



Title	Nuclear and mitochondrial DNA polymorphisms suggest introgression contributed to garden beet (<i>Beta vulgaris</i> L.) domestication
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2 **Nuclear and mitochondrial DNA polymorphisms**
3 **suggest introgression contributed to garden beet (*Beta***
4 ***vulgaris* L.) domestication**

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10 **Keywords:** Domestication, garden beet, introgression, mitotype, population structure

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11 **Abstract**

12 Garden beet is the ancestor of fodder beets and sugar beets, but the origin of garden beet's genetic potential
13 to evolve novel beet types is debatable. In this study, we analyzed nuclear and mitochondrial DNAs in 47
14 garden beet accessions using DNA markers. Multiple analytical methods revealed a unified population
15 structure with subpopulations evident in the European and Caucasian accessions. We diagnosed
16 mitochondrial genome types (mitotypes) based on mitochondrial minisatellite loci in 541 plants from the
17 47 accessions, revealing a major mitotype and 11 minor mitotypes in garden beets from Europe and the
18 Caucasus region that were also present in endemic leaf beets and wild beets. Our data indicate that European
19 and Caucasian garden beets include genetically differentiated subpopulations. Provided that the occurrence
20 of minor mitotypes is a vestige from crosses with leaf beets and wild beets, the notion that introgression
21 contributed to increasing the genetic diversity in the garden beet gene pool is substantiated at the molecular
22 level.

23

24 **Introduction**

25 Garden beet, or table beet, is a root crop that constitutes one of the cultivar groups belonging to the
26 cultivated beet complex (*Beta vulgaris* L. ssp. *vulgaris*) that has three other intercressable groups: leaf beets,
27 fodder beets, and sugar beets (Lange et al. 1999). The cultivated beet complex is morphologically diverse,
28 e.g., swollen roots and hypocotyls are seen in garden beet, fodder beet, and sugar beet groups but not in the
29 leaf beet group (Biancardi 2020).

30 The ancestor of cultivated beet is its wild relative sea beet (*B. vulgaris* L. ssp. *maritima*), from
31 which leaf beet was first domesticated in a region spanning the Middle East to the Mediterranean coast
32 (Biancardi and Lewellen 2020; Goldman and Navazio 2003). Garden beet was selected from leaf beet for
33 its swollen root (Goldman and Navazio 2003). Roman writers described uses of beet root without clearly
34 mentioning that the root was swollen (Ford-Lloyd and Williams 1975). Zossimovic (1940) proposed that
35 the origin of swollen-root type beets is a region that includes Iraq, Iran, and Turkey (Galewski and McGrath
36 2020). A taxonomic group of presumptive common progenitors of swollen- and nonswollen-root-type beets
37 was found in Turkey (Ford-Lloyd and Williams 1975). Swollen-root type beets were described in the 12th
38 century, in Arabic (de Bock 1986). Therefore, the notion that garden beet originated in the Middle East and
39 spread north-westward is a possible scenario (Galewski and McGrath 2020) but may be an

40 oversimplification. Crop domestication generally invokes a reduction in genetic diversity (Hancock 2003);
41 however, garden beet landraces exhibit a range of morphological variations in traits such as root shape and
42 root color (Baranski et al. 2001). The expansion of phenotypic diversity in the garden beet group is inferred
43 to have occurred during its dissemination within Europe when crosses with leaf beet could have occurred
44 (Goldman and Navazio 2008). This phenotypic expansion was associated with the appearance of fodder
45 beet, whose establishment was no later than the 18th century (Goldman and Navazio 2003). According to
46 historical records, sugar beet evolved from fodder beet by artificial selection in the 19th century (Goldman
47 and Navazio 2003). Therefore, the cross between garden beet and leaf beet groups in Europe was a critical
48 factor in the evolution of the two more recently established cultivated beets. In other words, the genetic
49 potential to evolve a novel crop was provided by crosses between two cultivar groups; however, the
50 molecular evidence for this notion has not been assembled.

51 A molecular understanding of genomic diversity in beet has been advanced in the sugar beet
52 group; nuclear DNA polymorphisms were analyzed by several population-genetic methods, such as those
53 based on genetic distance and allelic frequency difference, to provide results consistent with the history of
54 the breeding lines (e.g., Galewski and McGrath 2020; Schneider et al. 1999; Adetunji et al. 2014; Laurent
55 et al. 2007; Li et al. 2010; Mangin et al. 2015; Simko et al. 2012; Stevanato et al. 2014; Andrello et al.
56 2017). Mitochondrial genome types (mitotypes) in sugar beet have been analyzed to show selection based
57 on mitotype should be adopted for hybrid breeding (Cheng et al. 2009). A similar analysis was used to
58 investigate mitotypes in other cultivated beets. In summary, the greatest mitotype diversity was found in
59 European leaf beet, and the lowest mitotype diversity occurred in swollen-root type beets (Cheng et al.
60 2011; Nishizawa et al. 2007; Yoshida et al. 2012).

61 Genomes of garden beet cultivars were analyzed concomitantly with those of sugar beet, and
62 a puzzlingly large genetic diversity was revealed (Galewski and McGrath 2020; Mangin et al. 2015;
63 Andrello et al. 2017). The details behind this large diversity, however, were unclear. Although we had
64 previously examined mitotypes in garden beet (Cheng et al. 2011), the number of garden beet accessions
65 used was rather small, and a combined analysis of nuclear DNA polymorphism was missing. We proposed
66 that the evolutionary history of garden beet could be drawn by a combined analysis of nuclear and
67 mitochondrial genome diversity among garden beet genetic resources.

68 In this study, we have extended our analysis of nuclear and mitochondrial genome diversity to

69 a larger number of garden beet accessions. Our analysis of nuclear DNA polymorphism revealed the genetic
70 structure of garden beet genetic resources: one subpopulation included landraces with a broad origin,
71 including Europe, the Caucasus region, the Middle East, and West Asia. The origins of the other
72 subpopulations were confined to Europe and the Caucasus region. Our mitotype analysis revealed several
73 additional mitochondrial genome types (mitotypes) in garden beet, but they occurred infrequently, and the
74 general trend of mitochondrial DNA polymorphism was unchanged. To our surprise, minor mitotypes were
75 more frequent in European and Caucasian garden beet accessions. These minor mitotypes were identical to
76 those in European leaf- and wild beet accessions (Cheng et al. 2011). In conclusion, garden beet genetic
77 resources appeared to have experienced something that resulted in genetically differentiated subpopulations
78 in Europe and the Caucasus region. We propose that crosses with leaf beet or wild beet may explain how
79 these distinct subpopulations formed.

80

81

82 **Materials and Methods**

83 *Plant materials*

84 Forty-seven garden beet accessions were used in this study (Table 1). Although the mitotypes of BETA
85 1037, BETA 1058, BETA 1165, BETA 1343, BETA 1388, BETA 1478, BETA 1618, BETA 1795, BETA
86 1901, BETA 2040, BETA 2071, BETA 2129, and BETA 965 were first reported in Cheng et al. (2011), these
87 13 accessions were reanalyzed in this study. All 47 accessions were obtained from The Leibniz Institute of
88 Plant Genetics and Crop Plant Research (IPK), Germany. Three sugar beet lines were also used in this
89 study: NK-291mmBR-CMS, NK-195mmBR-CMS, and NK-315mmBR-O were developed at the Hokkaido
90 Agricultural Research Center, National Agriculture and Food Research Organization, Japan. Two wild beet
91 (*B. vulgaris* L. ssp. *maritima*) accessions were also used in this study: BETA 368, collected in Portugal and
92 obtained from IPK, and NGB 14676, collected in Denmark and obtained from The Nordic Genetic Resource
93 Center, Sweden.

94

95 *DNA markers and polymerase chain reaction*

96 Primers for 51 cleaved amplified polymorphic sequence (CAPS) markers for nuclear DNA analysis are
97 summarized in Table S1. The multiallelic nuclear DNA marker s17 was detailed in Taguchi et al. (2014).

98 The other nuclear DNA markers target gene-coding sequences or untranslated regions; ten DNA markers
99 are selections from Taguchi et al. (2019). Details about the development of the remaining 40 markers will
100 be described elsewhere. Mitochondrial minisatellite analysis and *orf129* detection were conducted as
101 described in previous studies (Cheng et al. 2011; Nishizawa et al. 2000). Total cellular DNA was isolated
102 from green leaves according to a standard procedure (Doyle and Doyle 1990). DNA fragments were
103 electrophoresed in 2% (w/v) agarose gels.

104

105 *Data analyses*

106 Accessions were clustered according to the neighbor-joining method (NJ) and the unweighted pair-group
107 method with arithmetic averages (UPGMA) using GENEPOP ver. 4.7 (Raymond and Rousset 1995) and
108 visualized using MEGA X (Kumar et al. 2018). STRUCTURE software (ver. 2.3.4) (Pritchard et al. 2000)
109 was run with an admixture model in which the Markov Chain Monte Carlo steps were set to 100000
110 following a burn-in period of 100000. Posterior probabilities for each K value were calculated using ten
111 replicates. Fisher's exact test was executed at a website ([http://aoki2.si.gunma-](http://aoki2.si.gunma-u.ac.jp/exact/fisher/getpar.html)
112 [u.ac.jp/exact/fisher/getpar.html](http://aoki2.si.gunma-u.ac.jp/exact/fisher/getpar.html)). Analysis of molecular variance (AMOVA) was conducted using GenAlEx
113 6.2 (Peakall and Smouse 2006, 2012) with the permutation number set at 999. Wright's fixation index (F_{st}),
114 a measure of population differentiation, was also calculated by GenAlEx.

115

116

117 **Results and Discussion**

118 *Nuclear DNA polymorphism in garden beet*

119 We investigated nuclear DNA polymorphism in garden beet genetic resources, most of which are landraces
120 or old cultivars. Each of the 47 garden beet accessions was represented by a single plant. We also analyzed
121 two wild beets collected in Portugal and Denmark, both of which are distant from the area where garden
122 beet was domesticated (i.e., the Middle East). In addition, three sugar beet lines were included in the
123 investigation. The CAPS markers used in this study were distributed among the nine beet chromosomes;
124 each chromosome was covered by 4 to 12 markers. In summary, 109 alleles were identified from the 51
125 markers, i.e., each marker yielded 2.14 alleles on average (Table S2).

126

A total of 52 accessions/lines were clustered according to their genetic distance based on the

127 polymorphisms detected by the CAPS markers. Figure 1 shows the result of the NJ. We divided accessions
128 into three groups, Group I, Group II, and Wild Beet. Group I consisted of 36 garden beet accessions. All
129 three sugar beet lines were clustered into Group II with 11 garden beet accessions. UPGMA resulted in a
130 different dendrogram (Fig. S1), but the members in each group were identical to those of the NJ.

131 We took another approach to infer the number of subpopulations (K) by a model-based
132 clustering method. The posterior probability increased when K was one to five but declined as K increased
133 (Fig. S2). A bar plot of $K = 5$ is shown in Fig. 2; the two wild beet accessions formed a distinct group,
134 shown as Pop C. Sugar beet lines were grouped into Pop A except for NK-315mmBR-O that was
135 incorporated into a garden beet group. Garden beet accessions were divided into Pops B, D, and E. Pop B
136 was a group with seven garden beet accessions and the sugar beet line NK-315mmBR-O. Pop D, the largest
137 group, consisted of 35 garden beet accessions. Pop E contained five garden beet accessions. Note that, of
138 the 35 accessions in Pop D, 34 were shared with Group I (Table 1). Accordingly, Group II well represented
139 the sum of Pops B and E. The exceptional garden beet accessions were BETA 187, K 7136, and BETA
140 1681, whose ancestry was complex as shown in the bar plots of Fig. 2.

141 We inferred the genetic diversity of Groups and Pops by AMOVA (Table 2). In the case of
142 Groups, the amount of variance among the groups was 11%, and F_{st} was 0.107. A permutation test
143 implemented with GenAlEx software reported a probability (P) value of 0.005. Pairwise population F_{st}
144 values were 0.066 to 0.306 with P values of 0.001 to 0.002 (Table S3). In the case of Pops, the variance
145 among Pops was 14%, and F_{st} was 0.139 with a P value of 0.001 (Table 2). In the matrix of pairwise F_{st}
146 values shown in Table S3, the value for Pop A vs. Pop B was given as 0 because the calculated value was
147 negative, and $P = 0.430$. This result may be related to the composition of these Pops since both Pop A and
148 Pop B contain sugar beet lines (Fig. 2). All the other pairwise F_{st} values were 0.110 to 0.290 with P values
149 of 0.001 to 0.0028 (Table S3). Collectively, these results provided a population structure for our garden
150 beet accessions. The distribution of Pops B, D, and E in Europe to West Asia is shown in Fig. 3. Pop B and
151 Pop E accessions were collected in Europe and the Caucasus region. Pop D was distributed widely from
152 Europe and the Middle East to West Asia. The distribution of Group I accessions corresponded to that of
153 Pop D, except for BETA 187 (Group I/ Pop B, collected in Georgia), BETA 1681 (Group II/ Pop D, Greece)
154 and K 7136 (Group I/ Pop B, Georgia). Group II is the merger of Pop B and Pop E. Accessions from Canada,
155 China, Cuba, and the USA belonged to either group.

156 The link between garden beet genetic diversity and its geographic origin was missing from
157 previous studies. In our study, we combined nuclear DNA polymorphism data with information about the
158 origin of the genetic resources. The results favor the notion that garden beet genetically diverged in Europe
159 and the Caucasus region, although a more in-depth analysis will be necessary to make a definitive
160 conclusion. This notion is consistent with the expansion of morphological variation of European garden
161 beet varieties (Baranski et al. 2001). How genetic diversity increased is an intriguing question, for which
162 introgression may be one of the possibilities (Goldman and Navazio 2008; see below).

163 The sugar beet lines used in this study were grouped into a garden beet group termed Group II
164 or Pop B. Considering the domestication history of sugar beet, i.e., sugar beet was selected from fodder
165 beet whose ancestor is garden beet, this result is not surprising. Interestingly, Group II and Pop B are the
166 groups that occur in Europe and the Caucasus region. This finding implies that the genetically diversified
167 garden beet contributed to the development of a novel type of swollen-root beet. The genetic diversity of
168 garden beets is the key to understanding the domestication of sugar beet.

169

170 *Mitotypes in garden beet*

171 We raised the question of whether the garden beet subpopulations were characterized by their mitotypes.
172 Mitotypes were determined according to haplotypes that were defined by the number of repeat units in four
173 minisatellite loci of the beet mitochondrial genome (Nishizawa et al. 2000, 2007) (Table S4). One of the
174 mitotypes, min06, was further investigated to determine whether the plants possessed mitochondrial gene
175 *orf129* that encodes a protein associated with male sterility (Yamamoto et al. 2008). All the min06 plants
176 in this study were PCR positive for *orf129* and were designated as min06/+*orf129*. A summary of the
177 mitotypes found in 541 plants from the 47 accessions is shown in Table 1. We found 12 mitotypes, of which
178 seven (min07, min10, min11, min15, min17, min33 and min37) were newly discovered as garden beet
179 mitotypes. Mitotype frequency is summarized in Table 3. We tested whether the distribution of mitotypes
180 was different between Groups or Pops. Our results showed that pairwise combinations of Groups or Pops
181 differed significantly in terms of mitotype frequency (Table S5).

182 The most predominant mitotype in garden beet was min18 that was found in 88% of the
183 examined plants, or it occurred in 37 of the 47 accessions (79%) (Table 1). Mitotypes min09, min15, and
184 min18 were present in fodder beets and sugar beets; min18 was especially common among accessions of

185 both beet types (Cheng et al. 2009; Yoshida et al. 2012). The predominance of min18 is likely a common
186 feature among swollen-root beets. These mitotype features were consistent with the historical record and
187 currently proposed domestication hypothesis that all swollen root-type beets have a common ancestor (see
188 Introduction). The remaining nine mitotypes include those found in leaf- and wild beets of Europe and the
189 Middle East, where the mitotypes of these beets are the most diverse (Cheng et al. 2011; Nishizawa et al.
190 2007) (Table 4). The minor mitotypes in European garden beets were min07, min09, min10, min11, min15,
191 min17, min19, min33 and min37, of which all but min15, min17, min33 and min37 were identified
192 previously in European leaf beets (Cheng et al. 2011) (Table 4). The four previously unidentified mitotypes
193 were found in European wild beets (Nishizawa et al. 2007 and our unpublished data). We focused on non-
194 min18 mitotypes because they are the principal contributors to garden beet mitotype diversity. As shown in
195 Fig. 4, the occurrence of non-min18 in garden beets was seen mainly in Europe and the Caucasus region,
196 suggesting that diversification of garden beet mitotypes predominated in these areas. In fact, multiple non-
197 min18 mitotypes were found in France, Slovakia, Romania, and Greece. Multiple non-min18 mitotypes
198 also occurred in accessions from Pakistan and China; min06/+orf129 and min08 found in accessions from
199 Pakistan and China were absent from our European accessions (Table 1).

200 The origins of the minor mitotypes in garden beets identical to those of European leaf- and wild
201 beets are likely due to past introgression. Another possible origin may be contamination of the germplasm
202 collection. We cannot exclude this possibility but note that at maturity all 47 garden beet accessions had a
203 garden beet-like phenotype when grown in our field. Illegitimate pollination during germplasm
204 multiplication could not have contributed to mitotype introduction since mitochondria are maternally
205 inherited.

206 Goldman and Navazio (2008) proposed a hypothesis that hybridization with leaf beet is
207 associated with the phenotypic diversity of garden beet cultivars. This proposal reminds us of BETA 2056,
208 whose origin is recorded as the French garden beet cultivar ‘Crapaudine.’ BETA 2056 belongs to Group II/
209 Pop B, and the plants have either min19 or min33. Interestingly, ‘Crapaudine’ is known for its carrot-like
210 root; the roots of BETA 2056 plants are thin and long, unlike typical garden beets that have a globular root-
211 hypocotyl (our unpublished observations), a characteristic reminiscent of leaf-beet roots. Further study
212 taking morphology into account will be necessary.

213 Another possibility is that the leaf beet-like mitotype occurred in garden beet *de novo*. TR1,

214 one of the minisatellite loci, is highly polymorphic in *B. vulgaris* genetic resources (Nishizawa et al. 2007).
215 Multiple independent occurrences of the same number of repeated sequence units at this locus are possible.
216 On the other hand, sugar beet mitotypes are stable enough to diagnose cytoplasm in the breeding program
217 (Cheng et al. 2009). Moreover, some minor mitotypes such as min10, min19 and min33 differ from min18
218 at two or three loci, making their independent occurrence unlikely. The stability of mitotypes and detailed
219 analyses of mitochondrial DNA and plastid DNA should be investigated in the future.

220

221

222 **Conclusions**

223 In garden beet, significant genetic diversity at the molecular level had been recognized before our study
224 (Galewski and McGrath 2020; Mangin et al. 2015; Andrello et al. 2017), although how diversity was
225 generated or maintained was unknown. In this study, we found a population structure in garden beet that
226 was supported by multiple analytical methods. What these subpopulations reflect is unknown because no
227 pedigree data are available from landraces; however, the evidence shows that subpopulations occurred in
228 Europe and the Caucasus region, a result supporting the notion that garden beet diversified in these areas.
229 Our analysis of mitochondrial DNA polymorphism revealed major and minor mitotypes in garden beet.
230 The minor mitotypes in Europe were also the subset of mitotypes in leaf beets and wild beets in Europe,
231 leading us to propose that these minor mitotypes are the vestiges of past introgressions. In summary, our
232 molecular data from nuclear and mitochondrial DNA analyses support the notion that the European garden
233 beet was genetically diversified by crosses with endemic leaf beet or wild beet populations, although other
234 possibilities cannot be excluded. The occurrence of subpopulations, minor mitotypes, as well as some
235 botanical studies (e.g. Ford-Lloyd and Williams 1975), led to our hypothesis that such crosses occurred
236 north-westward from an area in the Caucasus region toward Asia Minor and Greece.

237

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242

243 **Declarations**

244 **Funding**

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246

247 **Competing interests**

248 The authors declare no competing interest.

249

250 **Availability of data and material**

251 All data generated or analyzed during this study are included in this published article and its supplementary

252 information files.

253

254 **Code availability**

255 Not applicable.

256

257 **Authors' contributions**

258 Conceptualization: Yohei Kanomata and Tomohiko Kubo; Methodology: Yohei Kanomata, Kosuke Satoh

259 and Kazuyoshi Kitazaki; Formal analysis and investigation: Yohei Kanomata, Ryo Hayakawa, Jun

260 Kashikura, Kazuyoshi Kitazaki; Writing - original draft preparation: Yohei Kanomata; Writing - review and

261 editing: Tomohiko Kubo; Funding acquisition: Hiroaki Matsuhira, Kazuyoshi Kitazaki and Tomohiko

262 Kubo; Resources: Hiroaki Matsuhira and Yosuke Kuroda; Supervision: Tomohiko Kubo.

263

264 **Ethics approval**

265 Not applicable.

266

267 **Consent to participate**

268 Not applicable.

269

270 **Consent for publication**

271 Not applicable

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350 **Tables**

351 Table 1. Groups, Pops and mitotypes of garden beet accessions

Accession	Group	Pop	Number of plants in the mitotype													Country of origin	
			min06/ +orf129	min 07	min 08	min 09	min 10	min 11	min 15	min 17	min 18	min 19	min 33	min 37	Total		
BETA 1032	I	D										12				12	Turkey
BETA 1037	I	D										20				20	Georgia
BETA 1058	I	D										21				21	Germany
BETA 1065	I	D										11				11	Iran
BETA 1159	I	D										15				15	Greece
BETA 1165	I	D										12				12	Greece
BETA 1229	I	D										18				18	Turkey
BETA 1257	I	D										8				8	Uzbekistan
BETA 1285	I	D										15				15	Azerbaijan
BETA 1306	I	D		7				1								8	Greece
BETA 1343	I	D	19		4											23	Pakistan
BETA 1388	I	D										14				14	Soviet Union
BETA 1463	I	D										8				8	Greece

BETA 1468	I	D									10				10	Greece
BETA 1478	I	D									19				19	Greece
BETA 155	II	E									9				9	Russia
BETA 1594	I	D									11				11	Turkey
BETA 1618	I	D									23				23	Unknown
BETA 1681	II	D		5		5									10	Greece
BETA 1723	II	B					4								4	Italy
BETA 1744	I	D									7				7	Greece
BETA 1774	II	E									1				1	Germany
BETA 1777	I	D									9			1	10	Germany
BETA 179	I	D	2				8								10	China
BETA 1795	I	D									5				5	The Netherlands
BETA 184	I	D									6	4			10	Slovakia
BETA 187	I	B									13				13	Georgia
BETA 1901	II	E								3			20		23	France
BETA 2040	I	D									13				13	The Netherlands

BETA 2056	II	B									3	8		11	France
BETA 2071	I	D								21				21	Italy
BETA 2129	I	D								9				9	USA
BETA 222	I	D								8				8	Poland
BETA 223	II	B								16				16	Georgia
BETA 245	I	D								12				12	Iraq
BETA 248	II	B				4								4	Spain
BETA 273	I	D								12				12	Tajikistan
BETA 33	II	E								7				7	Canada
BETA 334	I	D						2	4					6	Romania
BETA 336	II	B				4								4	Slovakia
BETA 340	I	D								11				11	Romania
BETA 355	II	E								3				3	Georgia
BETA 3818	I	D								7				7	Cuba
BETA 3861	I	D								4				4	Georgia
BETA 3881	I	D								12				12	Georgia
BETA 965	I	D								15				15	Unknown
K 7136	I	B					11			5				16	Georgia

Total			21	12	4	13	12	12	2	7	422	7	28	1	541	
(# of accessions with the mitotype)			(2)	(2)	(1)	(3)	(2)	(2)	(1)	(2)	(37)	(2)	(2)	(1)		

352

353 Table 2. Summary of AMOVA statistics

Groups					
	df	Mean squares	Variance	%	F_{st} (<i>P</i>)
Among Groups	2	46.954	1.376	11	0.107 (0.005)
Within a Group	49	14.997	3.465	27	
Between accessions	52	8.067	8.067	62	
Total	103		12.908	100	
Pops					
	df	Mean squares	Variance	%	F_{st} (<i>P</i>)
Among Pops	4	38.475	1.815	14	0.139 (0.001)
Within a Pop	47	14.359	3.146	24	
Between accessions	52	8.067	8.067	62	
Total	103		13.028	100	

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357 Table 3. Summary of mitotype frequency in garden beet subpopulations

Group/ Pop	Mitotypes												Total
	min06	min07	min08	min09	min10	min11	min15	min17	min18	min19	min33	min37	
Group I	21	7	4	0	8	1	2	4	381	4	0	1	433
Group II ¹	0	5	0	13	4	11	0	3	41	3	28	0	108
Total	21	12	4	13	12	12	2	7	422	7	28	1	541
Pop B ¹	0	0	0	8	4	11	0	0	34	3	8	0	68
Pop D	21	12	4	5	8	1	2	4	368	4	0	1	430
Pop E	0	0	0	0	0	0	0	3	20	0	20	0	43
Total	21	12	4	13	12	12	2	7	422	7	28	1	541

358 ¹ Sugar beet lines were excluded.

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363 Table 4. Comparison of mitotypes between garden beets and leaf beets ¹

Garden beet

Origin		# of acc.	Mitotypes and the number of plants																Total
			min 04	min 06/+ orf 129	min 06/- orf 129	min 07	min 08	min 09	min 10	min 11	min 15	min 17	min 18	min 19	min 20	min 21	min 33	min 37	
Europe		26				12		13	4	1	2	7	202	7			28	1	277
Non-Europe	Georgia and Azerbaijan	7								11			75						86
	Turkey	3											41						41
	Iran and Iraq	2											23						23
	Pakistan, Uzbekistan, and Tajikistan	3		19			4						20						43
	Other countries	6		2					8				61						71

	Total	47	0	21	0	12	4	13	12	12	2	7	422	7	0	0	28	1	541
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Leaf beet

Europe		13	2	3	1	20	2	51	2	1			28	4	10	7			131
Non-Europe	Georgia	2			1	1		4					12						18
	Turkey	2			3		1		5				10						19
	Iraq and Israel	7		1	32			1	3	1			26						64
Total		24	2	4	37	21	3	56	10	2	0	0	76	4	10	7	0	0	232

364 ¹Leaf beet data were reported in Cheng et al. 2011

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368 **Figure legends**

369 **Fig. 1** Dendrogram of accessions based on the results of the NJ. Black bars on the right identify Groups
370 I and II and the Wild Beet group

371

372 **Fig. 2** Accessions resulting from the analysis using STRUCTURE software ($K=5$). Red, green, blue,
373 yellow, and purple bars represent different ancestries. A scale bar on the top indicates the proportion of
374 ancestry for each accession

375

376 **Fig. 3** Countries of origin for garden beet accessions. Filled circles represent accessions: green, yellow,
377 and purple indicate Pop B, Pop D, and Pop E, respectively

378

379 **Fig. 4** Countries of origin for garden beet accessions. Blue filled circles and white open circles indicate
380 the presence or absence of non-min18 mitotypes in the accessions, respectively.

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382

383 **Supporting information**

384 Figure S1 Dendrogram of accessions based on UPGMA results. Black bars on the right identify the three
385 groups resulting from this analysis.

386 Figure S2 Posterior probability (vertical axis) for each K value (horizontal axis) calculated by
387 STRUCTURE software.

388 Table S1 Summary of DNA markers used in this study

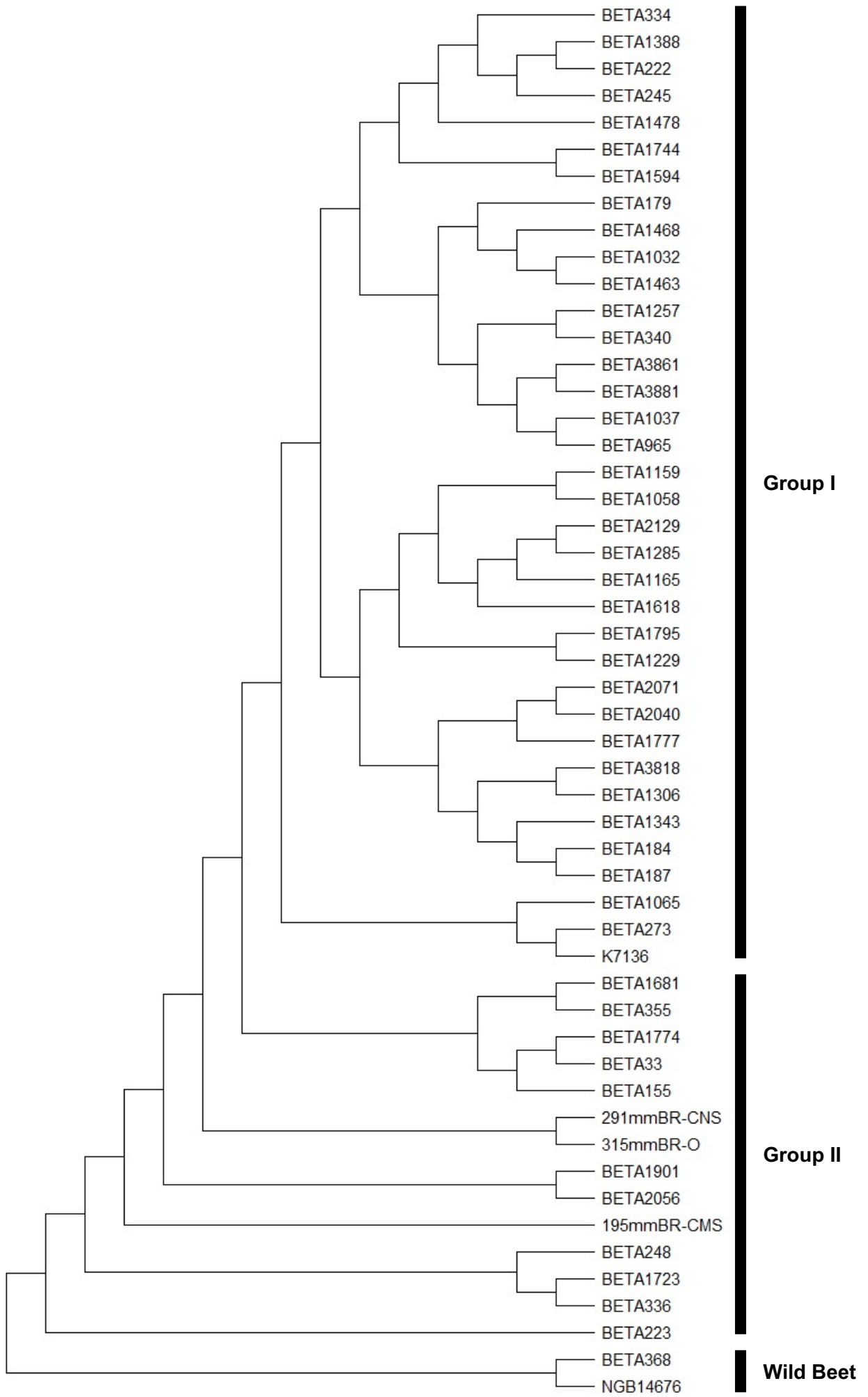
389 Table S2 Alleles detected in the accessions used in this study

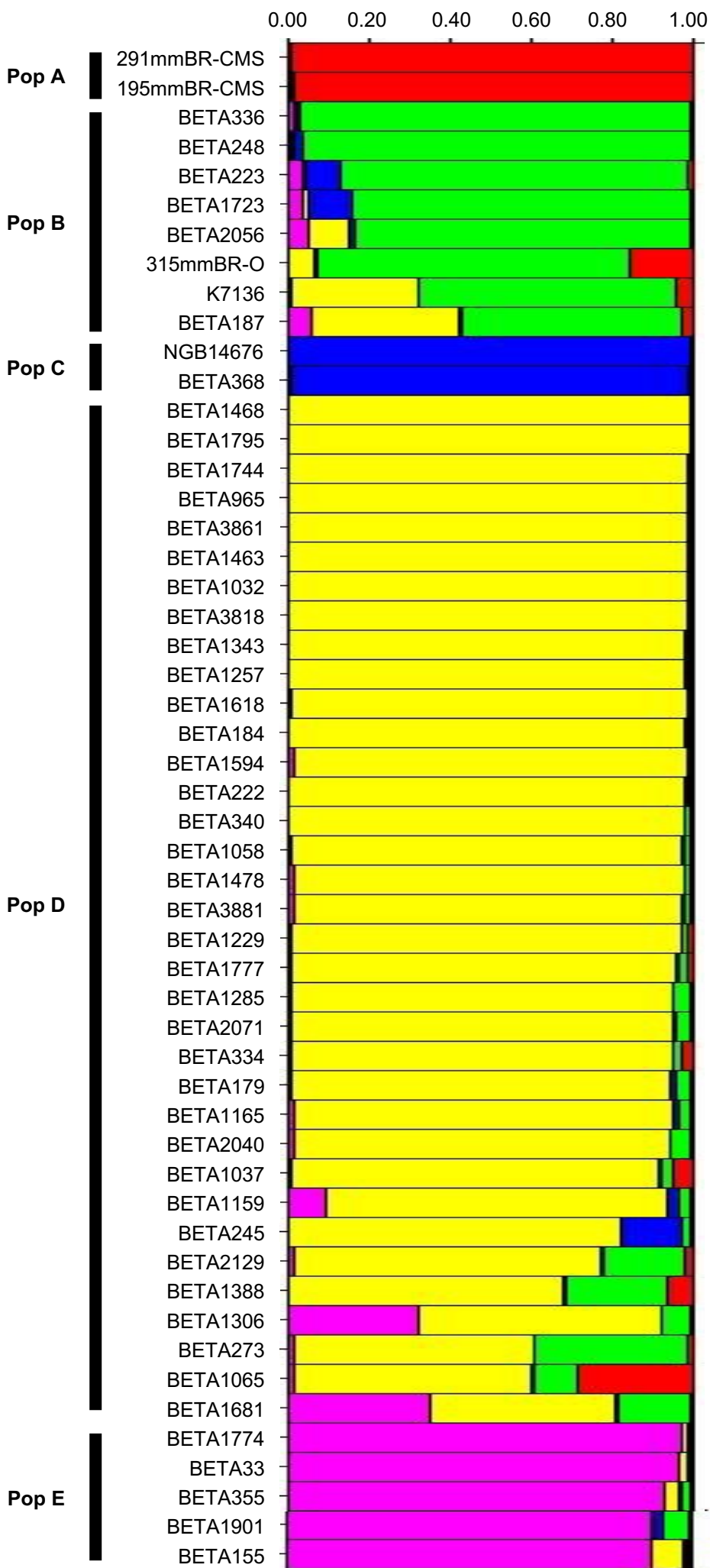
390 Table S3 Pairwise population F_{st} values among Groups and Pops. P values are shown in parentheses.

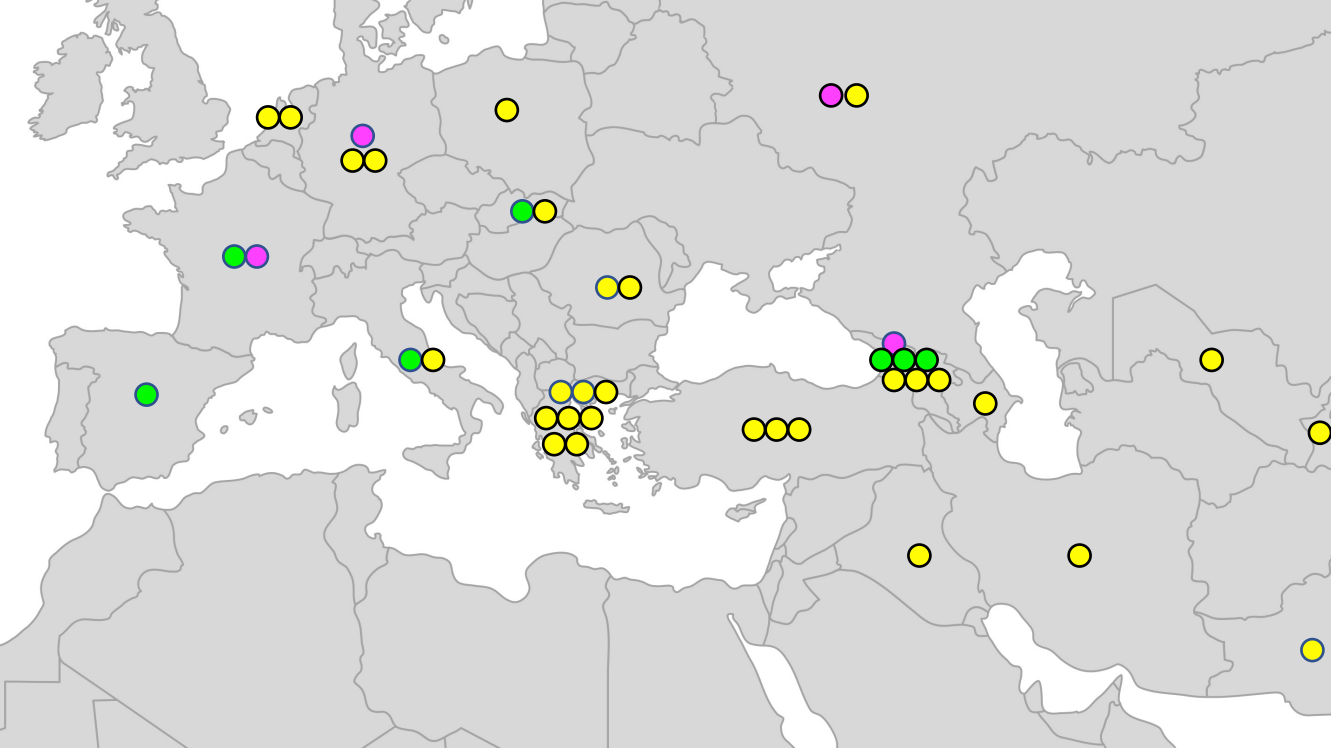
391 Table S4 Numbers of repeat units in the minisatellite loci of mitotypes

392 Table S5 Probabilities of mitotype differentiation for each pair of Groups and Pops calculated using
393 GENEPOP software

394







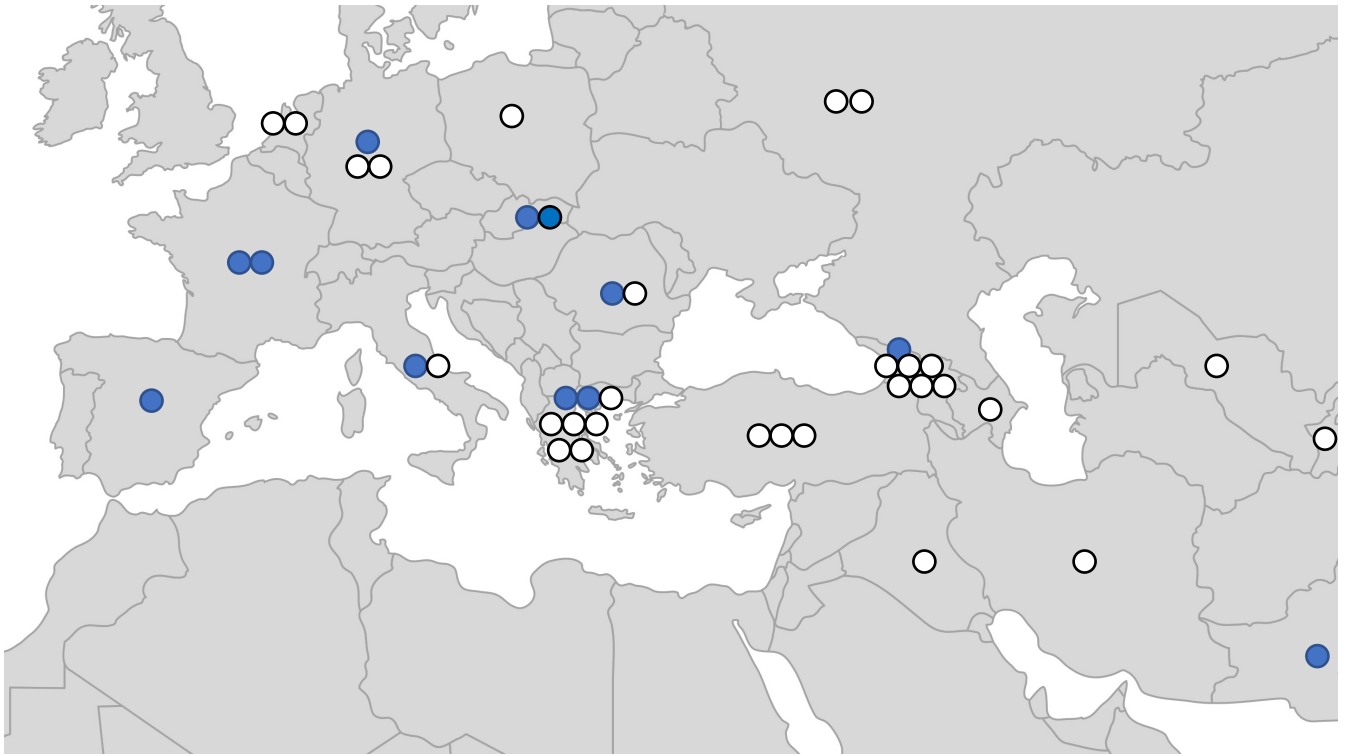


Table S1 Summary of DNA markers used in this study

Genomic location	Name of marker ¹	Fw primer	Rv primer	Restriction endonuclease	Reference
Chr 1	Chr1_1105371"BamH1	5'-TGCTTGTTGAACGGATGACC-3'	5'-TCTCTCCCAAGGCGTTGTTT-3'	Bam HI	This study
Chr 1	Chr1_5062537"EcoR1	5'-TTGAAACCATGGCACTCCAC-3'	5'-CAACCCATTCCATGGCACTA-3'	Eco RI	This study
Chr 1	Chr1_10739771"EcoR1	5'-TCCTGGCAGAGTTGCTCAA-3'	5'-AGACCTCACCTGGCTTGCAT-3'	Eco RI	This study
Chr 1	Chr1_15721785"BamH1	5'-GACGATAGACTCGACTCCGTATGA-3'	5'-TCGTCCCTTAAACGAGCGTA-3'	Bam HI	This study
Chr 1	Chr1_20340637"EcoR1	5'-CGGCTATGACAGGGTGAAGA-3'	5'-GGGGAAGATGTTGGTGTGCT-3'	Eco RI	This study
Chr 1	Chr1_24038662"EcoR1	5'-CTGACATTGACATGGCAGCA-3'	5'-CTTCAAGCAGCAGGAGCTGA-3'	Eco RI	This study
Chr 1	Chr1_30462492"EcoR1	5'-GCATGACCCTTCATCACTGC-3'	5'-CCTGCTGGATCTGAACCTCTCA-3'	Eco RI	This study
Chr 1	Chr1_35823465"EcoR1	5'-TAAAGTCGCATGGGTTGTGG-3'	5'-TGAAGCACTATCTCCCCACT-3'	Eco RI	This study
Chr 1	Chr1_40893642"EcoR1	5'-GCGTGGGAAAGTGA AAAAGG-3'	5'-ACCGAGGGTCTCAAGAACA-3'	Eco RI	This study
Chr 1	Chr1_47187792"Hind3	5'-GGGATATGGATTTGGGGTGA-3'	5'-GGATCCTTGGCTTCTTTTCC-3'	Hin dIII	This study
Chr 1	Chr1_51277414"BamH1	5'-GCGATAGATGCCACATTGGA-3'	5'-GCAAGCGGTGAACAAACAAG-3'	Bam HI	This study
Chr 1	Chr1_56725886"Hind3	5'-TCAATTCAGGCAAGCTGCAC-3'	5'-ACAATTTGGCAGGGAGCAAG-3'	Hin dIII	This study
Chr 2	MP0180	5'-AAAGGCTCCAACCTACCTCC-3'	5'-ACAGGTTTCATCGTGCTACAC-3'	Hae III	Taguchi et al. 2019 ²
Chr 2	Chr2_1408494"EcoR1	5'-GCCTCTCCAGTATTTGGCTTC-3'	5'-CTCGATTTGCAAAGGGGATG-3'	Eco RI	This study
Chr 2	Chr2_10970256"EcoR1	5'-TTTGCGTCTACCGCTACCAC-3'	5'-AGGGGATGGGTTGGTTTTTC-3'	Eco RI	This study
Chr 2	Chr2_36165939"BamH1	5'-GGGAGGGTTGTTCTAGTTTT-3'	5'-GATTTTGGTCTTCTGGACACC-3'	Bam HI	This study
Chr 2	Chr2_50304382"EcoR1	5'-GATGAAATGACGCTCGCTTG-3'	5'-GCCGGAATCACACTTCACA-3'	Eco RI	This study
Chr 3	s17	5'-CAATCTGTGGTGCTGACCAA-3'	5'-GATTAAGAGGGGCTGCTGAAGCCGAGA-3'	Hap II + Hin dIII	Taguchi et al. 2014 ³
Chr 3	tk	5'-GGTTTTGGSTCTCCTAACAAAG-3'	5'-GAGCATMAGAATGTTGGGCAT-3'	Hha I	Taguchi et al. 2019
Chr 3	Chr3_10122025"Hind3	5'-CCATGATAATTGGCGGGTTG-3'	5'-TTCGGCAACTCTGGGAGAAT-3'	Hin dIII	This study
Chr 3	Chr3_25572418"EcoR1	5'-AGACAACGCCGGAGAAGTA-3'	5'-TGGATACCCTGCATTCACCA-3'	Eco RI	This study
Chr 3	Chr3_39227764"Hind3	5'-TAAGGAAGGTGGAGGCTGGA-3'	5'-TCCCAACAGCGATTACATC-3'	Hin dIII	This study
Chr 3	Chr3_53929307"EcoR1	5'-CAACTAAAAGGCGCTGCAAG-3'	5'-TGGACTATGACCGACCCTCA-3'	Eco RI	This study
Chr 4	nir	5'-GTTAGRCTCAAGTGGCTTGG-3'	5'-GGCATTCTCTTCTCWACCTC-3'	Hae III	Taguchi et al. 2019
Chr 4	Chr4_15005279"Hind3	5'-AGAACTCTCCCTCTGTGGCCTA-3'	5'-TCAACCGGTGTTCTGCATTC-3'	Hin dIII	This study
Chr 4	Chr4_29996367"Hind3	5'-GCATCGAACCCGAAGAAGAA-3'	5'-AGGACTTCCCAGGGATTG-3'	Hin dIII	This study
Chr 4	Chr4_46068100"Hind3	5'-TGCAATCCAATGCACTACGC-3'	5'-GTGGCGCTTCGAAATTCTCT-3'	Hin dIII	This study
Chr 4	Chr4_60476329"EcoR1	5'-GCACGTTCTACTTCTGCAATG-3'	5'-CAAGCCACCTAGCCAGAAAA-3'	Eco RI	This study
Chr 5	invvac ⁶	5'-TTACCAGTACAACCCTGCAG-3'	5'-CAATGGCAGGCTTCTCAGGC-3'	Hae III	Taguchi et al. 2019
Chr 5	Chr5_15697877"EcoR1	5'-TTGGCACTTGAGGAGAGTGG-3'	5'-TCCGTCTTCTGCTGTTGCTC-3'	Eco RI	This study
Chr 5	Chr5_30062100"EcoR1	5'-AACTCTCGGTTCTTCTCCAAGG-3'	5'-TTTCCAGCCTCCAGGTTCTC-3'	Eco RI	This study
Chr 5	Chr5_45072440"BamH1	5'-CAATGGCCAATCTGTCTGA-3'	5'-GCGCACAGTTGGAGTTGTTC-3'	Bam HI	This study
Chr 6	cmo	5'-TTCTTGCTTGTGGAAGTGGC-3'	5'-AGGATCAAAGCATGGGCCT-3'	Afa I	Taguchi et al. 2019
Chr 6	Chr6_15454218"EcoR1	5'-CGATAGAGCATCGGCATCAA-3'	5'-AGCCAGCAGGGTCTCTCAA-3'	Eco RI	This study
Chr 6	Chr6_28786817"EcoR1	5'-GAGTGCCTGCTGTGTGTTT-3'	5'-TTCGGGGGAAGGACAGATAG-3'	Eco RI	This study
Chr 6	Chr6_60470324"Hind3	5'-CAAGTTCAGCTCCGCGTACA-3'	5'-ATTGGCAAGGGAGATGCTGT-3'	Hin dIII	This study
Chr 7	7M20	5'-GCTGATCTCCTAGGTTGG-3'	5'-GCATGAGTAATGCTCTCAGG-3'	Hae III	Taguchi et al. 2019
Chr 7	2G14	5'-GGTTTGCACCTTTCTTAGATGG-3'	5'-GAGCCAATCAATCTCAGCC-3'	Hha I	Taguchi et al. 2019
Chr 7	ss	5'-CTCTGAACTGAATGTGGAGC-3'	5'-GGAGCCTGAAGGATATCTAG-3'	Xsp I	Taguchi et al. 2019
Chr 7	Chr7_11043867"Hind3	5'-TGTAACCGTCGTCCCTTCA-3'	5'-CATGGAAGCTCCTTCTGTGG-3'	Hin dIII	This study

Chr 7	Chr7_26398346"Hind3	5'-GCGCGAGATTCGAAGGAAA-3'	5'-GGCTATCATCGCTAGTCCATTG-3'	Hin dIII	This study
Chr 7	Chr7_42026336"EcoR1	5'-GGCTGCCGGTGTCTGAATTA-3'	5'-ATGCAACCTGCTGATGCACT-3'	Eco RI	This study
Chr 7	Chr7_57077272"Hind3	5'-TTTGAGCCACCAACTCCAGA-3'	5'-CTGCGCATGAAGGTCAAAAG-3'	Hin dIII	This study
Chr 8	sps	5'-AGCTGTTATGGAAGGTTTCATG-3'	5'-TCGGGTCAGGCCTAGCAA-3'	Hae III	Taguchi et al. 2019
Chr 8	Chr8_9906841"Hind3	5'-CATGGTCTCCAAGTCCCACA-3'	5'-CATGGGTGCTTGCAGGATTA-3'	Hin dIII	This study
Chr 8	Chr8_25436072"EcoR1	5'-TTGAGCAGTTGCACGATCAG-3'	5'-CCTACTGTGCATCCATCACCT-3'	Eco RI	This study
Chr 8	Chr8_43259046"EcoR1	5'-AGCGTGTTTTCCAGTTCAGA-3'	5'-CAGTGGCTGCAAAGTGGAC-3'	Eco RI	This study
Chr 9	mp0018	5'-AAGCAAACACAGCATTAGCC-3'	5'-GTATGCAAAGTCCAGACAGAAG-3'	Hae III	Taguchi et al. 2019
Chr 9	Chr9_8948514"Hind3	5'-CGCCAAAATCAGATCACAG-3'	5'-GTCTCCAATCACCCCTTGCT-3'	Hin dIII	This study
Chr 9	Chr9_22312475"EcoR1	5'-ATGCAGTTCCTTTCCAGA-3'	5'-CTGCTGGACTTTGCTTACC-3'	Eco RI	This study
Chr 9	Chr9_36154592"BamH1	5'-TCCTTCTCCATATCCCAACACC-3'	5'-GATCGTGGTGGGAAGCTGATG-3'	Bam HI	This study
Mt	mt-TR1	5'-AGAACTTCGATAGGCGAGAGG-3'	5'-GCAATTTTCAGGGCATGAACC-3'	NA	Nishizawa et al. 2000 ^{*4}
Mt	mt-TR2	5'-TTAATTGCGAGACCGGAGGC-3'	5'-GAGCTTGCTCGCAGCTTATG-3'	NA	Nishizawa et al. 2000
Mt	mt-TR3	5'-AGATCCAAACAGAGGGACTG-3'	5'-CGGATCACCTATTCATTTG-3'	NA	Nishizawa et al. 2000
Mt	mt-TR4	5'-AATGAGACCCGATTCTCTTC-3'	5'-GTTAAAAGCCCTTCTATGCC-3'	NA	Nishizawa et al. 2000
Mt	orf129	5'-ATCCATGGTGATGAATCCTTATATTCTGC-3'	5'-CTAGAGCTCTCACTGTGAGAGATAG-3'	NA	Cheng et al. 2011 ^{*5}

*1 The number following the chromosome coordinates to the nucleotide sequence of sugar beet nuclear genome (Funk et al. Plant J 2018;95: 659-671.).

*2 Taguchi K, Kuroda Y, Okazaki K, Yamasaki M. Breed Sci 2019;69: 255-265.

*3 Taguchi K, Hiyama H, Yui-Kurino R, Muramatsu A, Mikami T, et al. Crop Sci 2014;54: 1407-1412.

*4 Nishizawa S, Kubo T, Mikami T. Curr Genet 2000;37: 34-38.

*5 Cheng D, Yoshida Y, Kitazaki K, Negoro S, Takahashi H, et al. Genet Resour Crop Evol 2011;58: 553-560.

*6 Invvac marker was assigned to chr 2 in Taguchi et al. (2019)^{*2} but it should be assigned to chr 5 according to the alignment of its primer sequences to the reference sequence of sugar beet^{*1}.

Table S3 Pairwise population F_{st} values among Groups and Pops. P values are shown in parentheses.

Groups

	Group II	Wild Beet
Group I	0.066 (0.002)	0.306 (0.001)
Group II	/	0.168 (0.002)

Pops

	Pop B	Pop C	Pop D	Pop E
Pop A	0.000 (0.430)	0.290 (0.028)	0.168 (0.001)	0.190 (0.003)
Pop B	/	0.172 (0.001)	0.086 (0.001)	0.110 (0.001)
Pop C	/	/	0.312 (0.001)	0.278 (0.002)
Pop D	/	/	/	0.126 (0.001)

Table S5 Probabilities of mitotype differentiation for each pair of Groups and Pops calculated using GENEPOP

Pairs	ρ *
Group I and Group II	0.00
Pop B and Pop D	0.00
Pop B and Pop E	0.00
Pop D and Pop E	0.00

* Exact G test

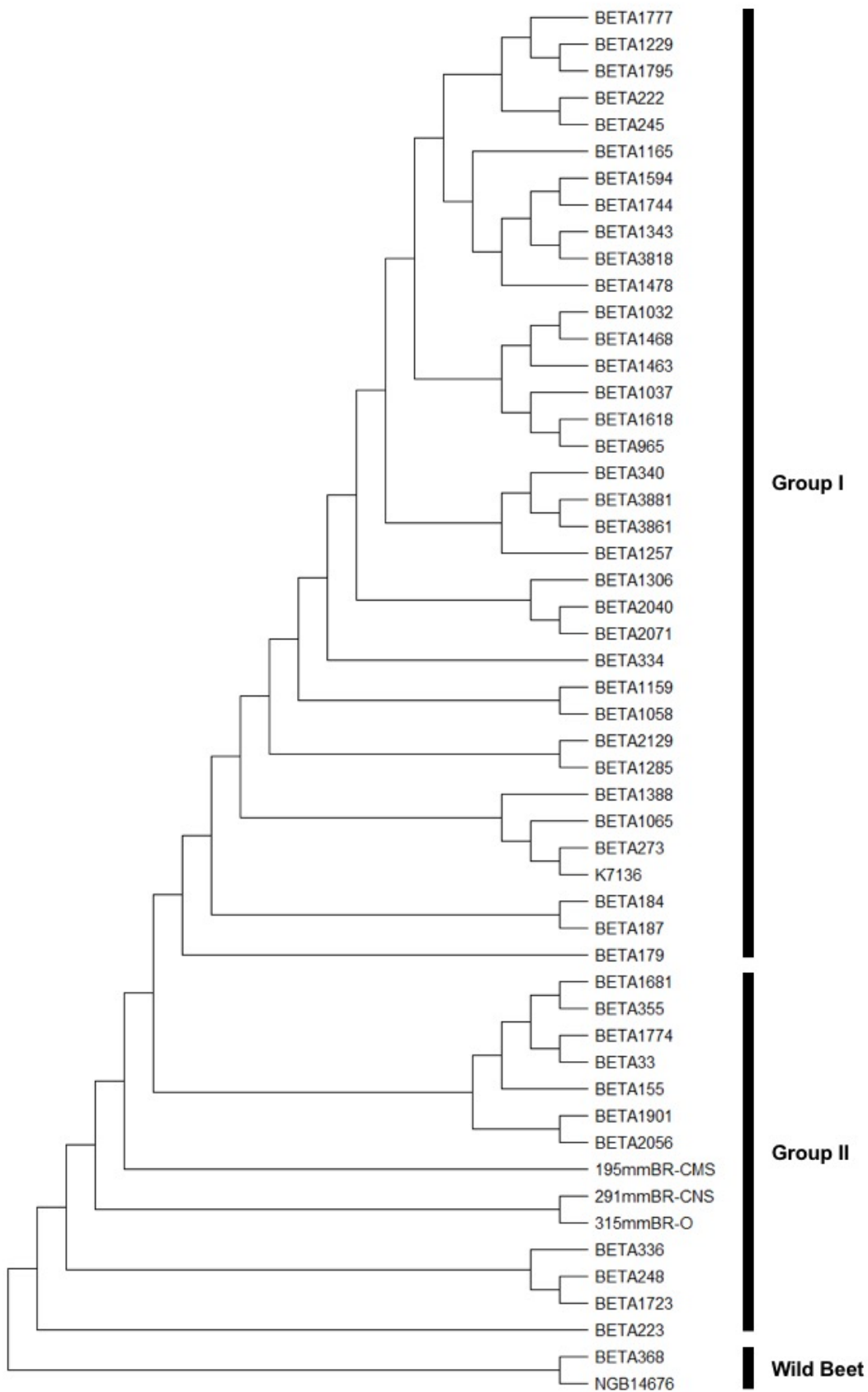


Fig S1 Dendrogram of accessions based on the result of UPGMA. Three groups in this dendrogram are shown by black bars.

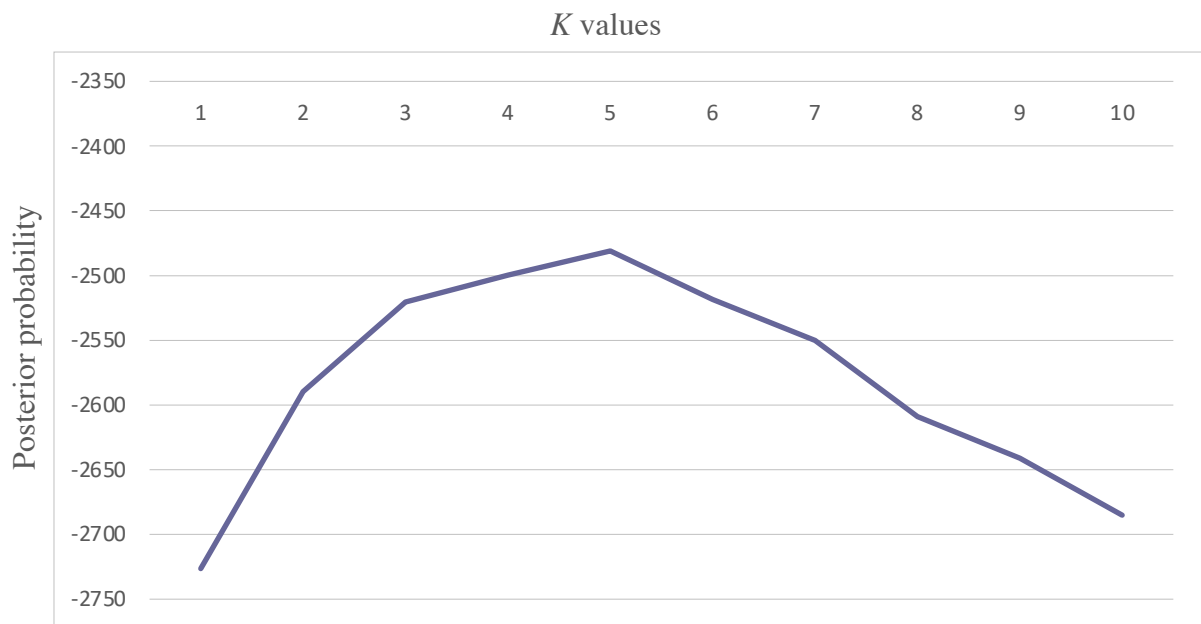


Fig S2 Plots of posterior probability (vertical axis) for each K values (horizontal axis) calculated by STRUCTURE.