maritima*	Wild-Greece	Presumed SF
23	Wild beet	PI504196	*B. vulgaris* spp. *maritima*	Wild-Italy	

[a] mm, Monogerm seed, SF, accessions with dominant self-fertility allele, O-type, accessions doubly recessive at *X* and *Z* loci required for maintenance of cytoplasmic male sterling breeding lines; cms, cytoplasmic male sterility, NR = not released

be the same allele at a locus, although it was unlikely that this assumption was satisfied in all cases since their genetic basis has not yet been determined. All alleles scored in this data set showed polymorphism, and most were present in 2 or more of the 23 accessions. However, in three cases (two in the sugarbeet accession GW59; AE09-2100 and AF05-1800 and one in EL48; AE09-500; Table 2) accession-specific loci were observed, excluding the wild accessions. Additional fragments were amplified in the wild accessions but not observed among sugarbeet accessions (data not shown). Although well resolved, these wild accession-specific loci were not included in the diversity estimates since their inclusion did not substantially affect conclusions regarding sugarbeet *per se*. Thus, diversity between wild accessions and sugarbeet was underestimated. Similarly, exclusion of monomorphic amplified fragments from the sugarbeet accessions, by design, likely overestimated their diversity scores.

Average heterozygosity (i.e. gene diversity) within accessions is given in Table 3. Heterozygosity was lowest in those accessions with a known or presumed self-fertility allele (Table 2 and accession PI 523 where seed set was observed on isolated plants) with the exception of C6869. C6869 also segregated for a Mendelian male-sterility gene, and this mechanism forced some degree of out-crossing. Heterozygosity was highest in those accessions deposited by Great Western Sugar, Co (prefix GW) with the exception of GW59, which originated in large part from a single plant selection (Brewbaker et al. 1950). Heterozygosity tended to be low in accessions homozygous for the recessive allele for monogerm seed (genetic symbol *mm*, Table 1), with the exception of SLC133. Most of these monogerms also carry alleles at the *X* and *Z* loci, which when homozygous recessive, prevent pollen fertility in sterile cytoplasms. Reduced heterozygosity as the result of inbreeding for these essential modern agronomic characters was expected.

Accessions with related characteristics were used to determine a group heterozygosity over all loci (Table 4). Characteristics considered were (1) approximate date of introduction or release as reported in Doney (1995) and Lewellen (1992), (2) selection pressure applied to accessions which corresponds to geographic location of the breeding stations where these groups were released (see below), (3) monogerm versus multigerm seed character (note many monogerms are also O-type, the shorthand designation for the normal cytoplasm, male-fertile maintainer line counterpart in cytoplasmic male steriles) and (4) self-fertile (SF) lines with a dominant SF allele occasionally used to enforce inbreeding. Breeding station releases included a set of curly top virus-resistant accessions selected predominantly in the Western and Inter-mountain areas of the US (Group 1, Table 4), the *Cercospora beticola* caused leaf spot-resistant materials predominantly from the Great Western Sugar Co (Group 2), the Eastern US

Table 2 List of primers used, the amplified fragments scored, the frequency of fragments amplified and the number of plants per accession scored

	NB1 $n = 12$	SP6822 12	US401 12	SLC101 12	US22/3 12	US33 12	GW304 12	GW359 12	GW85 12	US75 12	CO1 12	SLC133 12	FC701 12	SLC129 12	C6869 10	R&G 12	KleinE 12	GW59 12	EL48 6	P1409 10	P1625 10	P1523 10	P1196 10
Primer-marker																							
AC15-1800	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.917	0.917	1.000	1.000	0.917	1.000	1.000	0.300	0.900	1.000	1.000
AC15-1400	1.000	1.000	1.000	1.000	1.000	1.000	0.833	1.000	1.000	1.000	0.917	0.750	0.667	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.900
AC15-750	–[a]	–	0.333	–	–	0.083	–	0.182	0.182	–	0.083	–	0.250	–	–	0.083	–	0.250	–	0.400	–	–	–
AC15-700	0.583	0.500	0.833	–	0.333	0.667	0.250	0.455	0.455	0.182	0.167	0.667	–	0.417	1.000	0.750	0.583	0.667	0.333	0.100	–	–	0.400
AC15-675	–	0.300	0.167	–	0.083	–	0.083	0.182	–	–	0.500	–	–	–	–	–	–	–	–	–	0.300	–	–
AC15-600	–	0.500	0.583	–	–	0.167	–	–	0.091	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AD04-1800	1.000	0.700	1.000	1.000	0.917	1.000	1.000	1.000	1.000	1.000	1.000	0.917	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.800	0.800	–	0.800
AD04-1550	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.800	0.500	1.000	1.000
AD04-1500	1.000	1.000	1.000	1.000	1.000	0.833	0.417	0.500	0.500	1.000	0.833	1.000	0.500	1.000	0.800	1.000	0.583	0.667	0.833	0.700	1.000	0.100	1.000
AD04-1100	1.000	1.000	1.000	1.000	1.000	1.000	0.750	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.917	1.000	1.000	0.700	0.400	1.000	1.000
AD04-1050	0.500	–	–	0.917	0.833	0.500	0.750	0.583	0.500	1.000	0.583	0.417	1.000	1.000	1.000	1.500	0.667	0.833	1.000	1.000	0.300	1.000	0.900
AD04-950	0.167	0.500	–	–	0.167	0.417	0.167	0.333	0.417	–	0.417	0.417	–	–	–	0.583	–	0.167	–	–	–	–	–
AD04-900	0.833	1.000	0.333	–	1.000	1.000	1.000	0.833	1.000	1.000	0.750	0.583	1.000	0.909	1.000	1.000	0.750	1.000	1.000	0.400	0.900	–	0.400
AD04-850	–	–	–	–	0.583	0.083	0.250	–	–	0.167	0.083	–	0.083	–	–	–	0.167	–	–	0.900	0.800	–	0.400
AD04-725	–	0.500	0.417	–	0.250	0.250	0.250	0.545	0.333	0.833	0.417	0.417	–	–	0.700	0.636	0.250	0.250	0.167	0.100	0.400	0.900	0.300
AD04-700	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.636	0.750	0.833	0.833	0.667	1.000	1.000	1.000	1.000	0.750	0.833	1.000	–	–	0.100	–
AE09-2100	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.000	–	–	–	–	–
AE09-2000	1.000	1.000	1.000	1.000	0.917	0.917	0.917	1.000	0.833	0.909	1.000	1.000	1.000	1.000	1.000	1.000	1.000	–	1.000	–	0.400	–	–
AE09-1800	1.000	0.900	1.000	1.000	0.583	0.833	0.583	0.818	0.417	0.636	0.750	0.750	0.583	1.000	0.500	0.667	0.833	–	1.000	–	–	–	0.778
AE09-1750	–	1.000	0.583	–	0.833	0.333	–	0.545	0.750	0.818	0.750	0.667	–	–	0.700	0.833	0.583	–	0.667	–	–	1.000	1.000
AE09-1300	–	–	–	1.000	0.250	0.667	0.500	0.455	0.333	0.636	0.417	–	–	0.917	–	0.667	0.417	–	0.667	–	–	–	0.556
AE09-1200	1.000	0.900	0.667	1.000	1.000	0.917	0.833	0.500	0.833	0.909	0.833	0.500	1.000	0.833	0.700	0.500	0.750	1.000	0.833	1.000	1.000	0.700	0.600
AE09-1100	1.000	0.400	–	–	–	–	0.583	0.917	0.667	1.000	1.000	1.000	1.000	1.000	0.800	1.000	0.833	–	0.833	–	–	–	0.444
AE09-1000	0.917	0.800	0.833	–	1.000	1.000	0.833	0.917	0.750	1.000	0.667	0.500	–	0.917	1.000	0.917	0.583	–	–	0.300	0.100	–	–
AE09-950	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.800	–	–	–	–	0.500	0.500	1.000	1.000
AE09-900	–	0.600	0.583	–	–	–	–	–	–	–	–	–	–	–	0.700	–	–	–	–	–	–	–	–
AE09-800	1.000	1.000	0.833	–	0.917	0.917	0.750	1.000	0.500	1.000	1.000	0.750	1.000	1.000	1.000	1.000	1.000	0.917	–	0.200	0.400	1.000	1.000
AE09-700	–	–	–	–	–	–	0.083	0.167	–	–	0.250	0.167	0.083	–	–	0.333	0.250	–	–	–	–	1.000	–
AE09-600	1.000	–	–	1.000	0.750	0.500	0.583	0.500	–	–	0.667	0.833	0.833	–	0.600	0.417	0.500	–	0.500	0.800	0.900	0.600	0.333
AE09-500	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.833	–	–	–	–
AF05-3000	1.000	1.000	1.000	0.917	1.000	0.917	1.000	1.000	0.917	0.909	1.000	0.917	1.000	1.000	1.000	0.818	0.667	1.000	1.000	0.714	0.667	0.750	1.000
AF05-2900	1.000	0.700	0.545	–	0.833	0.833	0.500	0.636	0.833	0.545	0.750	0.917	0.636	1.000	0.667	0.818	0.417	0.750	1.000	1.000	1.000	1.000	0.556
AF05-2500	1.000	1.000	1.000	–	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.750	–	1.000	–	–	0.100	0.778
AF05-2000	1.000	1.000	0.909	0.333	0.667	0.583	1.000	0.727	0.583	0.636	0.167	0.583	0.636	0.900	1.000	0.833	0.750	0.417	1.000	0.571	0.889	0.778	0.500
AF05-1800	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.500	–	–	–	–	–
AF05-1700	0.900	1.000	1.000	0.917	0.417	0.250	0.750	0.909	0.667	0.727	0.667	1.000	0.667	1.000	1.000	0.833	0.667	0.750	0.833	–	0.333	–	–
AF05-1600	–	–	–	–	0.250	0.167	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.800	–
AF05-1300	0.909	1.000	1.000	–	0.333	0.917	0.583	0.818	0.917	0.818	0.667	0.583	0.400	0.300	0.889	0.917	0.333	1.000	0.600	–	–	–	–
AF05-1200	–	0.100	–	–	1.000	–	0.333	0.273	0.417	0.182	–	0.500	–	0.500	0.333	0.182	0.083	1.000	1.000	–	–	–	–
AF05-900	0.600	1.000	1.000	0.917	0.545	1.000	0.917	0.636	0.583	0.818	0.750	0.917	0.750	1.000	1.000	0.833	0.583	0.750	1.000	0.500	0.900	–	0.700
AF05-800	1.000	0.800	1.000	0.917	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.833	0.417	1.000	1.000	0.900	–	0.800
AB09-1800	–	1.000	1.000	1.000	0.917	1.000	0.714	0.417	0.583	1.000	1.000	1.000	0.250	1.000	1.000	1.000	0.833	0.833	1.000	–	0.300	0.100	–
AB09-1600	1.000	0.600	0.667	0.417	1.000	1.000	1.000	0.750	0.917	1.000	0.667	0.167	0.083	1.000	1.000	0.636	1.000	1.000	0.833	0.700	0.800	–	0.900
AB09-1200	0.917	0.500	0.583	1.000	0.583	0.500	0.429	0.417	0.083	0.500	0.500	0.417	0.417	1.000	1.000	1.000	0.500	0.500	0.500	1.000	0.900	0.900	–
AB09-900	1.000	1.000	1.000	1.000	0.917	0.917	1.000	0.750	0.917	1.000	0.667	1.000	1.000	1.000	0.500	1.000	1.000	1.000	0.833	0.800	0.900	1.000	0.600
AB11-2200	1.000	1.000	0.917	1.000	0.909	0.727	–	0.500	0.750	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	1.000	1.000	0.100	1.000

AB11-2000	–	–	0.083	0.917	0.545	–	0.833	0.250	0.250	–	0.667	0.417	0.250	0.583	0.600	0.364	0.750	0.750	0.333	–	–	0.900	0.700
AB11-1800	1.000	1.000	1.000	1.000	0.818	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.917	0.750	1.000	0.909	0.750	1.000	–	0.500	0.500	0.100	0.700
AB11-1550	1.000	1.000	0.667	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.400	1.000	1.000	1.000
AB11-1500	–	0.400	0.167	–	0.545	0.667	0.167	0.500	0.167	0.667	0.833	0.333	0.417	–	0.200	0.909	0.583	0.583	–	–	–	–	–
AB11-1300	1.000	0.200	0.417	–	0.364	0.417	0.667	0.583	0.500	0.250	0.583	0.250	0.250	–	0.800	0.273	0.250	0.167	0.333	–	–	–	–
AB11-1200	1.000	0.900	0.500	0.667	0.455	0.500	0.417	0.167	0.750	0.917	0.750	0.333	0.667	1.000	0.700	0.636	0.667	0.417	0.833	0.571	0.800	0.100	0.800
AB11-1000	–	0.800	0.583	–	0.182	0.833	0.750	0.500	0.250	0.167	0.583	0.500	0.083	–	0.400	0.455	0.250	0.583	–	–	0.600	0.100	0.100
AB11-950	1.000	0.900	0.833	1.000	1.000	–	0.667	0.917	0.833	1.000	0.667	0.917	0.917	1.000	0.700	0.909	1.000	1.000	1.000	–	0.600	0.900	0.700
AB11-550	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.917	1.000	1.000	1.000	0.909	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AG15-2050	1.000	1.000	1.000	1.000	0.500	1.000	1.000	0.917	1.000	0.833	0.833	1.000	1.000	0.917	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.900	1.000
AG15-1900	–	–	–	1.000	–	0.583	–	0.083	–	–	–	0.167	0.750	–	–	–	–	–	–	–	–	1.000	–
AG15-1825	–	0.700	–	–	0.250	0.500	1.000	0.500	0.833	0.750	–	0.333	0.417	0.727	1.000	0.818	0.833	0.917	0.250	0.100	0.300	0.100	–
AG15-1800	1.000	1.000	1.000	1.000	1.000	1.000	0.750	0.750	0.750	1.000	1.000	1.000	0.833	0.917	1.000	0.818	0.667	1.000	1.000	1.000	1.000	0.700	1.000
AG15-1650	–	0.800	0.667	1.000	0.583	0.583	0.833	0.583	0.833	0.917	0.750	0.667	0.667	0.545	1.000	0.727	0.750	0.750	1.000	–	–	1.000	1.000
AG15-1600	–	0.600	0.333	0.833	0.667	0.750	0.417	0.500	0.917	0.667	1.000	0.250	0.917	0.400	0.100	0.273	–	0.500	–	–	–	–	–
AG15-1400	1.000	0.900	0.917	1.000	0.833	0.667	0.750	0.667	0.833	1.000	0.667	0.917	1.000	0.083	0.900	0.909	0.833	0.667	1.000	0.200	0.400	–	–
AG15-1300	–	1.000	0.333	–	–	0.583	0.333	0.333	0.667	0.833	0.583	0.167	0.083	0.667	0.700	0.091	0.250	–	1.000	0.900	0.800	–	–
AG15-1225	1.000	0.600	0.917	1.000	1.000	0.417	0.833	0.583	0.500	0.917	1.000	0.833	1.000	0.500	0.500	0.455	0.500	0.417	1.000	0.500	0.700	1.000	0.100
AG15-1200	1.000	0.600	0.917	1.000	1.000	0.417	0.833	0.583	0.500	0.917	1.000	0.833	1.000	0.500	0.500	0.545	0.500	0.417	1.000	0.200	0.100	1.000	1.000
AG15-950	–	0.400	0.583	–	1.000	0.917	0.750	0.583	0.583	1.000	0.750	0.250	1.000	–	0.500	0.818	1.000	1.000	–	0.900	1.000	0.900	0.100
AG15-800	1.000	1.000	1.000	1.000	0.750	0.917	1.000	0.750	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.917	1.000	1.000	–	–	–	0.300
AG15-725	–	–	–	–	0.500	0.083	–	0.583	–	0.167	0.583	–	0.083	0.167	–	0.182	0.167	0.250	0.400	0.100	–	–	0.600
AG15-700	0.917	0.700	0.833	1.000	1.000	0.750	0.500	0.417	1.000	0.500	–	0.833	0.333	0.500	1.000	0.545	0.250	0.167	0.800	1.000	0.700	–	0.900

[a] A dash (–) indicates that an allele was not detected in a particular population

Table 3 Average heterozygosity (i.e. gene diversity) and standard errors within accessions ranked from lowest observed heterozygosity to highest

Accession	Gene diversity	SE	Gene identity[a]	Group[b]
SLC101	0.040	0.014	0.960	SF
NB1	0.045	0.015	0.955	SF
PI 523	0.084	0.017	0.916	SF
SLC129cms	0.117	0.024	0.883	1
EL48	0.136	0.026	0.864	3
US75	0.149	0.023	0.851	1
C6869	0.160	0.027	0.840	SF
FC701	0.164	0.025	0.836	4
PI 409	0.168	0.026	0.832	5
PI 196	0.173	0.027	0.827	5
GW59	0.177	0.027	0.823	2
US401	0.179	0.027	0.821	3
SP6822	0.179	0.028	0.821	3
PI 625	0.188	0.027	0.812	5
US22/3	0.205	0.027	0.795	1
R&G Old Type	0.210	0.026	0.790	4
US33	0.218	0.027	0.782	1
SLC133	0.230	0.027	0.770	1
CO1	0.232	0.027	0.768	4
GW304	0.235	0.026	0.765	2
GW85	0.248	0.027	0.752	2
Klein E	0.263	0.026	0.737	4
GW359	0.303	0.028	0.697	2

[a] Gene identity equals 1-gene diversity

[b] Groups were based on location of release (see text, used in Table 4) or presence of the self-fertility allele (SF)

group selected primarily for resistance to *Aphanomyces cochlioides*-caused seedling disease (Group 3), a miscellaneous set of germplasm which included the presumed ancestral varieties 'Klein E' and 'R&G Old Type' and 2 accessions selected for either virus yellows or *Rhizoctonia solani* root rot resistance (Group 4), and the 4 *Beta vulgaris* ssp. *maritima* accessions (Group 5). Results indicate that a continuum of variability is found within and among these accessions (Table 4).

Regarding release date, it was apparent that average heterozygosity between populations (Dst) increased while heterozygosity within populations (Hs) decreased over time (Table 4). While the total heterozygosity (Ht) remained similar, greater differentiation of accessions occurred over the time period assessed in these lines (e.g. Gst = Dst/Ht increased). With regard to location where an accession originated, the greatest differentiation between accessions (Gst, Table 4) was seen among the wild species, followed by the Eastern (Group 3), Western and Inter-mountain (Group 1) set the miscellaneous set (Group 4) and the Great Western set (Group 2). Again, the total heterozygosity was roughly equivalent between each of these groups as was the component of heterozygosity partitioned between accessions within a group (Dst), with the exception of the wild species. Heterozygosity within populations (Hs), however, showed an inverse relationship with Gst, indicating that selection and

Table 4 Summary of genetic diversity scores for groups pooled by date of release, location of release, seed characteristic or presence of the self-fertility allele

Group	Accessions[a]	Ht[b]	Hs[c]	Dst[d]	Gst[e]
All accessions	All	0.311	0.162	0.149	0.479
By release date:					
1940s	1, 2, 3	0.258	0.211	0.047	0.180
1950s	4, 6, 10, 11, 12, 13	0.277	0.201	0.075	0.272
1960s	8, 9, 14, 15, 16, 17, 18	0.257	0.160	0.097	0.379
By regional groups:					
Group 2 – Great Western	10, 11, 12, 13	0.286	0.221	0.066	0.229
Group 4 – Misc.	1, 2, 17, 18	0.260	0.199	0.060	0.233
Group 1 – Western	3, 4, 6, 8, 9	0.248	0.168	0.080	0.321
Group 3 – Eastern	14, 15, 16	0.223	0.146	0.077	0.345
Group 5 – Wild beets	20, 21, 22, 23	0.272	0.138	0.134	0.493
By seed type:					
Monogerm	8, 9, 15	0.214	0.144	0.070	0.327
Multigerm sugarbeet	All but mm and wild	0.280	0.195	0.086	0.306
Multigerm	All but mm	0.324	0.181	0.143	0.441
By self-fertility:					
SF – sugarbeet	5, 7, 19	0.202	0.074	0.128	0.635
SF – all	5, 7, 19, 22	0.281	0.074	0.207	0.735

[a] Accession ID numbers are listed in Table 1
[b] Ht, Total heterozygosity (i.e. genetic diversity) with a group of accessions
[c] Hs, Heterozygosity within populations
[d] Dst = Ht–Hs = Nei's standard genetic distance = heterozygosity among populations
[e] Gst = Dst/Ht = differentiation of accessions with a group

inbreeding were practiced to different extents with these programs as represented by these accessions. It should be noted that diversity in the wild species may have been artificially altered in this report by virtue of scoring only those amplified fragments also found in sugarbeet.

Similar trends were seen with both monogerm accessions and accessions with the self-fertility allele, that is both have been subject to fairly strong selection or inbreeding. With the exception of heterozygosity within populations (Hs), monogerm and multigerm accessions of sugarbeet were remarkably similar in their degree of differentiation (Gst) and heterozygosity between populations (Dst). These results agree with conclusions drawn by Kraft et al. (1997). However, within populations (Hs) there appears to have been a greater amount of inbreeding or selection among the monogerm seed types. The degree of inbreeding is highest in the self-fertile accessions, which showed the least heterozygosity within populations (Hs) and the greatest differentiation among themselves compared with all other accessions and groups, as perhaps may have been expected.

Standard genetic distances (Table 5) were used to compute a dendrogram of similarity using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Fig. 1). Results were concordant with some expectations based on accession passport information. That is, wild accessions were well resolved from sugarbeet accessions and the progenitors 'R&G Old Type' and 'Klein E' (identical varieties with different names, Lewellen 1992) grouped together. Highly selected accessions as well as inbreds tended to occupy distal places on the dendrogram, while the core, highly heterozygous (i.e. diverse) accessions showed a central tendency around 'R&G Old Type'/'Klein E'. The exact relationships within this core, based on the available pedigree data, did not appear to parallel their breeding location history but rather may be considered to have been derived from a common ancestor without large selection pressures changing allele frequencies at the loci examined.

Discussion

Gene frequency analysis suggests that genetic differentiation of US germplasm occurred during the first 50 years of sugarbeet breeding in the United States. Interestingly, the total diversity has not declined in this collection but has been partitioned between accessions, as might be expected from their narrow germplasm base origin. The trend towards homozygosity within germplasm releases over time has, in part, been the result of strong selection for disease resistance within a narrow germplasm pool and to the use of recessive characters required for crop and hybrid seed production world-wide. Generally, mass selection for disease resistance appears to have reduced diversity to a lesser extent than breeding for the recessive agronomic characters.

Table 5 Matrix of pairwise Nei's standard genetic distances (above diagonal) and their standard errors (below diagonal)

	NB1	SP6822	US401	SLC101	US22/3	US33	GW304	GW359	GW85	US75	CO1	SLC133	FC701	SLC129	C6869	R&G	KleinE	GW59	EL48	P1 409	P1 625	P1 523	P1 196
NB1	–	0.160	0.121	0.242	0.147	0.170	0.137	0.096	0.136	0.161	0.156	0.096	0.130	0.133	0.140	0.132	0.131	0.296	0.166	0.321	0.262	0.516	0.271
SP6822	0.04	–	0.039	0.291	0.144	0.082	0.123	0.080	0.054	0.070	0.104	0.077	0.179	0.130	0.085	0.066	0.104	0.216	0.161	0.421	0.275	0.530	0.298
US401	0.03	0.02	–	0.214	0.126	0.093	0.125	0.086	0.095	0.113	0.123	0.071	0.174	0.157	0.110	0.098	0.113	0.224	0.173	0.367	0.281	0.482	0.270
SLC101	0.06	0.06	0.05	–	0.209	0.242	0.210	0.242	0.243	0.252	0.214	0.168	0.162	0.204	0.293	0.261	0.194	0.300	0.197	0.346	0.312	0.441	0.273
US22/3	0.04	0.03	0.03	0.05	–	0.103	0.098	0.085	0.093	0.088	0.094	0.093	0.133	0.144	0.137	0.099	0.086	0.175	0.144	0.293	0.236	0.381	0.215
US33	0.04	0.02	0.02	0.05	0.03	–	0.081	0.071	0.068	0.093	0.096	0.117	0.152	0.141	0.123	0.074	0.099	0.190	0.208	0.293	0.233	0.516	0.273
GW304	0.04	0.04	0.03	0.05	0.03	0.02	–	0.038	0.059	0.090	0.103	0.094	0.105	0.102	0.093	0.086	0.051	0.161	0.139	0.338	0.245	0.399	0.264
GW359	0.02	0.02	0.02	0.05	0.02	0.03	0.01	–	0.034	0.056	0.043	0.042	0.093	0.095	0.085	0.030	0.034	0.175	0.139	0.327	0.253	0.370	0.196
GW85	0.04	0.01	0.02	0.05	0.02	0.02	0.02	0.01	–	0.047	0.092	0.071	0.116	0.101	0.086	0.065	0.066	0.149	0.117	0.304	0.249	0.447	0.212
US75	0.04	0.02	0.03	0.06	0.03	0.03	0.03	0.02	0.01	–	0.060	0.097	0.117	0.094	0.090	0.045	0.054	0.194	0.131	0.370	0.283	0.472	0.252
CO1	0.04	0.03	0.03	0.05	0.03	0.03	0.04	0.01	0.03	0.02	–	0.072	0.093	0.137	0.147	0.070	0.073	0.216	0.167	0.385	0.297	0.419	0.236
SLC133	0.03	0.02	0.03	0.04	0.03	0.03	0.03	0.01	0.02	0.03	0.02	–	0.090	0.114	0.093	0.046	0.066	0.196	0.107	0.342	0.251	0.393	0.208
FC701	0.04	0.04	0.04	0.05	0.04	0.04	0.03	0.02	0.03	0.04	0.02	0.02	–	0.163	0.193	0.136	0.090	0.202	0.178	0.305	0.251	0.370	0.255
SLC129	0.04	0.04	0.04	0.05	0.04	0.04	0.03	0.02	0.03	0.03	0.03	0.03	0.04	–	0.116	0.095	0.079	0.216	0.106	0.334	0.273	0.505	0.226
C6869	0.04	0.03	0.03	0.06	0.03	0.03	0.03	0.02	0.03	0.02	0.04	0.02	0.04	0.03	–	0.068	0.084	0.212	0.153	0.371	0.292	0.488	0.261
R&G	0.04	0.02	0.03	0.06	0.03	0.03	0.03	0.01	0.02	0.01	0.02	0.02	0.03	0.03	0.02	–	0.031	0.178	0.159	0.404	0.294	0.457	0.268
KleinE	0.03	0.02	0.03	0.04	0.03	0.03	0.02	0.01	0.02	0.02	0.03	0.02	0.03	0.02	0.02	0.01	–	0.137	0.135	0.288	0.208	0.329	0.175
GW59	0.06	0.05	0.05	0.07	0.05	0.05	0.04	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.04	–	0.271	0.329	0.259	0.388	0.293
EL48	0.05	0.04	0.04	0.05	0.04	0.05	0.04	0.04	0.03	0.04	0.05	0.03	0.05	0.03	0.04	0.04	0.04	0.06	–	0.363	0.297	0.475	0.245
Pl409	0.07	0.08	0.07	0.07	0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.06	0.06	0.07	0.07	0.07	0.05	0.06	0.07	–	0.048	0.289	0.207
Pl625	0.06	0.06	0.06	0.07	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.05	0.05	0.06	0.06	0.06	0.04	0.05	0.06	0.02	–	0.298	0.220
Pl523	0.09	0.09	0.09	0.09	0.07	0.09	0.08	0.07	0.08	0.09	0.07	0.07	0.07	0.09	0.09	0.08	0.06	0.07	0.09	0.06	0.06	–	0.233
Pl 196	0.06	0.06	0.06	0.06	0.05	0.06	0.06	0.04	0.05	0.06	0.05	0.05	0.06	0.06	0.06	0.06	0.04	0.06	0.06	0.05	0.05	0.05	–

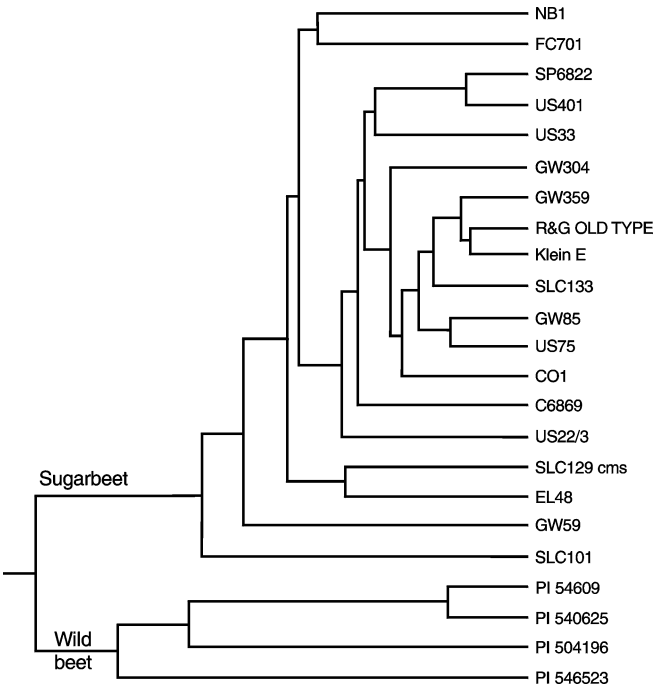

Fig. 1 UPGMA dendrogram based on Nei's standard genetic distances

Pedigree information is available for some of these accessions (Lewellen 1992) but often obscured by time, particularly regarding direct ancestral relationships between germplasm releases. In a few cases, such as sequential release after selection of 'R&G Old Type', US22/3, US33 and US75, a direct ancestry can be traced. This sequence is not reflected in the dendrogram (Fig. 1). In others, such as US401 and SP6822, direct ancestry is inferred from historical data and supported by the dendrogram, but an unbroken pedigree is currently unavailable. Similarly, SLC129 and EL48 could share a common ancestor. Although the result was surprising initially, it is known that SLC129 was used in the East Lansing breeding program during the 1960s (Hogaboam and Schneider 1982). Thus, accessions from at least two sources have been used at East Lansing. It is also known that GW59 and GW85 preceded release of GW304 and GW359, and this is not reflected in our data. It may be significant that GW359, the accession with the highest heterozygosity, included wide array of germplasm in its lineage, including *Cercospora* leaf spot resistance from *B. vulgaris* ssp. *maritima* (Lewellen 1992). None of our *B. vulgaris* ssp. *maritima* alleles we scored were shared with GW359 (data not shown), but this may not be surprising since leaf spot resistance was drawn from accessions geographically and genetically distinct from the ones we examined here. Finally, two of the wild beet accessions showed a close relationship with each other, concordant with their (presumed) similar geographic collection points either side of the English Channel.

There are a number of limitations to the approach taken here. First, the genetic basis of the amplified fragments scored is unknown. Amplification of DNA with RAPD technology can produce spurious results (e.g. Nilsson et al. 1997 and references therein). Second, maximizing the number of individuals per accession tested needed to be balanced against the number of markers which could be assayed. Nei (1987) argued that the balance should favor a greater number of loci over the number of individuals per accession where resources are limiting. Third, the exclusive scoring of polymorphic markers in the sugarbeet data set overestimated diversity in absolute terms. For discerning relationships among these more or less closely related accessions, amplified fragments which were monomorphic would not have provided additional information. Similarly, scoring bands in the wild species which were also present in sugarbeet (assuming allelism) to the exclusion of all others underestimated the relative diversity of the wild accessions relative to sugarbeet. However, the greatest differentiation was observed between wild and cultivated types.

Our goal was not to reconstruct a phylogeny of the US sugarbeet breeding history but rather to assess to what extent breeding has reduced genetic variability and increased genetic vulnerability. In this respect, this data has value as a baseline of genetic diversity from which to assess recent and future loss. Without sufficient diversity, selection will be ineffective. From our data, sufficient diversity may be present to maintain breeding progress, providing that parental material for hybrids is sampled from diverse accessions and not resampled from progressively more elite breeding lines. Indeed, the parents of the highly successful three-way Michigan hybrid, USH20, come from a cross between derivatives of SLC129 and SLC130 (Western, curly top resistance gene pool) with SP6822 (Eastern, Aphanomyces resistance gene pool) (Coe and Hogaboam 1971). This study also suggests that sugarbeet may profit from additional sampling of wild beets for desirable characters. Utilization of wild beet diversity will have a cost by temporarily reducing the agronomic potential of elite breeding germplasm, but application of molecular markers in recovering elite stocks could efficiently facilitate the introgression of beneficial characters while simultaneously removing detrimental material, particularly if the location of genes contributing to the agronomic phenotype of sugarbeet were adequately known.

Acknowledgments We thank Drs. Robert Lewellen, Lee Panella and Joseph Saunders for their helpful suggestions and advice.

References

Bosemark NO (1979) Genetic poverty of the sugarbeet in Europe. In: Zeven AC, Van Harten AM (eds), Proc Conf Broadening Genet Base Crops. PuDoc, Wageningen, the Netherlands, pp 29–35

Bosemark NO (1993) Genetics and breeding. In: Cooke DA, Scott RK (eds) The sugarbeet crop: science into practice. Chapman and Hall, London, pp 67–119

Brewbaker HE, Bush HL, Wood RR (1950) A quarter century of progress in sugarbeet improvement by the Great Western Sugar Co. Proc Am Soc Sugar Beet Technol 6 : 202–207

Coe GE, Hogaboam GJ (1971) Registration of US H20 Sugarbeet. Crop Sci 11 : 942

Coons GH (1936) Improvement of the sugarbeet. In: USDA Yearbook of Agriculture. US Gov Printing Office, Washington, D.C., pp 625–656

Dale MFB, Ford-Lloyd BV, Arnold MH (1985) Variation in some agronomically important characters in a germplasm collection of beet (*Beta vulgaris* L.). Euphytica 34 : 449–455

de Bock ThSM (1986) The genus *Beta*: domestication, taxonomy and interspecific hybridization for plant breeding. Acta Hortic 182 : 335–343

Doney DL (1993) Broadening the genetic base of sugarbeet. J Sugar Beet Res 30 : 209–219

Doney DL (1995) USDA-ARS sugarbeet releases. J Sugar Beet Res 32 : 229–257

Eagen KA, Goldman IL (1996) Assessment of RAPD marker frequencies over cycles of recurrent selection for pigment concentration and percent solids in red beet (*Beta vulgaris* L.) Mol Breed 2 : 107–115

Fischer HE (1989) Origin of the 'Weisse Schlesische Rübe' (white Silesian beet) and resynthesis of sugarbeet. Euphytica 41 : 75–80

Ford-Lloyd BV, Williams JT (1975) A revision of *Beta* section *Vulgares* (Chenopodiaceae), with new light on the origin of cultivated beets. Bot J Linn Soc 71 : 89–102

Hecker RJ, Helmerick RH (1985) Sugarbeet breeding in the United States. In: Russell GE (ed), Progress in plant breeding – 1, Butterworths, London, pp 37–61

Hogaboam GJ, Schneider CL (1982) Registration of EL44 and EL44CMS sugarbeet parental lines. Crop Sci 22 : 700

Jung C, Pillen K, Frese L, Fähr S, Melchinger AE (1993) Phylogenetic relationships between cultivated and wild species of the genus *Beta* revealed by DNA "fingerprinting". Theor Appl Genet 86 : 449–457

Kraft T, Fridlund B, Hjerdin A, Säll T, Tuvesson S, Halldén C (1997) Estimating genetic variation in sugarbeets and wild beets using pools of individuals. Genome 40 : 527–533

Kubis S, Heslop-Harrison JS, Schmidt T (1997) A family of differentially amplified repetitive DNA sequences in the genus *Beta* reveals genetic variation in *Beta vulgaris* subspecies and cultivars. J Mol Evol 44 : 310–320

Letschert JPW, Lange W, Frese L, Van Den Berg RG (1993) Taxonomy of *Beta* section *Beta*. J Sugar Beet Res 31 : 69–85

Lewellen RT (1992) Use of plant introductions to improve populations and hybrids of sugarbeet. In: Shands HL, Wiesner LE (eds) Use of plant introductions in cultivar development, part 2. Crop Sci Soc Am, Madison, WI, pp 117–136

McGrath JM, Wielgus SM, Uchytil TF, Kim-Lee H, Haberlach GT, Williams CE, Helgeson JP (1994) Recombination of *Solanum brevidens* chromosomes in the second backcross generation from a somatic hybrid with *S. tuberosum*. Theor Appl Genet 88 : 917–924

Mita G, Dani M, Casciari P, Pasquali A, Selva E, Minganti C, Piccardi P (1991) Assessment of the degree of genetic variation in beet based on RFLP analysis and the taxonomy of *Beta*. Euphytica 55 : 1–6

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Nilsson N-O, Halldén C, Hansen M, Hjerdin A, Säll T (1997) Comparing the distribution of RAPD and RFLP markers in a high density linkage map of sugarbeet. Genome 40 : 644–651

Ota T (1993) DISPAN: Genetic distance and phylogenetic analysis. Institute of Molecular Evolution and Genetics, Pennsylvania State University University Park, Pa.

Owen FV (1945) Cytoplasmically inherited male-sterility in sugarbeets. J Agric Res 71 : 423–440

Panella L (ed) (1996) Report on the status of *Beta* germplasm in the United States. Sugarbeet Crop Germplasm Committee, USDA-ARS, Crops Research Laboratory, Fort Collins, Colorado

Santoni S, Bervillé A (1992) Characterization of the nuclear ribosomal DNA units and phylogeny of *Beta* L. wild forms and cultivated beets. Theor Appl Genet 83 : 533–542

Savitsky VF (1952) A genetic study of monogerm and multigerm characters in beets. Proc Am Soc Sugar Beet Technol 7 : 331–338

Schmidt T, Heslop-Harrison JS (1993) Variability and evolution of highly repeated DNA sequences in the genus *Beta*. Genome 36 : 1074–1079

Shen Y, Newbury HJ, Ford-Lloyd BV (1996) The taxonomic characterization of annual *Beta* germplasm in a genetic resources collections using RAPD markers. Euphytica 91 : 205–212

Smith GA (1980) Sugarbeet. In: Fehr WR, Hadley HH (eds), Hybridization of crop plants, Am Soc Agron, Madison, Wis., pp 601–616

Van Geyt JPC, Lange W, Oleo M, De Bock ThSM (1990) Natural variation within the genus *Beta* and its possible use for breeding sugarbeet: a review. Euphytica 49 : 57–76

Winner C (1993) History of the crop. In: Cooke DA, Scott RK (eds) The sugarbeet crop: Science into practice. Chapman and Hall, London, pp 1–35