| Title | Nuclear and mitochondrial DNA polymorphisms suggest introgression contributed to garden beet (Beta vulgaris L.) <br> domestication |
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Instructions for use

Nuclear and mitochondrial DNA polymorphisms suggest introgression contributed to garden beet (Beta vulgaris L.) domestication<br>Yohei Kanomata ${ }^{1}$, Ryo Hayakawa ${ }^{1}$, Jun Kashikura ${ }^{1}$, Kosuke Satoh ${ }^{1}$, Hiroaki Matsuhira ${ }^{2}$, Yosuke Kuroda ${ }^{2}$, Kazuyoshi Kitazaki ${ }^{1}$, Tomohiko Kubo ${ }^{1, *}$<br>${ }^{1}$ Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan ${ }^{2}$ Hokkaido Agricultural Research Center, National Agriculture and Food Research Organization, Memuro, Japan

Keywords: Domestication, garden beet, introgression, mitoype, population structure

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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 intercrossable 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 $2 \%(\mathrm{w} / \mathrm{v})$ 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.gunmau.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 $\left(F_{s t}\right)$, 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 $F_{s t}$ was 0.107 . A permutation test implemented with GenAlEx software reported a probability $(P)$ value of 0.005 . Pairwise population $F_{s t}$ 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 $F_{s t}$ was 0.139 with a $P$ value of 0.001 (Table 2). In the matrix of pairwise $F_{s t}$ 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 $F_{s t}$ 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 $(\min 07, \min 10, \min 11, \min 15, \min 17, \min 33$ and $\min 37)$ 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 $\min 18$ 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 $\min 18$ were present in fodder beets and sugar beets; min 18 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 $\min 07, \min 09, \min 10, \min 11, \min 15$, $\min 17, \min 19, \min 33$ and $\min 37$, of which all but $\min 15, \min 17, \min 33$ and $\min 37$ 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$\min 18$ 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$\min 18$ 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 roothypocotyl (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 $\min 10, \min 19$ and $\min 33$ 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; Andrello 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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## Declarations

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## Competing interests

The authors declare no competing interest.

## Availability of data and material

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

## Code availability

Not applicable.

## Authors' contributions

Conceptualization: Yohei Kanomata and Tomohiko Kubo; Methodology: Yohei Kanomata, Kosuke Satoh and Kazuyoshi Kitazaki; Formal analysis and investigation: Yohei Kanomata, Ryo Hayakawa, Jun Kashikura, Kazuyoshi Kitazaki; Writing - original draft preparation: Yohei Kanomata; Writing - review and editing: Tomohiko Kubo; Funding acquisition: Hiroaki Matsuhira, Kazuyoshi Kitazaki and Tomohiko Kubo; Resources: Hiroaki Matsuhira and Yosuke Kuroda; Supervision: Tomohiko Kubo.

## Ethics approval

Not applicable.

## Consent to participate

Not applicable.

## Consent for publication

Not applicable

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

| Accession | Group | Pop | Number of plants in the mitotype |  |  |  |  |  |  |  |  |  |  |  |  | Country of origin |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | min06/ <br> +orf129 | $\min$ <br> 07 | $\begin{gathered} \min \\ 08 \end{gathered}$ | $\begin{gathered} \min \\ 09 \end{gathered}$ | $\begin{gathered} \min \\ 10 \end{gathered}$ | $\min$ <br> 11 | $\min$ $15$ | $\min$ <br> 17 | $\min$ <br> 18 | $\begin{gathered} \min \\ 19 \end{gathered}$ | $\min$ <br> 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 <br> 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 <br> Netherlands |




Table 2. Summary of AMOVA statistics

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

Table 3. Summary of mitotype frequency in garden beet subpopulations

| Group/ Pop | Mitotypes |  |  |  |  |  |  |  |  |  |  |  | Total |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | min06 | $\min 07$ | min08 | min09 | $\min 10$ | min11 | $\min 15$ | $\min 17$ | $\min 18$ | min 19 | min33 | $\min 37$ |  |
| 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 |

${ }^{1}$ Sugar beet lines were excluded.

Table 4. Comparison of mitotypes between garden beets and leaf beets ${ }^{1}$
Garden beet

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



Leaf beet

${ }^{1}$ 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 $F_{s t}$ 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

 291mmBR-CMS 195 mmBR -CMS

BETA336 BETA248 BETA223 BETA1723 BETA2056 315 mmBR -

K7136 BETA187 NGB14676 BETA368 BETA1468 BETA1795 BETA1744 BETA965 BETA3861 BETA1463 BETA1032 BETA3818 BETA1343 BETA1257 BETA1618 BETA184 BETA1594 BETA222 BETA340 BETA1058 BETA1478 BETA3881 BETA1229 BETA1777 BETA1285 BETA2071 BETA334 BETA179 BETA1165 BETA2040 BETA1037 BETA1159 BETA245 BETA2129 BETA1388 BETA1306 BETA273 BETA1065 BETA1681
 BETA177 BETA33 BETA355 BETA1901 BETA155




| 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’-TCCTGGCAGAGTTGCTTCAA-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'-CCTGCTGGATCTGAACTTCTCA-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'-GCGTGGGAAAGTGAAAAAGG-3' | 5'-ACCGAGGGTCCTCAAGAACA-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'-AAAGGCTCCAACTAACCTCC-3' | 5'-ACAGGTTCATCGTGCTACAC-3' | Hae III | Taguchi et al. 2019*2 |
| Chr 2 | Chr2_1408494"EcoR1 | 5'-GCCTCTCCAGTATTTTGGCTTC-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'-GGGAGGGGTTGTTCCTAGTTT-3' | 5'-GATTTTGGTCCTTCTGGACACC-3' | Bam HI | This study |
| Chr 2 | Chr2_50304382"EcoR1 | 5'-GATGAAATGACGCTCGCTTG-3' | 5'-GCCGGAAATCACACTTCACA-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'-AGACAACGCCGGAGAAGGTA-3' | 5'-TGGATACCCTGCATTCACCA-3' | Eco RI | This study |
| Chr 3 | Chr3_39227764"Hind3 | 5'-TAAGGAAGGTGGAGGCTGGA-3' | 5’-TCCCAACAGCGATTCACATC-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'-AGGACTTCCCCAGGGATTTG-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'-GCACGTTCTACTTCCTGCAATG-3' | 5'-CAAGCCACCTAGCCAGAAAA-3' | Eco RI | This study |
| Chr 5 | invvac* ${ }^{\text {6 }}$ | 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'-CAATGGCCAATCTGTCCTGA-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'-CGATAGAGCATCGGCATCAA-3' | 5'-AGCCAGCAGGGTCTCTTCAA-3' | Eco RI | This study |
| Chr 6 | Chr6_28786817"EcoR1 | 5'-GAGTGCGTGCCTGTGTGTTT-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'-GCTGATCTTCCTAGGTTGG-3' | 5'-GCATGAGTAATGCTCTCAGG-3' | Hae III | Taguchi et al. 2019 |
| Chr 7 | 2G14 | 5'-GGTTTGCACTTTTCTTAGATGG-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'-TGTAAACCGTCGTCCCTTCA-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'-AGCTGTTATGGAAGGTTCATG-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'-CCACTGTGCATCCATCACCT-3' | Eco RI | This study |
| Chr 8 | Chr8_43259046"EcoR1 | 5'-AGCGTGTTTCCCAGTTCAGA-3' | 5'-CAGTGGCTGCAAAAGTGGAC-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'-CGCCCAAAATCAGATCACAG-3' | 5'-GTCCTCCAATCACCCTTGCT-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'-TCCTTCTCСATATCCCAACACC-3' | 5'-GATCGTGGTGGAAGCTGATG-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'-CGGATCACCCTATTCATTTG-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.
${ }^{*}{ }^{*}$ 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
${ }^{*}$ 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 ${ }^{* 1}$.

|  |  | \％ | ${ }^{\prime \prime}$ |  | ${ }_{27} 7^{\text {ancos }}$ |  |  |  |  |  |  |  | 边 | 2 |  |  |  | ${ }^{4}$ | 隹 |  |  |  |  |  |  |  |  |  |  |  |  | aman | an |  |  | ， | Nas． |  |  |  |  |  |  | aream |  |  |  |  |  |  |  |
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|  |  |  |  |  |  |  |  |  |  |  |  |  | ${ }_{2}^{22}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| momen |  |  |  |  | $\frac{23}{23}$ | －${ }^{2}$ |  |  | $\frac{2}{2}$ | $\frac{3}{2}$ |  | 吕 | ${ }_{2}^{23}$ | ${ }_{2}^{23}$ | ， |  |  | $\frac{\square}{22}$ | $\frac{1}{2}$ |  |  |  |  |  |  |  |  | － | ${ }^{11}$ | ＂ | ${ }^{22}$ | － |  | ， |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  | $\stackrel{\frac{2}{2}}{2}$ |  |  |  | ${ }^{2}$ | ${ }_{3}^{23}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
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|  |  |  |  |  |  | ${ }_{2}^{2}$ |  |  | ${ }^{22}$ | － | $\stackrel{1}{1}$ | － | － | ${ }^{-2}$ | ${ }_{\text {c }}$ |  |  | ， |  |  | ＂ |  |  | ， |  |  | － |  | $\stackrel{\square}{1}$ | $\stackrel{\square}{\square}$ | ${ }_{2}^{2}$ | ＂11 | ， | $\stackrel{2}{21}$ |  |  | $\xrightarrow{\substack{20}}$ |  |  | ＂ |  |  |  |  |  |  |  | 18 |  |  |  |
|  |  |  |  |  |  |  |  |  | $\frac{22}{22}$ | $\frac{2}{23}$ |  |  | $\frac{12}{22}$ | $\frac{\square}{2}$ | $\frac{12}{1}$ |  |  |  |  |  |  |  |  |  |  |  | ${ }^{2}$ |  | 品 | 品 |  | ＂11 | ＂${ }^{\prime \prime}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |  | $\frac{12}{22}$ |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  | ${ }_{3}^{33}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 隹 |  |  |  |  |  |  |  |  |  | ${ }_{2}^{2}$ |  |  |  | ${ }_{\text {a }}^{3}$ | 品 |  |  |  |  |  |  |  |  |  |  |  |  | \％ | ， | ， |  | ＂110 | ， |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 隹 |  |  |  |  |  | \％ |  |  | ${ }^{22}$ | ${ }_{23}^{2}$ | ， | $\stackrel{12}{18}$ | ${ }_{2}^{22}$ | ${ }^{\frac{2}{2}}$ | ${ }^{\text {che }}$ |  |  | ${ }^{2}$ | ＂110 |  | ${ }^{11}$ |  |  | － |  |  | － | 告 | ${ }^{\text {\％}}$ | ， | $\cdots$ | ${ }^{\prime \prime}$ | $\stackrel{11}{*}$ | $\stackrel{3}{3}$ |  |  | ＂ |  |  |  |  |  |  | － |  |  |  | － |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  | － | ${ }^{\frac{2}{2}}$ | ${ }^{\text {che }}$ |  |  |  |  |  |  |  |  |  |  |  | $\stackrel{2}{2}$ | ${ }^{2}$ | ， | $\stackrel{11}{41}$ |  | ${ }^{\text {il }}$ | ＂${ }^{11}$ |  |  |  | － |  |  |  |  |  |  |  |  |  |  | ${ }^{2}$ |  |  |  |
| 隹 |  |  |  |  |  |  |  |  |  |  |  | ， | － | $\stackrel{5}{2}_{\frac{2}{20}}^{8}$ | ${ }^{1}$ |  |  |  |  |  |  |  |  |  |  |  | ${ }^{\frac{2}{32}}$ |  | $\stackrel{\text {＂}}{\text {＂}}$ | $\stackrel{1}{ }$ |  | ${ }^{\text {i11 }}$ | ＂${ }^{\text {＂1 }}$ |  |  |  | $\stackrel{\square}{18}$ |  |  |  |  |  |  | ${ }^{\prime \prime}$ |  |  |  | $\stackrel{\text { 22 }}{\substack{12}}$ |  |  |  |
|  |  | ， |  |  | $\frac{23}{2 .}$ | ＂12 | ， |  | ${ }_{2}^{22}$ | ${ }_{\text {2 }}^{2}$ | ， | － | \％ | ， | ， |  | － | $\frac{22}{22}$ |  | ， | ＂I＇ |  | $\stackrel{21}{21}$ | ＂ | － | ＂${ }^{1 / 2}$ | $\frac{22}{22}$ | ${ }^{\frac{20}{2}}$ | ， | $\stackrel{2}{2}$ | 22 | ＂11． | ， |  |  | － | $\frac{12}{32}$ | 㫛 |  |  |  |  |  | ${ }^{11}$ |  |  |  |  |  |  |  |
| 隹 |  |  |  |  |  |  |  |  |  |  |  |  |  | ${ }^{2}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 隹 |  |  |  |  | ${ }^{2}$ | － | 2 | 号 | $\frac{23}{22}$ | －${ }^{\frac{23}{22}}$ | \％ | $\stackrel{\text { in }}{ }$ | － | $\frac{3}{28}$ | ${ }^{\circ}$ |  | ， | $\stackrel{28}{\text {＋18 }}$ | － | ${ }^{2}$ | ＂ |  | ${ }^{2}$ | ${ }^{12}$ | ${ }^{22}$ | ＝ | － | $\frac{\square}{\frac{3}{2}}$ | ＂ | $\stackrel{4}{4}$ | $\frac{2}{2}$ | ＂ | ＂ | ${ }_{-}^{2}$ |  | ＂ | $\stackrel{\square}{28}$ | $\stackrel{1}{\square}$ |  | ＂ |  |  |  | ＂1＂ |  |  | ＂ | $\frac{28}{22}$ | 2 |  |  |
| 隹 |  |  |  |  | ${ }^{17}$ | －${ }_{2}^{22}$ |  |  | $\frac{22}{22}$ | （220 | 2 | －${ }_{\text {in }}^{1 / 2}$ | － | $\stackrel{3}{2}_{2}^{2}$ |  |  |  | ${ }_{2}$ | $\stackrel{12}{12}$ | ${ }_{2}^{2}$ | ， |  |  | － |  |  | $\frac{22}{22}$ |  | ${ }^{\prime \prime}$ | ＂ | ${ }^{2}$ | ${ }^{11}$ | ${ }^{1}$ | ， 2 |  |  | ${ }^{1}$ | $\stackrel{1}{\square}$ |  |  |  |  |  | ＂ |  |  |  | － |  |  |  |
|  |  |  |  |  |  |  |  |  | ${ }_{2}$ | $\frac{23}{\frac{23}{3}}$ | ， | 吅 | $\frac{22}{18}$ | $\frac{3}{2}$ | \％ |  |  | $\frac{12}{22}$ |  |  |  |  |  |  |  |  | ${ }_{2}^{22}$ | ${ }^{\frac{2}{20}}$ | ${ }^{1}$ | 㫛 | －${ }^{2}$ | ${ }^{11}$ | ， | 3 |  | ＂ | －${ }^{12}$ | ， |  |  |  |  |  | ${ }^{\text {in }}$ |  |  |  | ${ }^{2}$ |  |  |  |
|  |  |  |  |  |  |  |  |  | $\frac{22}{22}$ | （2）${ }_{2}^{2}$ | ， | $\stackrel{\square}{12}$ | $\frac{22}{22}$ | $\frac{3}{23}$ | ${ }_{-1}^{\square}$ |  |  | ${ }_{2}^{21}$ | ， |  | ${ }^{\prime \prime}$ |  |  |  |  |  | ${ }_{2}^{2}$ | \％ | ＂ | ＂ |  | － | ， | ${ }^{\frac{3}{2}}$ |  |  |  | ， |  |  |  |  |  | ＂ |  |  |  | ${ }^{2}$ |  |  |  |
| 隹 |  |  |  |  |  | ${ }_{2}^{2}$ |  |  | ${ }^{2}$ | ${ }^{2 .}$ | － | 㫛 | ${ }_{2}^{22}$ | ${ }^{2}$ | ${ }_{\text {c }}$ |  |  | ${ }^{22}$ | ${ }_{5}^{3}$ |  | ${ }^{11}$ |  | 2 | ${ }^{2}$ | ${ }_{2}^{28}$ |  | － | ${ }^{\frac{2}{2}}$ | ＂ | ${ }_{4}^{4}{ }^{\text {in }}$ | ，${ }_{2}{ }^{\frac{2}{2}}$ | ${ }^{12}$ | ， | ，${ }_{\text {，}}^{3}$ |  |  | ＂ | $\stackrel{1}{\square}$ |  |  |  |  |  | ${ }^{11}$ |  |  |  |  |  |  |  |
| 为 |  |  |  |  | ， | ${ }_{2}^{23}$ | ， |  | ${ }_{2}^{2}$ | ${ }_{2}^{2}$ | 号 | $\stackrel{i_{2}^{2}}{\square}$ | ${ }_{2}^{22}$ | ${ }^{\frac{2}{3}}$ |  |  |  | ${ }_{\text {in }}$ |  |  | ${ }^{11}$ |  |  | － | ${ }_{\text {in }}^{\stackrel{2}{2}}$ |  | $\stackrel{2}{2}$ |  | ${ }^{\prime \prime}$ | $\stackrel{14}{i_{2}^{23}}$ | ${ }_{2}^{2}$ | － | $\stackrel{11}{*}$ | ${ }^{-1}$ |  |  | $\xrightarrow{\substack{23 \\ 23}}$ |  |  |  |  | ${ }^{11}$ |  | ${ }^{\text {＂11 }}$ |  |  | $\stackrel{1}{1}$ | ${ }_{\text {\％}}^{\substack{20}}$ |  |  |  |
|  |  |  |  |  |  | ${ }_{2}^{22}$ |  |  |  |  |  | 㫛 | － | ${ }^{\frac{2}{2}}$ | \％ |  |  |  |  |  | － |  |  |  |  |  | $\stackrel{\text { 22 }}{22}$ | cren | ${ }^{\text {che }}$ |  |  |  | ＂ |  |  |  |  |  |  |  |  |  |  | ${ }^{\prime \prime}$ |  |  |  | ${ }_{2}^{22}$ |  |  |  |
|  |  |  |  |  |  |  |  |  |  | $\stackrel{y}{\square}$ |  |  |  | $\stackrel{1}{\square}$ |  |  |  |  |  |  |  |  |  |  |  |  | ${ }^{\frac{22}{22}}$ | ${ }^{\text {and }}$ | $\stackrel{\square}{\square}$ |  |  |  | $\stackrel{\frac{2}{2}}{2}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 隹 |  |  |  |  |  |  |  |  | $\stackrel{11}{12}$ | $\stackrel{11}{\square}$ | － | ${ }_{12}^{12}$ |  | 退 | ， |  |  |  |  |  | ${ }^{1 \prime}$ |  |  | ， |  |  | ${ }^{\frac{22}{2}}$ |  | $\stackrel{1}{11}$ |  | － | ＂ | I＇ |  |  |  | $\stackrel{2}{18}$ |  |  |  |  |  |  | ${ }^{\prime \prime}$ |  |  |  |  |  |  |  |
| 为 |  |  |  |  | ${ }_{2}^{2}$ | ${ }^{2}$ | ， |  | $\stackrel{1}{4}$ | ${ }^{11}$ | － | $\cdots$ | ${ }_{22}$ | － | － |  |  | $\stackrel{\square}{\square}$ | － |  | ＂ |  |  | $\square$ | ${ }_{-1}$ | 边 | $\stackrel{2}{2}$ | $\stackrel{3}{ }{ }^{\text {a }}$ | ＂ | ${ }^{-2}$ | $\stackrel{2}{2}$ | － | ， | ${ }^{22}$ |  |  |  |  |  |  |  |  |  | ， |  |  |  | ${ }^{22}$ |  |  |  |
|  |  | ， |  |  | － | ${ }_{\text {\％}}^{12}$ |  |  | $\stackrel{22}{22}$ | ${ }^{2}$ | ${ }^{\frac{2}{1}}$ |  | － | ${ }^{2}$ | \％ |  |  | ＂ | $\stackrel{11}{12}$ | 2 | ${ }^{11}$ |  | － | － | ${ }^{11}$ | ， | ${ }^{11}$ | ${ }^{\prime \prime}$ | ＂${ }^{\text {II }}$ | $\stackrel{11}{1 / 2}$ | ${ }_{2}{ }^{2}$ | ${ }^{\frac{3}{3}}$ | $\stackrel{11}{11}$ | ${ }^{2}$ |  | ${ }^{2}$ | $\stackrel{12}{12}$ | ＂ |  | ， |  | － |  | －1 |  |  |  | 2 |  |  |  |
| 隹 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ${ }_{22}$ |  |  |  |  |  |  |  |
| mencememo bemmex |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

Table S3 Pairwise population $F_{s t}$ 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.


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