Introduction

Sugar beet (Beta vulgaris subsp. vulgaris L.) accounts for around 20% of the world sugar production (http://www.fao.org/faostat/en/). The history of domestication of B. vulgaris began with the use of leaf beet in Greek Mediterranean countries some 3,000 years ago, with the expanded hypocotyl and root later becoming an important vegetable. During the Middle Ages, larger roots suitable for livestock fodder were developed in northern Europe (Biancardi et al. 2010). The discovery that variants of fodder beets contained the same type of sugar as sugar cane (Saccharum officinarum L.) led to the selective breeding of the sugar beet in Germany from the end of the 1700s. This selection led to the first sugar beet variety, “Weisse Schlesische Rube” (White Silesian beet) (Fischer 1989). The selection of a single high-sugar-accumulating fodder beet line made the genetic diversity of the initial population extremely small through founder effects. Thus the onset of beet improvement for sugar is quite recent, c.a. 200 years ago at the most, compared to other major crops.

From the inception of sugar beet cultivation until some 100 years ago, breeding lines developed from European and the United States germplasm were largely open-pollinated. 

Genetic and phenotypic assessment of sugar beet (Beta vulgaris L. subsp. vulgaris) elite inbred lines selected in Japan during the past 50 years

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Genetic diversity of Japanese sugar beet elite inbred line diversity (JSBDIV) set consisting of 63 lines was investigated using 33 cleaved amplified polymorphic sequence and 38 simple sequence repeat analyses. JSBDIV set was significantly subdivided into six (pedigree information), seven (Neighbor-Joining method) or 12 (population structure analysis) groups. The highest value of a pairwise population differentiation estimate, \(\Phi_{PT}\), value, among groups was yielded from population structure analysis with explained variation 32%. Some of the groups defined in this study exhibited close association with ancestral open-pollinated varieties (OPVs), suggesting that inter-OPV cross was rare during the establishment of JSBDIV set. On the other hand, low \(\Phi_{PT}\) values between some groups suggest that genetic backgrounds of ancestral OPVs had historically overlapped to some extent. Phenotypic traits showed significant differences both among and within groups. A nearly identical group was identified as the highest sugar content group irrespective of the grouping methods. Groups with Aphanomyces root rot resistance are associated with an OPV ‘Tmm-1’, suggesting it as a source of this trait. ‘Tmm-1’ is also associated with Cercospora leaf spot resistance, but an exceptional resistant line with no association of ‘Tmm-1’ supports a notion that different genetic resources exist for this trait.

Key Words: structure analysis, sugar beet, OPV, heterotic pool, breeding.

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Abbreviations: ADM: admixture, AMOVA: analysis of molecular variance, AR: Aphanomyces root rot, CAPS: cleaved amplified polymorphic sequence, CLS: Cercospora leaf spot, CMS: cytoplasmic male sterility, EST: expressed sequence tag, F: coefficient of parentage (probability of identity by decent), GD: genetic distance, GS: genetic similarity, HARC: Hokkaido Agricultural Research Center, HEGS: high efficiency genome scanning, JSBDIV: Japanese sugar beet elite inbred line diversity set, K: number of populations, NARO: National Agriculture and Food Research Organization (of Japan), NIT: \(\alpha\)-amino nitrogen, NJ: neighbor-joining (method), OPVs: open-pollinated variety, POT: potassium content, QTL: quantitative trait loci, RAPD: random amplified polymorphic DNA, RR: Rhizoctonia root rot, RW: root weight, SC: sugar content, SNP: single nucleotide polymorphism, SOD: sodium content, SSR: simple sequence repeat, SST: simple sequence repeat analysis, TBE: Tris/Borate/EDTA (buffer)
varieties (OPV). In the mid-20th century, new hybrid breeding programs were launched in both European countries and the United States, after the discovery of various Mendelian and non-Mendelian characters including cytoplasmic male sterility (CMS) (Owen 1945), monogermity gene ‘m’ (Savitsky 1950) and self-fertility gene ‘Sf’ (Owen 1942): CMS was necessary to obtain pure hybrids because sugar beet bears hermaphroditic flowers. Deploying m saved the labor for thinning the excess seedlings in the field. Sf made the production of inbred line possible otherwise sugar beet is self-incompatible. This breeding system principally aimed to utilize heterosis through the efficient production of hybrid seeds using a monogerm male sterile line, and it remains the main breeding strategy today. This system has been successfully introduced into Japan since 1960s, and breeders have developed many elite breeding lines. With 50 years of breeding records in Japan, the coefficient of parentage showed a clear pedigree kinship among the elite breeding lines (Taguchi et al. 2006). They were derived from ancestral cultivars originated from as few as ten OPVs and detailed pedigree information is unavailable for some lines, making genetic relationship between the elite breeding lines and the ancestral OPVs obscure. Hence, it is unclear how similar the genetic structure of current elite breeding lines are.

The coefficient of parentage (F) (Kempthorne 1957) is an important measure in estimating genetic diversity based on pedigree. It indirectly measures the genetic diversity among cultivars by estimating the probability that alleles at a locus are identical by descent. However, assumptions of ancestry, selection pressure, and genetic drift need to be considered when calculating F. Since these assumptions may be violated in whole or in part due to uncertainties, alternative measures of genetic diversity have been developed, such as genetic distance (GD) (Nei 1972) and the genetic similarity (GS) (Hedrick 1971) based on molecular marker analysis. These methods can be used to infer a phylogenetic relationships. From DNA polymorphisms, genetic structure can be discriminated as groups or subgroups (Pritchard et al. 2000). Such analyses have been conducted for maize (Zea mays L.), rice (Oryza sativa L.), and Aegilops species, among others (Garris et al. 2005, Liu et al. 2003, Mizuno et al. 2010, Reif et al. 2005, Yamazaki and Idet 2013). Recently, such analyses has become a promising approach for understanding genetic diversity within elite breeding lines in sugar beet (Adetunji et al. 2014, Li et al. 2010, 2011, Mangin et al. 2015, Simko et al. 2012, Stevanato et al. 2014). Structure analysis at the genome-wide level has proven to be useful in clarifying whether varieties and lines are derived from single or multiple origins. The degree of variation and the frequency of alleles help one to estimate genetic admixture in each variety or line, thereby serving to estimate contributions for genomic regions that have been mixed in the past. In the present study, the genetic structure and diversity of the elite sugar beet lines was analyzed to assess the extent of genetic contribution of each ancestral OPV.

The authors sought to (i) examine the population structure of Japanese elite inbred lines, (ii) quantify genetic diversity within and among subgroups of Japanese elite inbred lines, and (iii) assess the contribution of pedigree and molecular characteristics for the phenotypic variation against agronomically important traits in the Japanese elite inbred lines.

Materials and Methods

Plant material

A total of 63 inbred lines consisting of 61 inbreds (prefixed with ‘NK’) and two different sources of inbred lines were used in this study. Of the 63 inbreds, 62 lines are selections for monogerm and CMS maintainer genotypes (hence considered as seed parent). PS56 is a multigerm, non-CMS maintainer line. This suite of materials was designated the Japanese Sugar Beet inbred line DIVersity set (JSBDIV). These lines were chosen to encompass the diversity of seed parents available in Japanese hybrid breeding from the 1960’s to the present. Materials were developed by National Agriculture and Food Research Organization (NARO)-Hokkaido Agricultural Research Center (HARC) in Japan.

DNA isolation and genotyping with molecular markers

Total cellular DNA was extracted from fresh green leaves according to the procedure described by Roger and Bendich (1988). Cleaved Amplified Polymorphic Sequence (CAPS) markers were developed as follows: using primers of Single Nucleotide Polymorphism (SNP) marker sets (Mohring et al. 2004) and Expressed Sequence Tag (EST) marker sets (Schneider et al. 1999), PCR products were generated, then digested with one of thirteen restriction endonucleases: Hae III, Hha I, Taq I, Hap II, Mbo I, Afa I, Xsp I, Aiu I, Acc II (Takara Bio, Ohtsu, Japan), TspE I (TOYOBO, Osaka, Japan), Mse I, HpyCh4 IV and Nla III (New England BioLabs, Ipswich, MA) (Supplemental Table 1). Restriction digests were electrophoresed in 2% agarose gels. Thirty-two simple sequence repeat (SSR) markers used in this study were reported previously (Laurent et al. 2007, McGrath et al. 2007) (Supplemental Table 1). Cycling parameters were 94°C for one min, 40 cycles of 50 or 60°C for one min, followed by one cycle at 72°C for 10 min. Amplified products were electrophoresed in a High Efficiency Genome Scanning (HEGS) system (Kikuchi et al. 2004) using a discontinuous non-denatured acrylamide gel and Tris/Borate/EDTA (TBE) buffer. Gels were scanned after staining with Sybr green I (Molecular Probes, Eugene, OR) and photographed under a UV trans-illuminator (ATTO, Tokyo, Japan).

Calculation of coefficient of parentage and genetic distance

Based on Kempthorne (1957), Cox et al. (1985) and Taguchi et al. (2006), coefficient of parentage between two genotypes (F) was set at 0 for remote ancestors, and coefficient of inbreeding (fI) was set 1 as inbred lines and 0.5 as...
OPVs, respectively.
For each genotype, $F$ was calculated by using following equation:

$$F_{xy} = \sum_{i=1}^{n} \left( \frac{1}{2} \right)^{T_i} \left( 1 + f_i \right) / 2$$

where $S_i$, $T_i$, and $f_i$ denote total cross number of common ancestor OPV $(i)$ to cultivar $X$, total cross number of common ancestor OPV $(i)$ to cultivar $Y$, and coefficient of in-breeding of common ancestor OPV $(i)$, respectively.

Gene frequencies were tabulated and input into a phylogenetic analysis package PHYLIP (Felsenstein 1989) to calculate genetic distance (GD) and construct Neighbor-Joining (NJ) tree (Saitou and Nei 1987). Trees were drawn with TreeView X (http://darwin.zoology.gla.ac.uk/~rpage/treeviewx/download.html). Pearson’s correlations were determined between GD and $F$.

**Population structure**

STRUCTURE (ver 2.2) software (Pritchard et al. 2000) was used to detect population structure and assign cultivars to subgroups, using an admixture model and correlated allele frequencies. Posterior probabilities were estimated using a Markov chain Monte Carlo method based on $2 \times 10^6$ iterations following a burn-in period of $1 \times 10^6$ iterations. At least ten runs were performed by setting the number of populations $(K)$ at integer values from 1 to 15. Final subgroups were based on the likelihood plots of these models. The coefficients of each membership were estimated and compared with pedigree records.

**Analysis of molecular variance**

An analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was performed to partition variance converted from molecular divergence between ‘Pools’ (groups based on pedigree data, see Results), ‘Groups’ (groups inferred by neighbor-joining (NJ) method) or ‘Pops’ (groups inferred by population structure analysis), and within subpopulations or the subgroups. Pairwise population differentiation values $(\Phi_{PT})$ and Nei’s genetic distance between groups were calculated by GenAIEx 6.2 (Peakall and Smouse 2006). $\Phi_{PT}$, an Fst analog, suppresses within-population variance, and simply calculates population differentiation based on the genotypic variance. Probability values were estimated by 9999 permutations to determine whether the partitioning of variance components was significant.

**Evaluation of yield component traits**

JSBDIV set and standard commercial varieties were evaluated in routine plant breeding trials (randomized block design) with four replicates at NARO-HARC, Japan in 2004 and 2006. Field trials were carried out in NARO-HARC fields (Memuro, Japan). Materials were seeded in paper pots March 17, 2004. Seedlings were transplanted on April 25, 2004. Individual plot size was 4.05 m$^2$ and the final plant density was 30 plants per plot ($\approx$74,000 plants ha$^{-1}$). In total 120 plants (i.e., 30 plants/subplot) were evenly distributed into four replications. Roots (i.e., 26/subplot) were harvested by hand on October 10. RW (fresh weight/plant), SC (sucrose percentage of fresh weight), POT (potassium) (meq hg$^{-1}$), SOD (sodium) (meq hg$^{-1}$) and NIT (α-amino nitrogen) (meq hg$^{-1}$) were determined using a Venema automated analysis system Hokunoshi (Venema Installations B. V., Borkumwegm, Netherland) under a modified Sachs-Le Docte method (Le Docte 1927). Beet brei was clarified with $\mathrm{Al}_2(\mathrm{SO}_4)_3$. POT and SOD were determined by flame photometry, SC by polarimetry, and NIT with the OPA-method (Burba and Georgi 1976). Data were averaged across replicates. Statistical analyses were conducted using the statistical program package R.

**Evaluation of resistance to Aphanomyces root rot, Cercospora leaf spot and Rhizoctonia root rot**

Materials were seeded on paper pot at April 11, 2006 and seedlings were transplanted on May 16, 2006. The transplanted field was in an area of high *Aphanomyces* oomycete density in Ikeda, Hokkaido, Japan (details in Taguchi et al. 2009). Individual plot size was 1.49 m$^2$ and the final plant density was ten plants per plot ($\approx$6600 plants ha$^{-1}$). In total 40 plants (i.e., 10 plants/subplot) were evenly distributed into four replications. Roots were harvested by hand on October 3, and disease symptoms at the whole-plant level were assessed on an index of root rot severity (Taguchi et al. 2009) ranging from 0 (no symptoms) to 5 (fully decayed). The data were averaged across replicates.

For Cercospora leaf spot (CLS) resistance evaluation, individual plot size was 1.35 m$^2$ and the final plant density was ten plants per plot ($\approx$74000 plants ha$^{-1}$). In total 40 plants (i.e., 10 plants/subplot) of each line were evenly distributed into four replications. Cercospora *beticola* inoculum was prepared as follows: leaves expressing severe CLS symptom were collected from HARC fields, dried, and ground to a powder. In the subsequent year, inoculum (10 g, 2% powder/soil) was applied at the foot of each plant. Initial symptoms were observed roughly one month after inoculation and continued to be observed until September. Visual symptoms of CLS were rated on a range of 0 (no symptoms), to 5 (almost fully destroyed mature leaves) (Hokkaido Agricultural Research Station 1986). The data were averaged across replicates when the weakest genotypes reached around 5 on August 17, 2006.

For Rhizoctonia root rot (RR), *Rhizoctonia solani* AG II-2 inoculum was cultured on barley (*Hordeum vulgare* L.) grains (Gaskill 1968, Naito et al. 1993). The infested barley grains were dried and applied on the root surface of each plant (about 10 grains per plant) on July 3. Visual symptoms of RR were rated on a range from 0 (no symptoms), to 5 (fully decayed) (Hokkaido Agricultural Research Station 1986). The symptoms were observed roughly one month after inoculation on August 1st. Data were averaged across replicates and analyzed by R.
**Results**

**Grouping by pedigree information**

Japanese elite inbred lines that are the constituents of current hybrid cultivars were, in general, descended from OPVs, of which seven most important ones are ‘TA-15’, ‘TA-30’, ‘TA-36’, ‘TA-37’, ‘TA-27’, ‘Tmm-1’, and ‘Tmm-14’ (Taguchi *et al.* 2006). We re-analyzed the data of Taguchi *et al.* (2006), and the mean coefficient of parentage for 61 inbred lines constituting JSBDIV set was calculated as $F = 0.11$, a low value indicating a potentially high degree of diversity in Japanese hybrid breeding stock (Supplemental Fig. 1A). Taguchi *et al.* (2006) reported that each of the seven OPVs contributed unevenly to the inbred lines. For example, contribution of ‘TA-15’ is 66% in five inbreds, 33% in two inbreds and none in the others (Taguchi *et al.* 2006). In this study, we focused on the best contributed OPV in each of the inbreds. From JSBDIV set, we selected 50 inbreds that had the best contributed OPV with the score of 38% or more. The 50 lines were divided into 6 distinct groups according to the best OPV; ‘TA-15’ is the best OPV in five lines, ‘TA-30’ in 15 lines, ‘TA-36’ in ten lines, ‘TA-37’ in 11 lines, ‘Tmm-1’ in five lines, and ‘Tmm-14’ in four lines. Genetic contribution of ‘TA-27’ is less than 38% in any of the inbreds. The remaining 13 inbreds were considered to be admixture (ADM). We refer these groups as ‘Pools’, e.g. ‘TA-15’ Pool.

**Grouping by genetic distance**

JSBDIV set was analyzed by 33 SSR and 38 CAPS markers, respectively. The SSRs and CAPS gave 2.5 alleles per locus (ranging 2–4 alleles/locus) and 2.1 alleles per locus (ranging 2–4 alleles/locus), respectively (Supplemental Table 1). For further analysis, we selected markers that exhibited clear and discriminating finger print patterns, and excluded markers with rare and null alleles. We obtained a total of 4473 genotyping data, in which there were 30 heterozygous genotypes (0.7%). This indicated that each

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**Fig. 1.** Dendrogram for the JSBDIV produced by the neighbor joining method. The name of inbred lines describes abbreviate such as “NK***” from “NK-***mm-O”.

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inbred in JSBDIV set was highly homozygous. Mean GD in the JSBDIV set was 0.61, ranging between $0.01 \leq GD \leq 1.04$ (Supplemental Fig. 1B). Based on genetic distance matrices, we drew a phylogenetic tree using the NJ method (Saitou and Nei 1987). The phylogenetic tree generated classified inbred lines into seven groups and three solitaries (Fig. 1). The groups consisted of five to 14 inbred lines. We refer groups defined by the phylogenetic tree as ‘Groups’. e.g. Group I.

**Grouping by population structure**

To investigate the degree of relatedness, genetic diversity of inbred lines were assessed according to their population structure. A model-based clustering with maximum likelihood revealed that the optimum population $K$ value was 12 (Supplemental Data 1, 2). The posterior probability at $K = 12$ was by far the highest among the models assuming $1 \leq K \leq 15$. Thus, we defined 12 groups in JSBDIV set, which were designated as Pops A to L (Fig. 2) consisting of 2–8 inbred line: Pop K was the largest group ($n = 8$), whereas Pop H was the smallest ($n = 2$). The remaining 13 inbreds were gathered as admixture (ADM).

Fig. 2. Model-based clustering ($K = 12$) in JSBDIV with a total of 71 CAPS and SSR markers. Color codes indicate typical genotype of the inferred subgroup (A–L). The name of inbred lines describes abbreviate such as “NK***” from “NK-***mm-O”.

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Relationship among groups defined by different methods

The relationship among ‘Pools’, ‘Groups’ and ‘Pops’ is an interesting subject. We compared these groups in terms of genetic contribution of the seven OPVs (Table 1). A significant Pearson’s correlation coefficient ($p \leq 0.0001$) was evident between $F$ and Nei’s GD, indicating some relationship between ‘Pools’ and ‘Groups’. Genetic contribution of the seven OPVs were averaged in each Pool and Group. Group V was observed to bear a high (49%) contribution from ‘TA-15’, suggesting close relationship to ‘TA-15’ Pool. In fact, Group V contained all the five inbreds of ‘TA-15’. Group V contained all the five inbreds of ‘TA-15’, suggesting close relationship to ‘TA-15’ Pool. In fact, Group V contained all the five inbreds of ‘TA-15’. Genetic contribution was less than 38% from any of the seven OPVs. However, of note is that the greatest contributions for Groups VI and VII was than 38% from any of the seven OPVs. Among the seven OPVs, ‘TA-30’ contributed 5% or less for all the pops. Although figures of ‘Tmm-1’ contribution were less than 38%, its contribution appears to be unignorable to all the Pops (7–26%).

Genetic diversity, molecular variance, and relationships between groups

We inferred inter- and intra-group diversity in Pools, Groups and Pops by AMOVA analyses (Table 2). The appreciable amount of variation among Pools was obvious (16%), as well as within the Pools (84%). Probabilities of $\Phi_{PT}$ values (0 [no difference] to 1 [complete difference]) were significant ($P \leq 0.001$) between the six Pools, supporting the notion of diversity between them. The $\Phi_{PT}$ value between ‘TA-30’ and ‘Tmm-14’ Pools was the smallest (0.09) whereas that between the ‘TA-15’ and ‘Tmm-14’ Pools was the largest (0.46). The ‘TA-15’ Pool appeared to be the most differentiated from the other groups (Table 3a).

Similar approach was taken to infer the diversity in Groups. The AMOVA analyses of Groups indicated the appreciable amount of genetic variation among the Groups (26%), as well as within the Groups (74%). $\Phi_{PT}$ value between Groups III and VI was the smallest (0.14), whereas that between Groups II, IV and V, and between Groups VI and VII were the largest (0.36) (Table 3b).

As for Pops, AMOVA analyses indicated the appreciable amount of genetic variation among Pops (32%), as well as within Pops (68%). The average $\Phi_{PT}$ value was higher than those in Pools and Groups (Table 3c), suggesting that structure analysis is better to discriminate JSBDIV set. The $\Phi_{PT}$ value among Pops showed the delineation between Pop I and Pop H to be the weakest (0.11), whereas that between Pop G and Pop D was the strongest (0.74). $\Phi_{PT}$ values involving Pops B, D, and G were relatively high (their medians of $\Phi_{PT}$ are 0.56, 0.47, and 0.5, respectively), suggesting that they are well isolated groups.

Agronomically important traits of groups

Yield components (207 days after seedling) and three disease resistances were investigated in JSBDIV set. A wide range of variation was observed in each trait (mean ±
Breeding JSBDIV during the past 50 years

**Table 3.** Pairwise PhiPT values in three different classification methods

| a. Pedigrees information | TA-15 | TA-30 | TA-36 | TA-37 | Tmm-1 | Tmm-14 | ADM | Out.Group |
|--------------------------|-------|-------|-------|-------|-------|-------|-----|-----------|
| TA-15                    | 0.00  | 0.00  | 0.00  | 0.01  | 0.00  | 0.00  | 0.02|
| TA-30                    | 0.31  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.17|
| TA-36                    | 0.36  | 0.16  | 0.00  | 0.00  | 0.00  | 0.01  | 0.04|
| TA-37                    | 0.31  | 0.09  | 0.10  | 0.00  | 0.00  | 0.00  | 0.08|
| Tmm-1                    | 0.26  | 0.13  | 0.24  | 0.20  | 0.00  | 0.02  | 0.19|
| Tmm-14                   | 0.46  | 0.15  | 0.14  | 0.20  | 0.00  | 0.03  | 0.11|
| ADM                      | 0.30  | 0.08  | 0.02  | 0.10  | 0.16  | 0.13  | 0.17|
| Out.Group                | 0.33  | 0.04  | 0.10  | 0.07  | 0.08  | 0.14  | 0.07|

| b. NJ method |
|---------------|
| Group I | Group II | Group III | Group IV | Group V | Group VI | Group VII | Out.Group |
| 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.02    |
| 0.35    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    |
| 0.19    | 0.21    | 0.00    | 0.00    | 0.00    | 0.00    | 0.01    |
| 0.27    | 0.36    | 0.20    | 0.00    | 0.00    | 0.00    | 0.01    |
| 0.32    | 0.36    | 0.24    | 0.33    | 0.00    | 0.00    | 0.01    |
| 0.20    | 0.22    | 0.14    | 0.22    | 0.00    | 0.00    | 0.01    |
| 0.18    | 0.24    | 0.23    | 0.31    | 0.36    | 0.15    | 0.00    |
| 0.14    | 0.32    | 0.18    | 0.31    | 0.34    | 0.15    | 0.15    |

| c. Structure analysis |
|------------------------|
| Pop. A | Pop. B | Pop. C | Pop. D | Pop. E | Pop. F | Pop. G | Pop. H | Pop. I | Pop. J | Pop. K | Pop. L | ADM |
| 0.00    | 0.02    | 0.01    | 0.10    | 0.00    | 0.03    | 0.00    | 0.02    | 0.01    | 0.02    | 0.10    | 0.18    |
| 0.44    | 0.00    | 0.00    | 0.02    | 0.01    | 0.01    | 0.05    | 0.01    | 0.00    | 0.00    | 0.01    | 0.02    | 0.00    |
| 0.32    | 0.38    | 0.00    | 0.01    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.01    | 0.01    | 0.01    |
| 0.48    | 0.67    | 0.25    | 0.00    | 0.02    | 0.03    | 0.10    | 0.13    | 0.01    | 0.02    | 0.10    | 0.30    |
| 0.38    | 0.59    | 0.30    | 0.35    | 0.00    | 0.01    | 0.05    | 0.01    | 0.00    | 0.00    | 0.01    | 0.02    | 0.08    |
| 0.29    | 0.49    | 0.33    | 0.52    | 0.39    | 0.00    | 0.07    | 0.01    | 0.00    | 0.01    | 0.03    | 0.03    | 0.01    |
| 0.50    | 0.63    | 0.35    | 0.74    | 0.46    | 0.46    | 0.00    | 0.03    | 0.02    | 0.00    | 0.10    | 0.03    | 0.03    |
| 0.36    | 0.56    | 0.26    | 0.16    | 0.30    | 0.35    | 0.53    | 0.00    | 0.00    | 0.03    | 0.01    | 0.02    | 0.30    |
| 0.39    | 0.56    | 0.35    | 0.45    | 0.35    | 0.42    | 0.53    | 0.41    | 0.00    | 0.00    | 0.01    | 0.00    |
| 0.22    | 0.37    | 0.25    | 0.14    | 0.24    | 0.28    | 0.42    | 0.31    | 0.01    | 0.32    | 0.00    | 0.01    |
| 0.37    | 0.39    | 0.32    | 0.51    | 0.39    | 0.43    | 0.39    | 0.47    | 0.44    | 0.30    | 0.00    | 0.02    |
| 0.39    | 0.64    | 0.31    | 0.47    | 0.49    | 0.40    | 0.67    | 0.40    | 0.43    | 0.26    | 0.47    | 0.00    |
| 0.06    | 0.31    | 0.13    | 0.04    | 0.08    | 0.18    | 0.26    | 0.03    | 0.19    | 0.19    | 0.21    | 0.00    |

standard deviation): RW = 526 ± 143 g (range: 228–1020 g); SC = 15.80 ± 1.65 % (range: 11.81–20.29 %); POT = 4.53 ± 1.03 meq hg⁻¹ (range: 2.65–7.96 meq hg⁻¹), SOD = 0.34 ± 0.17 meq hg⁻¹ (range: 0.15–1.21 meq hg⁻¹), and NIT = 1.91 ± 0.79 meq hg⁻¹ (range: 0.48–4.52 meq hg⁻¹) (**Supplemental Fig. 2**). The *Aphanomyces* root rot (AR) disease index (DI) variation was 1.7 ± 1.0 overall, indicating a wide variation in resistance. On the other hand, RR-DI variation was narrow distributed, and was 4.5 ± 0.5 overall, although resistant lines were few amongst inbred lines. CLS-DI variation was 3.3 ± 0.4 overall, also showing a narrow distributed frequency of variation in resistance. ANOVA among JSBDIV set indicated significant differences between lines in all trials (**Table 4, Supplemental Data 3**).

We examined whether Pools exhibit any of the agronomic characteristics. Statistics of agronomic characters are summerized in **Table 4**. ‘TA-15’ Pool was characterized as high SC and low SOD and POT, while the ‘TA-30’ Pool was characterized by relatively high SC and low POT and NIT. On the other hand, ‘TA-36’ Pool exhibited low SC, high POT and NIT. ‘Tmm-1’ Pool was characterized by high POT and NIT. With respect to disease resistances, ‘Tmm-1’ Pool showed extremely high resistance to AR. ‘Tmm-14’ and ‘Tmm-1’ Pools were relatively tolerant to CLS and the ‘TA-30’ Pool was relatively tolerant to RR. All the inbreds in ‘Tmm-1’ Pool were highly resistant to AR.

We next saw the agronomic characters in Groups. Group V was characterized with high SC. The second and the third highest SC groups were Group VII and Group II, respectively. Group IV was characterized as low SC and relatively tolerant to AR, CLS and RR.

As for Pops, Pop I was characterized as high SC. Pops A, B, H and F comprised another high SC group. As for disease resistance, Pop E was relatively tolerant to AR, CLS and RR. The following Pops are potential alternative source of disease tolerance (but see Discussion): Pop I for AR, Pop G for CLS, and Pop F for both CLS and RR.

**Discussion**

Pedigree information is indispensable for breeding programs; however, record is often incomplete, and tracing back the exact pedigree is difficult. The present study shows potential of molecular genetic data complementing this caveat. For example, it is difficult to estimate a ‘fi’ of ancestral OPVs presisely. In this study, ‘fi’ was fixed 0.5 in calculation for
Standard varieties for RR as follows; ‘Strong’ is ‘TK-80-2BR’, ‘Medium ~ Weak’ is ‘Starhill’, ‘Medium’ is ‘Leland’ and ‘Weak’ is ‘Starhill’.

Standard varieties for CLS as follows; ‘Strong’ is ‘Yukihinode’, ‘Medium’ is ‘Monohikari’ and ‘Weak’ is ‘Monohomare’.

Standard varieties for AR as follows; ‘Strong’ is ‘Hokkai. 90’, ‘Medium’ is ‘Monohomare’ and ‘Medium ~ Weak’ is ‘Kabutomaru’.

‘***’ means significant differences at 0.1% level in ANOVA (Analysis of variance).

### Table 4. Summary of phenotypic variation of JSBDIV in 3 different classification method

| Sub-population | RWS | SC (%) | SOD (meq/100g) | POT (meq/100g) | NIT (meq/100g) | Ave. RR-DI index (0~5) | Ave. CLS-DI index (0~5) | Ave. RR-DI index (0~5) |
|----------------|-----|--------|----------------|----------------|----------------|------------------------|------------------------|------------------------|
| **Reference variety** |     |        |                |                |                |                        |                        |                        |
| Monohomare      | 992 | 15.59  | 0.45           | 5.49           | 1.53           | –                      | –                      | –                      |
| Standard variety ‘Strong’ | –   | –      | –              | –              | –              | 0.8                    | 2.5                    | 2.8                    |
| Standard variety ‘Medium’ | –   | –      | –              | –              | –              | 1.5                    | 3.1                    | 4.3                    |
| Standard variety ‘Medium ~ Weak’ | –  | –     | –              | –              | –              | 2.1                    | –                      | –                      |
| Standard variety ‘Weak’ | –   | –      | –              | –              | –              | 3.7                    | 5.0                    | –                      |

### Pedigree information

| Pedigree information | Ave. RR-DI index (0~5) | Ave. CLS-DI index (0~5) | Ave. RR-DI index (0~5) |
|----------------------|------------------------|------------------------|------------------------|
| **TA-15**            | 1.3 ± 0.7              | 3.1 ± 0.2              | 4.7 ± 0.2              |
| **TA-30**            | 1.2 ± 0.8              | 3.2 ± 0.4              | 4.0 ± 0.5              |
| **TA-36**            | 2.6 ± 1.1              | 3.4 ± 0.3              | 4.9 ± 0.1              |
| **TA-37**            | 2.3 ± 1.2              | 3.6 ± 0.6              | 4.3 ± 0.6              |
| **Tmm-1**            | 0.6 ± 0.6              | 3.0 ± 0.4              | 4.2 ± 0.5              |
| **Tmm-14**           | 2.2 ± 0.7              | 2.9 ± 0.5              | 5.0 ± 0.1              |
| **ADM**              | 1.4 ± 0.9              | 3.6 ± 0.4              | 4.7 ± 0.3              |
| **Out.Group**        | 2.4 ± 0.9              | 3.4 ± 0.4              | 4.8 ± 0.3              |

### NJ method

| NJ method | Ave. RR-DI index (0~5) | Ave. CLS-DI index (0~5) | Ave. RR-DI index (0~5) |
|-----------|------------------------|------------------------|------------------------|
| **Group I**       | 2.2 ± 1.1              | 3.4 ± 0.4              | 4.6 ± 0.4              |
| **Group II**      | 1.8 ± 1.1              | 3.3 ± 0.4              | 4.8 ± 0.3              |
| **Group III**     | 2.0 ± 1.1              | 3.7 ± 0.6              | 4.4 ± 0.6              |
| **Group IV**      | 1.0 ± 0.7              | 3.1 ± 0.4              | 3.9 ± 0.5              |
| **Group V**       | 1.1 ± 0.9              | 3.4 ± 0.6              | 4.7 ± 0.3              |
| **Group VI**      | 2.0 ± 1.3              | 3.0 ± 0.4              | 4.2 ± 0.7              |
| **Group VII**     | 1.3 ± 0.8              | 3.5 ± 0.2              | 4.6 ± 0.4              |
| **Out.Group**     | 1.5 ± 1.1              | 3.5 ± 0.2              | 3.8 ± 0.7              |

### Structure analysis

| Structure analysis | Ave. RR-DI index (0~5) | Ave. CLS-DI index (0~5) | Ave. RR-DI index (0~5) |
|-------------------|------------------------|------------------------|------------------------|
| **Pop. A**        | 2.2 ± 1.0              | 3.2 ± 0.5              | 4.2 ± 0.7              |
| **Pop. B**        | 1.7 ± 1.5              | 3.5 ± 0.4              | 4.8 ± 0.3              |
| **Pop. C**        | 2.2 ± 1.1              | 3.7 ± 0.6              | 4.4 ± 0.6              |
| **Pop. D**        | 2.5 ± 0.4              | 3.1 ± 0.4              | 4.6 ± 0.2              |
| **Pop. E**        | 1.0 ± 0.7              | 3.1 ± 0.4              | 3.9 ± 0.5              |
| **Pop. F**        | 2.2 ± 1.1              | 3.3 ± 0.4              | 4.7 ± 0.3              |
| **Pop. G**        | 2.0 ± 1.3              | 3.0 ± 0.4              | 4.2 ± 0.7              |
| **Pop. H**        | 1.3 ± 0.8              | 3.5 ± 0.2              | 4.6 ± 0.4              |
| **Pop. I**        | 1.5 ± 1.1              | 3.5 ± 0.2              | 3.8 ± 0.7              |

### Pedigree

| Pedigree | Ave. RR-DI index (0~5) | Ave. CLS-DI index (0~5) | Ave. RR-DI index (0~5) |
|----------|------------------------|------------------------|------------------------|
| **Out.Group**     | 2.2 ± 1.0              | 3.2 ± 0.5              | 4.2 ± 0.7              |
| **Group**        | 1.7 ± 1.5              | 3.5 ± 0.4              | 4.8 ± 0.3              |
| **Sub-population** | 2.2 ± 1.1              | 3.7 ± 0.6              | 4.4 ± 0.6              |
| **line**         | 2.0 ± 1.3              | 3.0 ± 0.4              | 4.2 ± 0.7              |

### Convenience, because it must be over-/under-estimation if 1 or 0 were used as ‘fi’. However, it was confirmed that there was no big difference in tendency for estimation of coefficient of parentage even if ‘fi’ was from 0.2 to 0.8 (Taguchi et al. 2006). This is because most pedigree information of Japanese sugar beet were recorded at least five cross generations, so that the effect of changes in ‘fi’ value was relatively small on the result.

Assessments using a phylogenetic approach or genetic structural analysis helped to elucidate relationship between inbreds of JSBDIV set. In our assessment, we adopted DNA markers independently developed by different research groups; they used their original populations in Europe or USA (Laurent et al. 2007, McGrath et al. 2007, Möhring et al. 2004, Schneider et al. 1999). We used CAPS and SSR to avoid the influence of marker type. We also note that these markers were developed not only from sugar beet × sugar beet crosses but also from sugar beet × wild beet and table beet × sugar beet crosses. Accordingly, our marker set is unbiased and fairly well covered the genome (see

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**Note:**

Table 4. Summary of phenotypic variation of JSBDIV in 3 different classification method.

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**Taguchi, Kuroda, Okazaki and Yamasaki**
Supplemental Table 1.

In sugar beet, several sets of diploid hybrid varieties and breeding lines were analyzed by using DNA markers. Simko et al. (2012) used Diversity Array-Technology (DArT), SNP and SSR markers, and described that SSR was the most efficient marker for polymorphism detection. In the present study, we observed that the number of alleles per locus was comparable between CAPS and SSR but slightly higher in SSR. Influence of marker type appears to be very small, if any, to infer the population structure of sugar beet (Adetunji et al. 2014, Inghelandt et al. 2010, Li et al. 2010, 2011, Mangin et al. 2015, Simko et al. 2012).

Population structure inferred from the polymorphic data has been associated with the breeding background. Simko et al. (2012) revealed three well-defined populations of varieties that generally correlates with their seed company origin. Li et al. (2010, 2011) distinguished two populations, seed-parents and pollen-parents, in 289 inbreds. We revealed 12 populations in JSBDIV set. Of note is that these populations more or less reflect their pedigree. A good example is Pop I: it was genetically contributed by mainly ‘TA-15’, which is consistent with the data that five of the seven Pop I members are shared with ‘TA-15’ Pool. Therefore, it is possible that population structure inferred by molecular data can complement pedigree information. This can be practically important when the missing pedigree data of a breeding material is needed. On the other hand, Groups, in general, less reflect pedigree information than Pops: strong association was seen only between Group V and ‘TA-15’ Pool. This may suggest that pedigree information is not always interconvertible with simple GD.

Our data suggest some underrealized aspects of Japanese sugar beet breeding. In its initial stage, OPVs from Europe and the United States were introduced and selected for the adaptability to Japanese climate and to cultivation methods (Hasegawa and Takeda 1982). Characters such as high sugar content, bolting tolerance, and disease resistance were favoured. From the 1960s, new breeding program using monogerm, CMS and self fertility was launched, and a high SC pool was created. From genetically admixed population such as OPVs, we observed that high SC groups arose from low SC breeding materials by recurrent selection conducted in early generations. It is likely that high SC groups arose from low SC breeding materials by recurrent selection conducted in early generations. On the other hand, other high SC groups are rather complex.

We have isolated a major gene Acr1 for AR resistance. Acr1 is a potential donor of resistance to AR. The ‘Tmm-1’ Pool is also fairly well resistant to CLS, making ‘Tmm-1’ Pool a potential donor of resistance to CLS. ‘Tmm-1’ Pool may also be used as another sources for CLS resistance. In fact, CLS resistant lines ‘NK-306mm-O’ and ‘NK-307mm-O’ are more resistant to CLS than the other populations. From the results of this study, we observed that the number of alleles per locus was comparable between CAPS and SSR but slightly higher in SSR. Influence of marker type appears to be very small, if any, to infer the population structure of sugar beet (Adetunji et al. 2014, Inghelandt et al. 2010, Li et al. 2010, 2011, Mangin et al. 2015, Simko et al. 2012).

Population structure inferred from the polymorphic data has been associated with the breeding background. Simko et al. (2012) revealed three well-defined populations of varieties that generally correlates with their seed company origin. Li et al. (2010, 2011) distinguished two populations, seed-parents and pollen-parents, in 289 inbreds. We revealed 12 populations in JSBDIV set. Of note is that these populations more or less reflect their pedigree. A good example is Pop I: it was genetically contributed by mainly ‘TA-15’, which is consistent with the data that five of the seven Pop I members are shared with ‘TA-15’ Pool. Therefore, it is possible that population structure inferred by molecular data can complement pedigree information. This can be practically important when the missing pedigree data of a breeding material is needed. On the other hand, Groups, in general, less reflect pedigree information than Pops: strong association was seen only between Group V and ‘TA-15’ Pool. This may suggest that pedigree information is not always interconvertible with simple GD.

Our data suggest some underrealized aspects of Japanese sugar beet breeding. In its initial stage, OPVs from Europe and the United States were introduced and selected for the adaptability to Japanese climate and to cultivation methods (Hasegawa and Takeda 1982). Characters such as high sugar content, bolting tolerance, and disease resistance were favoured. From the 1960s, new breeding program using monogerm, CMS and self fertility was launched, and objectives shifted to yield performance and the individual traits. Selection was conducted using the introduced OPVs. Japanese seed parent, ‘Tmm-1’ was one of such OPVs and the main donor of the monogerm trait, which explains the current data that all inbreds in JSBDIV set have a kinship to ‘Tmm-1’ (Table 2).

We detected 12 Pops and seven Groups, which outnumber the similar studies of sugar beet in other countries (see above). According to pedigree data, the mean F of JSBDIV set was low. This could happen if intentional crosses and/or backcrosses have been confined within each of the seven ancestral OPVs but rarely between the OPVs. We infer that early breeders avoided inter-crossing between inbreds originated from different OPVs and treated each OPV as if it were a hetroetic pool. Why? Perhaps they may be aware of the importance of keeping genetic diversity in breeding materials to proceed the breeding program of sugar beet, an outcrossing crop that shows heterosis. Hence, it was suspected that they attempted to avoid acute bottlenecks in the Japanese breeding population. This scenario is worth to be examined to explain the outnumbered Pops and Groups.

On the other hand, delineation between the ancestral OPVs might be not so strict because the first sugar beet (i.e., White Silesian beet) was very small population and there is not much time since then to accumulate genetic diversity. This notion comes from some low $\Phi_{PT}$ values between Pools, Groups, or Pops. Variation in $\Phi_{PT}$ values may suggest a possibility that genetic backgrounds of the ancestral OPVs are in part overlapped with each other.

Phenotypic variation was observed among Pools, Groups, or Pops. The ‘TA-15’ Pool, Group V and Pop I exhibited the highest SC values than the others. Interestingly, these are groups that received the greatest genetic contribution from ‘TA-15’. They also share most of the inbred constituents, and they appear to represent single well-defined group. This is supported by the high $\Phi_{PT}$ value to the others. On the other hand, other high SC groups are rather complex. The second highest SC Pool was ‘TA-30’ Pool. Some of the inbred constituents of ‘TA-30’ Pool are shared with Groups VI and VII, two high SC Groups, and Pop H, a high SC Pop. However, although the second highest SC Pop is Pop B, none of its inbred constituents is shared with ‘TA-30’ Pool. Inheritance of sugar content is additive gene effect in sugar beet (Smith et al. 1973). Schneider et al. (2002) identified five quantitative trait loci (QTL) associated with sucrose concentration. It is possible that source and constituents of high SC genes are diversified. In relation to this, although ‘TA-36’ Pool was a low SC Pool, it was associated with rather high SC Pool (Pop K). Pop B is the second highest SC Pool, but it was not genetically contributed by ‘TA-15’. It is likely that high SC groups arose from low SC breeding materials by recurrent selection conducted in early generations. If so, it would be a good example of transgressive segregation occurred in a breeding program involving selection from genetically admixed population such as OPV.

As for disease resistance, RR-DI showed a smaller variation than CLS-DI and AR-DI. It suggests that progress in RR resistance using JSBDIV set is unexpected. On the other hand, there are few Rrl lines in the JSBDIV set. This is because Rhizomania resistance is mainly introduced to hybrids from the pollen parent in Japan, whereas JSBDIV set consists of mainly seed parent.

We have isolated a major gene Acr1 for AR resistance from a breeding line originated from ‘Tmm-1’ (Taguchi 2014). In the present study, high AR resistance was observed in Groups IV and V, and Pops E and I, all of which received high genetic contribution from ‘Tmm-1’. It might be necessary to determine whether all the AR resistance observed in this study are governed by Acr1. ‘Tmm-1’ Pool is also fairly well resistant to CLS, making ‘Tmm-1’ Pool a potential donor of resistance to CLS. ‘Tmm-14’ Pool may also be used as another sources for CLS resistance. In fact, CLS resistant lines ‘NK-306mm-O’ and ‘NK-307mm-O’
were derived from ‘Tmm-1’ Pool and ‘Tmm-14’ Pool, respectively. Taken together, breeding resistant lines against diseases principally requires resources having resistant trait. On the other hand, ‘NK-237BRmm-O’ is an exceptional line associated with ‘TA-30’ Pool, a modest resistant group. Unlike AR, genetics of CLS resistance is complex and gene(s) responsible for CLS resistance is difficult to identify (Taguchi et al. 2011). It is possible that accumulation of minor genes makes ‘NK-237BRmm-O’ resistant to CLS, suggesting another strategy for the breeding of CLS resistant line.

In summary, JSBDIV set is proven to be genetically and phenotypically diversified population as a whole. Because sugar beet breeding is challenged by Rhizomania expansion, fungal infestations, Beet Cyst Nematode, and demand for increased yield, it is necessary to investigate whether the current genetic diversity is sufficient to proceed breeding. In the future, genomes of JSBDIV set should be scrutinized by re-sequencing to further investigate their potential.

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