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**Nuclear and mitochondrial DNA polymorphisms
suggest introgression contributed to garden beet (*Beta
vulgaris* L.) domestication**

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Abstract

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

Introduction

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

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

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

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

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

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

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

Materials and Methods

Plant materials

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

DNA markers and polymerase chain reaction

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

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

Data analyses

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

Results and Discussion

Nuclear DNA polymorphism in garden beet

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

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

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

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

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

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

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

Mitotypes in garden beet

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

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

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

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

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

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

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

Conclusions

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

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243 **Declarations**

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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			(2)	(2)	(1)	(3)	(2)	(2)	(1)	(2)	(37)	(2)	(2)	(1)		
accessions																
with the																
mitotype)																

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353 Table 2. Summary of AMOVA statistics

Groups					
	df	Mean squares	Variance	%	F_{st} (P)
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} (P)
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																
			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	Total
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

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

Figure legends

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

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

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

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

Supporting information

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

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

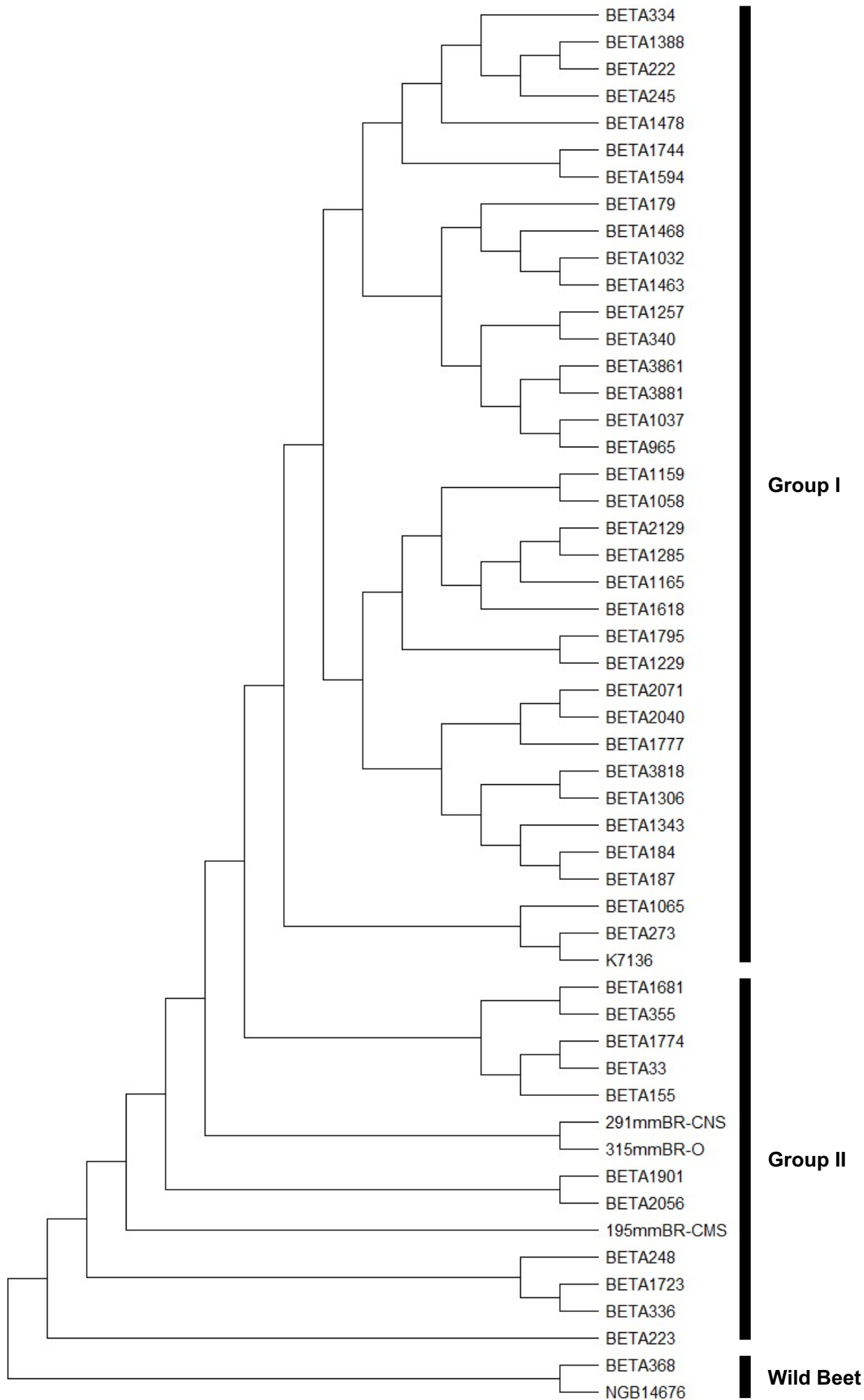
Table S1 Summary of DNA markers used in this study

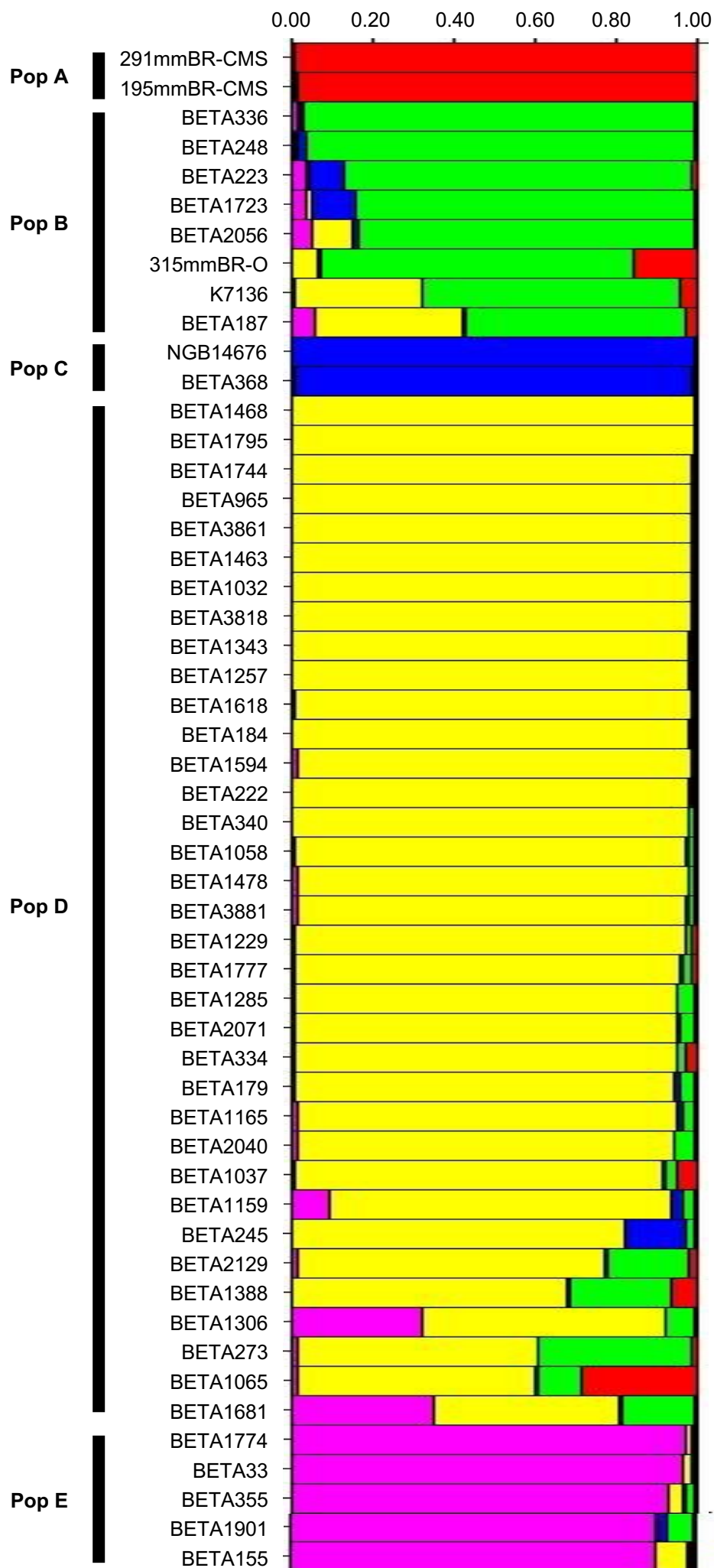
Table S2 Alleles detected in the accessions used in this study

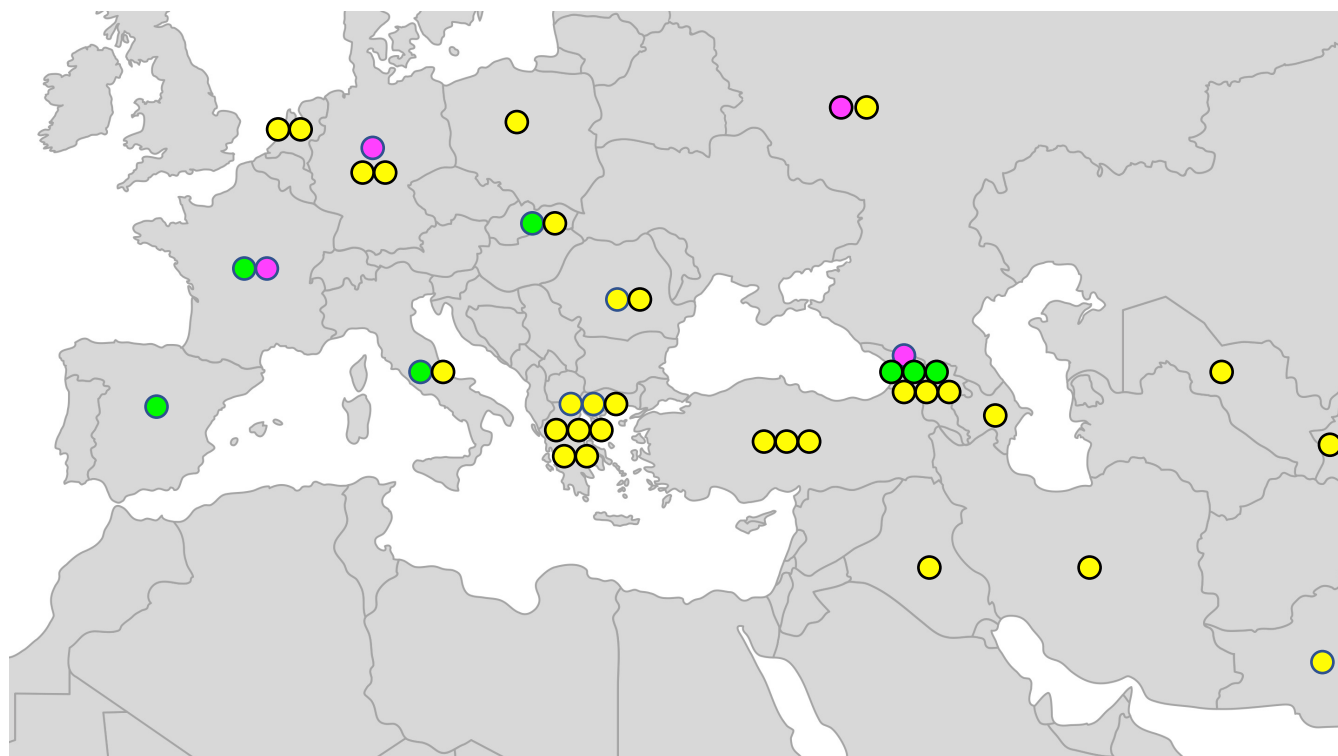
Table S3 Pairwise population F_{st} values among Groups and Pops. P values are shown in parentheses.

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

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







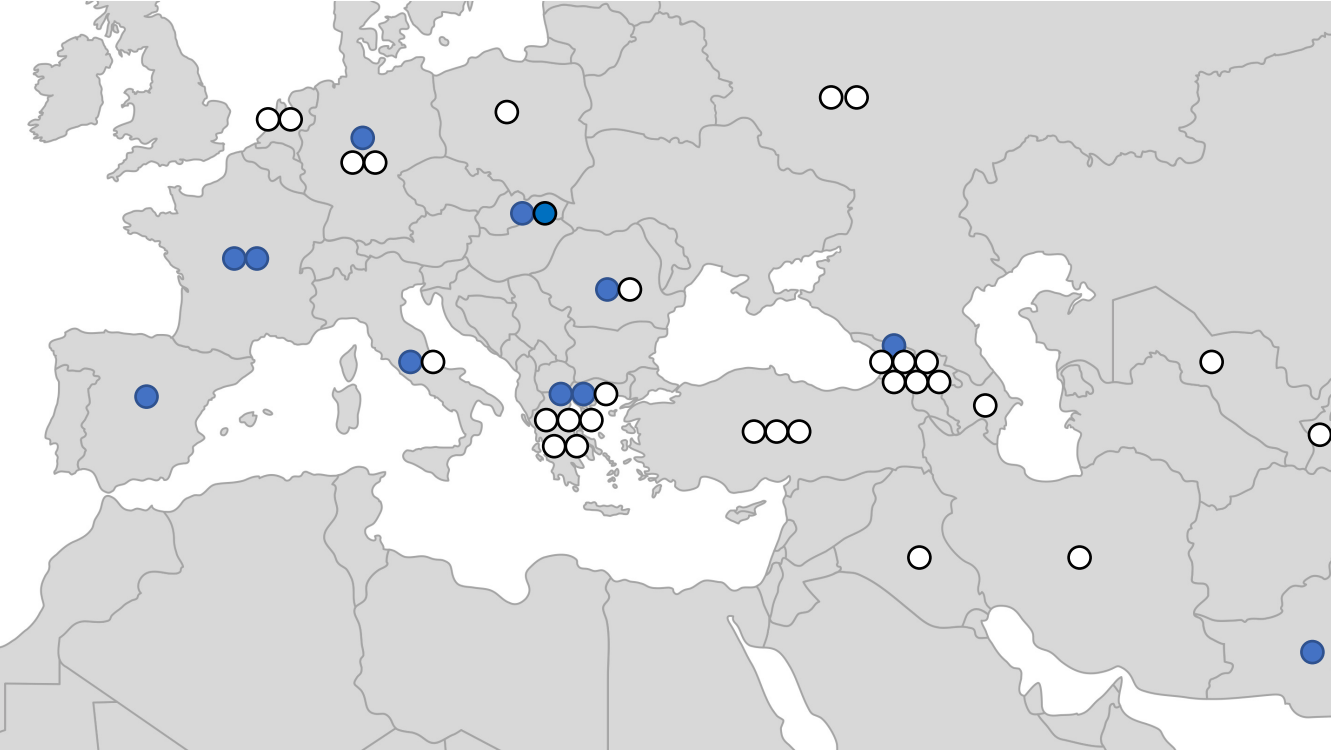


Table S1 Summary of DNA markers used in this study

Genomic location	Name of marker ^{*1}	Fw primer	Rv primer	Restriction endonuclease	Reference
Chr 1	Chr1_1105371"BamH1	5'-TGCTTGTGAACGGATGACC-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'-TGAAGCACTATCTCCCCCACT-3'	Eco RI	This study
Chr 1	Chr1_40893642"EcoR1	5'-GCGTGGGAAAGTGA AAAAGG-3'	5'-ACCGAGGGTCTCAAGACA-3'	Eco RI	This study
Chr 1	Chr1_47187792"Hind3	5'-GGGATATGGATTGGGGTGA-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'-GGGAGGGGTTGTTCTAGTTT-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'-GATTAAAGAGGGCTGCTGAAGCCGAGA-3'	Hap II + Hin dIII	Taguchi et al. 2014 ^{*3}
Chr 3	tk	5'-GGTTTTGGSTCTCCTAACAAG-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'-TGGATACCCTGCATTACCA-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'-TCAACCGGTGTTCTGCATTG-3'	Hin dIII	This study
Chr 4	Chr4_29996367"Hind3	5'-GCATCGAACCCGAAGAAGAA-3'	5'-AGGACTTCCCCAGGGATTG-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'-AACTCTCCGTTCTTCTCCAAGG-3'	5'-TTTCCAGCCTCCAGGTTCTC-3'	Eco RI	This study
Chr 5	Chr5_45072440"BamH1	5'-CAATGGCCAATCTGTCTCTGA-3'	5'-GCGCACAGTTGGAGTTGTTC-3'	Bam HI	This study
Chr 6	cmo	5'-TTCTTGCTTGTGGAAGTGGC-3'	5'-AGGATCAAAAGCATGGGCCT-3'	Afa I	Taguchi et al. 2019
Chr 6	Chr6_15454218"EcoR1	5'-CGATAGAGCATCGGCATCA-3'	5'-AGCCAGCAGGGTCTCTTCAA-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'-GGTTTGCACTTTCTTAGATGG-3'	5'-GAGCCAATCAATCTTCAGCC-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'-GCGCGAGATTCTGAAGGAAA-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'-TCGGGTTCAGGCCTAGCAA-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'-CCACTGTGCATCCATCACCT-3'	Eco RI	This study
Chr 8	Chr8_43259046"EcoR1	5'-AGCGTGTTTCCCAGTTCAGA-3'	5'-CACTGGCTGCAAAAGTGGAC-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'-CGCCCCAAATCAGATCACAG-3'	5'-GTCTTCCAATCACCTTGCT-3'	Hin dIII	This study
Chr 9	Chr9_22312475"EcoR1	5'-ATGCAGTTCCCCTTTCCAGA-3'	5'-CTGCTGGACTTTGCTTCACC-3'	Eco RI	This study
Chr 9	Chr9_36154592"BamH1	5'-TCCTTCTCCATATCCCAACACC-3'	5'-GATCGTGGTGGAAGCTGATG-3'	Bam HI	This study
Mt	mt-TR1	5'-AGAAGTTCGATAGGCGAGAGG-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'-CGGATCACCTATTCAATTTG-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 S2. Alleles detected in the accessions used in this study

	47	MPH00	R	mpd018	YMD0	2014	aps	lar	ss	revca	emo	Chr1_116	Chr1_368	Chr1_107	Chr1_157	Chr1_203	Chr1_249	Chr1_304	Chr1_358	Chr1_408	Chr1_474	Chr1_512	Chr1_567	Chr2_1	Chr2_109	Chr2_263	Chr2_308	Chr1_101	Chr1_255	Chr1_392	Chr1_539	Chr1_156	Chr1_259	Chr1_469	Chr1_604	Chr1_156	Chr1_306	Chr1_450	Chr1_154	Chr1_287	Chr1_468	Chr1_118	Chr1_263	Chr1_420	Chr1_570	Chr1_598	Chr1_254	Chr1_412	Chr1_88	Chr1_222	Chr1_341	1972	band11
pop.BETA1285_AZERBAIJAN	55	11	22	22	11	22	11	12	22	11	12	12	12	22	23	12	22	24	12	12	12	11	11	12	22	12	22	11	12	22	22	11	11	22	22	11	11	12	12	22	22	12	22	11	22	11	12	12	12	11	11	12	
pop.BETA1017_GEORGIA	55	11	11	22	11	22	11	12	22	11	12	12	12	22	23	12	22	24	12	12	12	11	11	12	22	12	11	22	11	11	22	22	11	11	22	12	22	12	22	12	22	11	22	11	22	11	12	12	12	11	11	12	
pop.BETA1081_GEORGIA	55	11	11	22	11	22	11	12	22	11	12	12	12	22	23	12	22	22	22	22	22	11	11	12	11	22	11	22	11	12	12	22	22	11	11	22	11	11	22	12	11	22	11	22	11	12	12	12	11	11	12		
pop.BETA1061_GEORGIA	55	11	11	22	11	22	11	12	22	11	12	12	12	22	23	12	22	22	22	22	22	11	11	12	11	11	22	11	11	22	11	11	22	11	11	22	11	11	22	12	11	22	11	22	11	12	12	11	11	12			
pop.BETA1071_GEORGIA	55	11	11	22	12	12	11	11	22	13	12	11	12	22	23	12	22	22	12	22	22	11	11	12	11	11	22	11	12	11	12	11	12	11	11	12	11	11	12	12	11	22	11	22	11	12	11	11	12				
pop.KT106_GEORGIA	55	11	12	22	11	22	11	11	23	22	12	12	22	22	12	12	11	11	12	11	11	11	12	11	11	11	11	22	12	22	12	22	22	11	11	23	11	12	12	12	11	11	11	12	12	12	11	11	12				
pop.BETA221_GEORGIA	22	11	11	22	11	22	11	11	22	22	44	12	22	11	12	12	13	12	12	11	11	12	12	11	12	22	22*	22	22	12	11	23	11	22	22	11	23	11	22	12	22	11	22	11	12	12	12	11	11	12			
pop.BETA1081_IRAN	55	11	12	22	11	22	11	11	22	22	12	12	12	22	23	12	22	11	11	12	11	11	12	11	11	12	11*	22	22	11	11	11	11	12	11	11	12	11	11	22	11	12	11	12	11	11	12	11	12				
pop.BETA245_IRAQ	55	11	12	22	11	22	11	22	22	22	12	12	22	23	12	22	33	12	12	22	11	11*	12	12	11	11	22	12	11	12	22	11*	33	11	11	12	12	22	11	22	11	23	22	12	11	22	11	12	12				
pop.BETA1041_PAKISTAN	55	11	11	22	11	12	11	22	22	22	12	12	22	23	12	22	24	22	22	22	11	11	22	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12			
pop.BETA1771_TAJIKISTAN	55	11	12	22	11	12	11	11	22	12	12	12	22	23	12	22	24	22	11	22	11	11	12	11	22	11	22	22	11	22	22	22*	22	11	22	11	22	12	12	11	22	11	22	11	12	12	12	11	11	12			
pop.BETA1041_TURKEY	55	11	11	22	11	22	11	22	22	12	12	12	22	23	12	22	24	11	12	22	11	11	12	11	22	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12		
pop.BETA1012_TURKEY	55	11	12	22	11	22	11*	22	22	12	12	12	22	23	12	22	24	12	12	22	11	11	22	22	12	11	22	22	11	22	22	11*	22	12	11	12	12	11	22	11	22	11	22	11	22	11	12	11	11	11	12		
pop.BETA129_TURKEY	45	11	12	22	11	22	11*	22	22	12	12	22	23	12	22	22	44	11	11	12	11	11	22	11	22	11	22	11	22	11	22	12	22	12	22	11	11	22	12	22	11	22	11	22	11	12	11	12	11	12			
pop.BETA121_PAKISTAN	55	11	12	22	11	22	11	22	22	11	22	11*	22	12	22	11	22	24	22	22	22	11	12	22	11	12	22	11	12	22	11	12	12	11	11	22	11	11	12	22	11	22	11	22	11	12	11	12	11	12			
pop.BETA206_FRANCE	55	11	12	22	11	11	12	22	22	12	12	12	12	12	12	12	11	14	22	22	11	11	12	11	11	11	22	22	11	11	22	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12		
pop.BETA1058_GERMANY	55	11	11	22	11	12	11	12	12	22	22	12	12	22	12	12	22	44	11	11	12	11	11	12	11	11	12	11	12	12	11	12	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12
pop.BETA1777_GERMANY	55	11	11	22	11	11	11	22	22	11	12	12	22	23	12	22	33	22	11	22	11	11	22	12	22	11	22	12	22	11	22	11*	22	12	22	11*	22	11	12	22	11	22	11	22	11	22	11	22	11	12	11	12	
pop.BETA1019_GREECE	55	11	11	12	11	11	22	22	22	12	12	22	22	12	12	12	22	11	11	12	11	11	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12	
pop.BETA1199_GREECE	35	11	11	22	11	11	22	22*	22	12	12	12	12	12	12	12	22	44	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12		
pop.BETA1188_GREECE	55	11	11	22	11	12	11	22	22	12	12	22	22	12	12	12	22	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12	
pop.BETA1041_GREECE	55	11	12	22	11	12	22	22	22	12	12	12	12	12	12	12	12	11	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12	
pop.BETA1741_GREECE	55	11	11	22	11	22	22	22	22	12	12	12	12	12	12	12	22	34	12	12	12	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12	
pop.BETA1468_GREECE	55	11	12	22	11	12	22*	22	22	12	12	12	12	12	12	12	22	24	12	12	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12	
pop.BETA1081_GREECE	55	11	12	22	11	12	22	22	22	12	12	12	12	12	12	12	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12	
pop.BETA1071_ITALY	35	11	11	22	12	11	11	22	22	12	12	12	12	12	12	12	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12	
pop.BETA1721_ITALY	55	11	12	22	11	12	11	22	22	12	12	12	11	12	12	12	33	22	11	12	11	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12	
pop.BETA1795_THE_NETHERLANDS	55	11	11	22	11	22	11	22	12	12	22	12	12	12	12	12	22	44	22	11	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12		
pop.BETA200198_THE_NETHERLANDS	55	11	12	22	11	12	11	22	22	12	12	12	12	12	12	12	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12	
pop.BETA2221_POLAND	55	11	12	22	11	12	11	12	22	12	12	11	12	12	12	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12	
pop.BETA3401_ROMANIA	55	11	12	22	11	22	11*	22	22	12	12	12	12	12	12	12	22	22	12	12	12	22*	*	*	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	12		
pop.BETA1041_ROMANIA	45	11	12	22	11	22	12	12	22	12	12	11	22	12	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	p^*
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

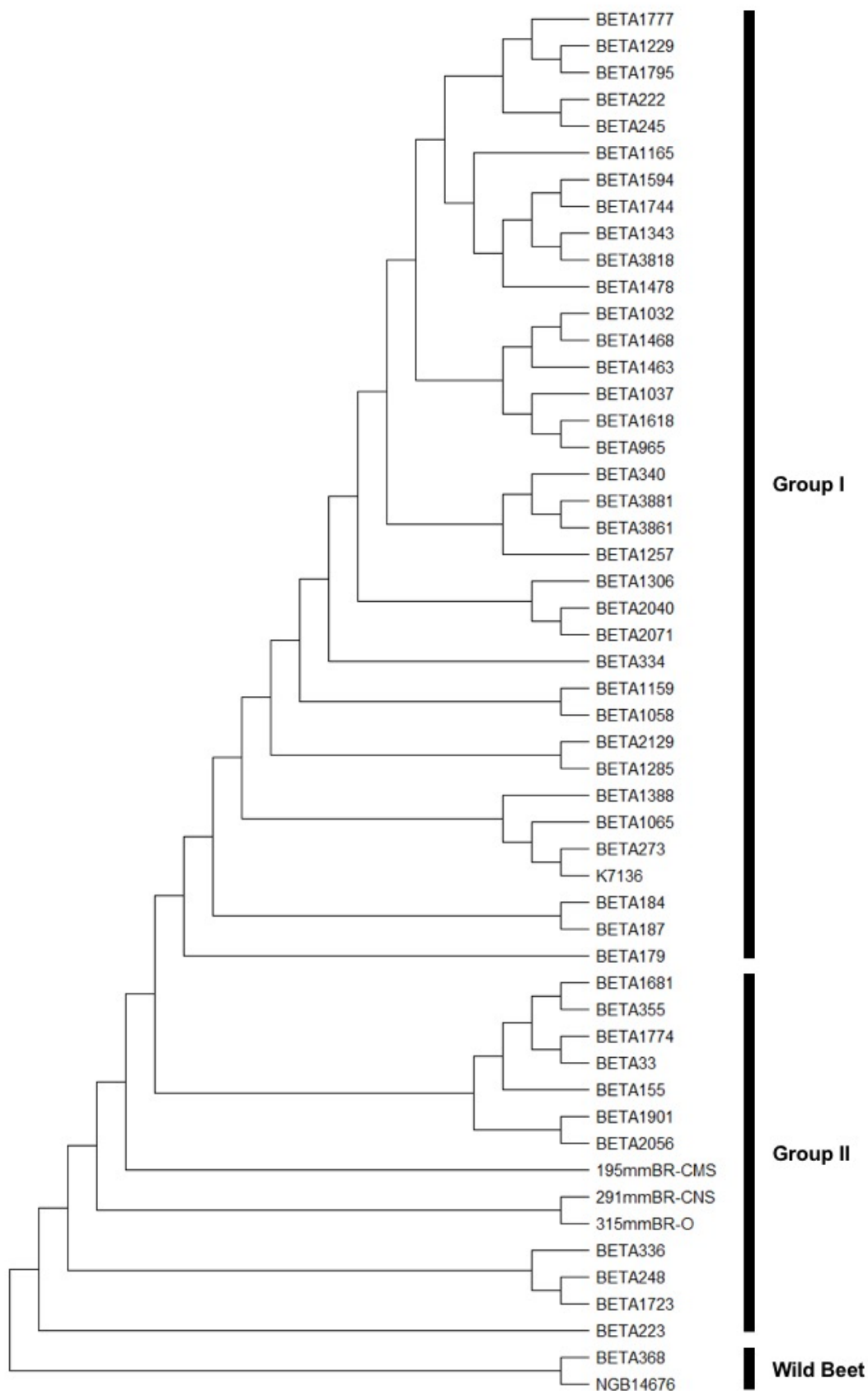


Fig S1 Dendrogram of accessions based on the result of UPGMA. Three groups in this dendrogram are shown by black bars.

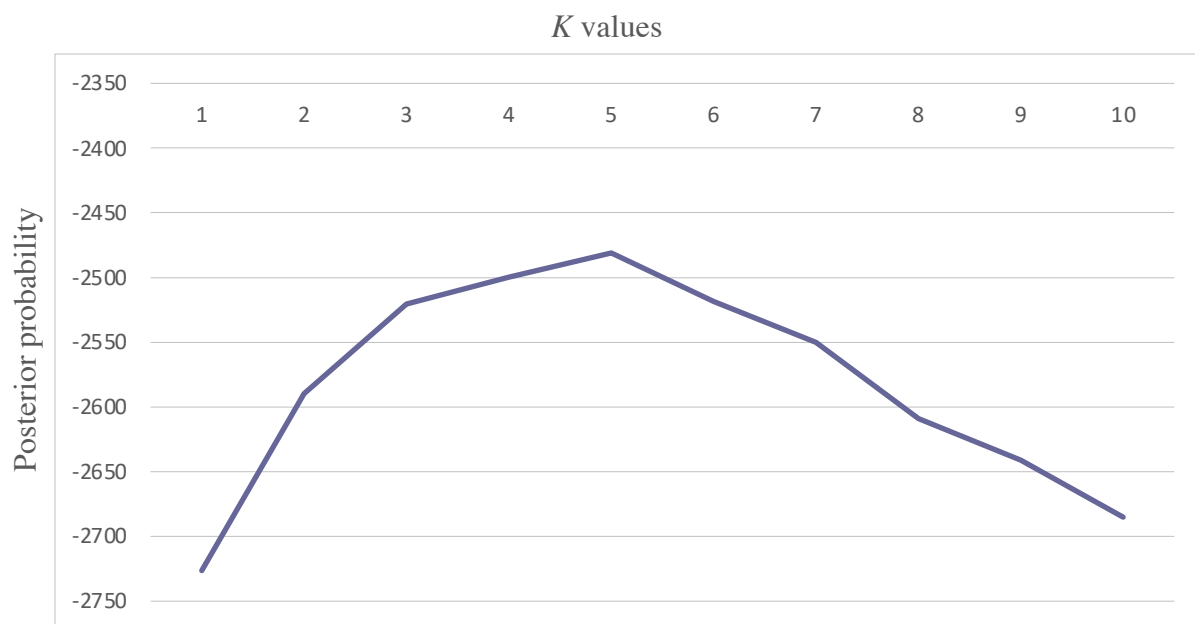


Fig S2 Plots of posterior probability (vertical axis) for each K values (horizontal axis) calculated by STRUCTURE.