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ORIGINAL ARTICLE

Deciphering the complex nature of bolting time regulation in *Beta vulgaris*

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Abstract

Key message **Only few genetic loci are sufficient to increase the variation of bolting time in *Beta vulgaris* dramatically, regarding vernalization requirement, seasonal bolting time and reproduction type.**

Abstract Beta species show a wide variation of bolting time regarding the year of first reproduction, seasonal bolting time and the number of reproduction cycles. To elucidate the genetics of bolting time control, we used three F_3 mapping populations that were produced by crossing a semelparous, annual sugar beet with iteroparous, vernalization-requiring wild beet genotypes. The semelparous plants died after reproduction, whereas iteroparous plants reproduced at least twice. All populations segregated for vernalization requirement, seasonal bolting time and the number of reproduction cycles. We found that vernalization requirement co-segregated with the bolting locus *B* on chromosome 2 and was inherited independently from semel- or iteroparous reproduction. Furthermore, we found that seasonal bolting time is a highly heritable trait ($h^2 > 0.84$), which is primarily controlled by two major QTL located on chromosome 4 and 9. Late bolting alleles of both loci act in a partially recessive manner and were identified in both iteroparous pollinators. We observed an additive interaction of both loci for bolting delay. The QTL region on chromosome 4 encompasses the floral promoter gene *BvFT2*, whereas the QTL on chromosome 9 co-localizes with the BR_1 locus, which controls post-winter bolting resistance. Our findings are applicable for marker-assisted sugar beet breeding regarding early bolting to accelerate generation cycles and late bolting to develop bolting-resistant spring and winter beets. Unexpectedly, one population segregated also for dwarf growth that was found to be controlled by a single locus on chromosome 9.

Introduction

The appropriate timing of flowering is essential for the reproductive success of plants especially in temperate regions. Furthermore, flowering is a prerequisite for crop

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production. It is essential for crops that are harvested for their seeds or fruits. In contrast, flowering should be avoided in crops that are harvested for vegetative parts such as tubers, roots or leaves. Regulation of flowering time was a main feature of plant domestication in respect of adaptation to long days or the change from annual to biennial life cycle (Blümel et al. 2015; Jung and Müller 2009). In addition, early and late flowering massively affect the crop yield. Thus, flowering time is a selection criterion in nearly all breeding programs as is also the case for sugar beet, *Beta vulgaris* ssp. *vulgaris* var. *altissima*.

Sugar beet is the only sucrose-storing crop cultivated in temperate regions. The worldwide sugar beet production is 267 million tons per year, which contributes to 30% of the world's annual sugar production (http://faostat3.fao.org, 2016). Sugar beets are biennials. Conventionally, they are cultivated as a spring crop in temperate regions and harvested in the vegetative stage before winter. After winter, sugar beets start bolting and flowering. Bolting is visible by stem elongation and indicates the transition to reproductive growth. Bolting drastically reduces the beet yield and is, therefore, completely undesired for farming. Thus, spring-cultivated sugar beets are bred for obligatory bolting resistance during the first season. Current breeding programs are also focusing on the development of post-winter bolting-resistant beets. The so-called winter beets will allow cultivation of sugar beet as winter crop, which might increase beet yields up to 26% due to pre-winter development and accelerated growth in spring (Hoffmann and Kluge-Severin 2011). Although bolting resistance is of major importance for farming, bolting is absolutely necessary for seed production. All modern sugar beet varieties are hybrids. Hybrid seed parents must flower at the same time to allow maximum seed production. Thus, to prevent or to synchronize bolting, bolting time control is an absolute requirement for sugar beet production.

The *B. vulgaris* taxon is highly variable for bolting time. It includes annual, biennial and even iteroparous plants (Hautekèete et al. 2001; Letschert 1993). While annual beets require only long-day conditions for bolting induction, biennial beets have additionally an obligatory vernalization requirement. Biennial beets acquire floral competence through extended cold-exposure during winter and start bolting under long days of the next season. Annual and biennial beets are semelparous and die after reproduction. In contrast, iteroparous beets revert to vegetative growth after reproduction and pass through a next cycle of bolting and flowering in the subsequent season (Hautekèete et al. 2001). Frequently, natural populations consist of a mixture of semel- and iteroparous plants (Hautekèete et al. 2002; Van Dijk 2009). Besides the variation regarding the year of first reproduction, beets also show a wide variation for bolting time within the season. This was observed for natural beet accessions (Van Dijk and Hautekèete 2014) as well as F_3 mapping populations, in which seasonal bolting time varied up to 18 weeks independently from the year of first reproduction (Dally et al. 2014; Pfeiffer et al. 2014).

Previous studies showed that vernalization requirement of most biennial beets is controlled by the bolting locus *B*, which encodes the pseudo-response regulator gene *BOLTING TIME CONTROL 1*, *BTC1* (Pin et al. 2012). A second bolting locus, *B2*, was identified that encodes the zinc finger transcription factor *BvBBX19* (Dally et al. 2014). Both loci act epistatically and promote annual growth through suppression of the bolting repressor gene *BvFT1* and activation of the floral promoter gene *BvFT2*, two homologues of the floral integrator gene *FLOWERING LOCUS T* (*FT*) of *Arabidopsis* (Pin et al. 2010). Biennial beets are homozygous for recessive, loss-of-function alleles of either *BvBTC1* or *BvBBX19* and show altered transcription patterns of both *FT* homologues. Before winter, *BvFT1* is expressed whereas *BvFT2* is not transcribed, which keeps beets in the vegetative stage. However, vernalization induces *BvFT1* suppression and *BvFT2* activation, and biennial beets start bolting (Dally et al. 2014; Pin et al. 2012). Besides vernalization, which controls the year of first reproduction, bolting time is also affected by external factors like actual day-length, light quality and vernalization temperature as well as internal factors such as seed dormancy and plant hormones (Mutasa-Göttgens et al. 2010; Ritz et al. 2010; Wagmann et al. 2010). Also DNA methylation affects bolting (Trap-Gentil et al. 2011). By comparing gene expression and DNA methylation profiles in shoot apical meristems of bolting-sensitive and bolting-resistant beet genotypes after vernalization, 169 differentially expressed genes and 111 differentially methylated regions were determined as putative bolting time loci (Hébrard et al. 2016). In the annual, long-day model plant *Arabidopsis thaliana*, flowering time is controlled by more than 150 flowering genes that promote or suppress floral transition (Andrés and Coupland 2012; Blümel et al. 2015; Capovilla et al. 2014; Pajoro et al. 2014; Wang 2014). Thus, it can be assumed that also in beet a high number of genes regulate bolting time.

In previous studies, bolting time was primarily measured to determine genetic loci controlling vernalization requirement or post-winter bolting resistance (Dally et al. 2014; Pfeiffer et al. 2014; Pin et al. 2012). However, it was not investigated whether these loci also affect seasonal bolting time or distinguish between semelparous and iteroparous reproduction. The purpose of this study was to elucidate if a common or independent regulatory mechanisms control these traits that contribute to the wide variation of bolting time. Furthermore, bolting time loci should be identified that will be beneficial for breeding bolting-resistant spring and winter sugar beets.

To dissect and genetically characterize the quantitative trait bolting time, we (1) crossed annual and iteroparous beets and developed mapping populations that segregated for vernalization requirement, seasonal bolting time and the number of reproduction cycles, (2) constructed genetic maps based on newly developed sequence-based markers, (3) determined the locus controlling vernalization requirement in our mapping populations, (4) analyzed co-segregation of vernalization requirement and reproduction type, and (5) mapped quantitative trait loci (QTL) for seasonal bolting time using F_2 and F_3 phenotypic data.

Materials and methods

Plant material and growth conditions

Three mapping populations were produced by crossing a semelparous, annual sugar beet (*B. vulgaris* ssp. *vulgaris*) with plants from two iteroparous wild beet populations, *B. vulgaris* ssp. *maritima*. The detailed crossing scheme is shown in Suppl. Figure 1. The annual parent is a selfing progeny of 93167P, which is homozygous for the dominant allele at the *B* locus (*BB*) (El-Mezawy et al. 2002). 93167P reproduces without vernalization and dies after reproduction. Plants develop green hypocotyls suggesting a *rr* genotype at the *R* locus (Hatlestad et al. 2012). The iteroparous wild beets were collected in the United Kingdom (Van Dijk and Hautekèete 2014; 95 Sea fam. 1; seed code 100849) and Ireland. The Irish accession was provided by the USDA (http://www.ars-grin.govs; PI 518412; seed code 080454). Both wild beets have red hypocotyls, suggesting a dominant allele at the *R* locus (*RR*/*Rr*), and require vernalization for bolting induction. After first reproduction, they revert back to vegetative growth and repeat reproduction in the subsequent season. The mean lifespan of the British accession is 3.8 years and unknown for the Irish accession. Pollination with the Irish and British accession gave 3 and 20 F_1 plants, respectively. F_1 plants were distinguished from selfing progenies of the annual seed bearing plant by red hypocotyls (*rR*) and confirmed using the co-dominant marker GJ1001c16 (Büttner et al. 2010). All F_1 plants bolted and flowered without vernalization. F_2 population A was derived from the Irish accession and consisted of 569 individuals that were obtained by selfing a single F_1 plant. The sibling populations B1 and B2 were derived from the British accession by selfing two F_1 plants. They consisted of 118 and 114 F_2 individuals (Suppl. Figure 1).

After sowing on April 18, 2012, 569 plants of F_2 population A germinated in the greenhouse under 20 °C and 16-h light (900 µmol m^{-2} s^{-1}, Son-T Agro 400 W, Koninklijke Philips Electronics N.V., Eindhoven, Netherlands). Five-week-old plants were planted to a field nursery in Kiel, Germany. Additionally, 30 plants of the semelparous, annual accession and 33 selfing progenies of the Irish accession were included in the field experiment as controls. The field experiment ended in June 2014. F_3 seeds were produced by bag-isolation of bolting F_2 plants. Since the F_2 population was segregating for vernalization requirement, seeds of 320 F_2 plants without vernalization requirement were obtained in 2012, whereas seeds of 62 F_2 plants with vernalization requirement were obtained in 2013.

To exclude the impact of winter on plant survival, F_2 plants of the sibling populations B1 and B2 were cultivated in a greenhouse of the Plant Breeding Institute, University of Kiel. After sowing on May 2, 2012, 118 and 114 F_2 plants were potted in 11 × 11 × 12 cm^3 pots (Hermann Meyer KG, Germany) and grown under 16-h artificial light (900 µmol m^{-2} s^{-1}, Son-T Agro 400 W, Koninklijke Philips Electronics N.V., Eindhoven, Netherlands) and 20 °C together with 24 potted plants of the annual accession, 15 selfing progenies of the British accession and 33 F_1 plants derived from the reciprocal cross. Bolting F_2 plants were bag-isolated to produce F_3 seeds. F_2 plants that had reproduced without vernalization were grown from October 17, 2012 under 12-h light to signal the end of the growing season. Since both populations segregated for vernalization requirement, plants without reproduction until mid of August 2012 were cold-treated in a cold chamber at 4 °C and 16-h light (200 µmol m^{-2} s^{-1}, Osram Lumilux T8 L 58W/840, Osram AG, München, Germany) for 14 weeks. Afterwards, they were grown under 22-h light at 20 °C. As soon as these plants started flowering, they were transferred to 12-h light. A second reproduction cycle was induced by supplying 16-h light from March 6 to April 4, 2013. Then all plants were transferred to a greenhouse without additional light supply. The greenhouse experiment ended in September 2013.

For phenotyping F_3 families, 155, 108 and 98 F_3 families of population A, B1 and B2 with sufficient numbers of seeds were selected that were assumed to start bolting without vernalization based on F_2 genotypic data of marker GJ1001c16. Seeds were sown on May 4, 2015. Eight to 24 F_3 plants per family were planted on May 18, 2015 as groups in 96-well multipot-plates (QuickPot QP 96T, Hermann Meyer KG, Germany) following a randomized complete block design with three replicates and groups as experimental unit. Then, F_3 plants were grown under natural light conditions until September 29, 2015 (Fig. 1i).

Phenotyping

F_2 plants were phenotyped for hypocotyls color, seasonal bolting time, vernalization requirement, and death after reproduction, reversion to vegetative growth after reproduction and repeated bolting/flowering. Hypocotyls color was

Fig. 1 Plant phenotypes of mapping populations A, B1 and B2. **a** 4-week-old F_2 plants of population A with normal (*white asterisk*) and dwarf growth (*red asterisk*). Normal beets developed big, plane leaves whereas dwarf plants developed small, elongated and acuminated leaves. **b** Dwarf beets were mostly non-bolting or **c** showed disturbed bolting, which did not result in flowering even 6 months after sowing. **d** In the year of sowing, F_2 plants of population A segregated for non-bolting (*left*), reversion to vegetative growth after bolting (*middle*) and bolting with subsequent death (*right*, photo October 2012). **e** After winter, the same individuals as in **d** bolted the first time after vernalization (*left*), repeated bolting (iteroparous growth, *mid*) or were dead (semelparous growth, *right*, photo May 2013). **f** The annual parental accession died directly after reproduction (photo September 2012). Greenhouse-grown F_2 plants of **g** B1 and **h** B2 segregated for iteroparous (*left*) and semelparous growth (*right*, photo April 2013). **i** Cultivation of F_3 plants in 96-well multipot-plates under seminatural conditions (photo July 2015). *white bar* 3 cm (color figure online)

recorded directly after germination. Bolting time was phenotyped twice a week and recorded as the date, at which stem elongation was visible for the first time. Days-to-bolting (DTB) were calculated as the sum of days from sowing to bolting. Bolting plants with flowers were scored as regular bolting. Plants that bolted without producing flowers were scored as incomplete bolting. When plants were non-bolting 3 months after sowing or showed incomplete bolting, they were assumed to require vernalization for reproduction. A plant was determined as dead, when all green areas had disappeared. Repeated bolting was recorded when field-grown plants had bolted and flowered in two subsequent seasons or when greenhouse-grown plants had a clear break of reproduction with vegetative growth of about 2 months until new bolting shoots with flowers emerged. F_2 population A segregated additionally for normal and dwarf growth which was distinguishable as soon as the first leaves emerged.

F_3 plants were phenotyped for bolting time weekly. Dwarfs and non-bolting F_3 plants were finally determined on September 29, 2015. The dwarf rate of F_3 families was calculated as number of dwarf plants out of the total plant number per family. The bolting rate was calculated per F_3 family as number of non-bolting, normal growing plants out of the total number of normal growing plants. The average seasonal bolting time was determined as average DTB for F_3 families that contained in sum at least 12 bolting plants in at least 2 replicates with minimum 3 bolting plants within a replicate.

Marker development

For the development of sequence-based markers, whole-genome next generation sequencing (NGS) of the parental plants and F_1 plant of population A was performed using the Illumina HiSeq 2000 system. Genomic DNA was extracted by a slightly modified CTAB method (Saghai-Maroof et al. 1984), barcoded and sequenced as 100 bp paired-end reads. The raw reads have been deposited in the NCBI Short Read Archive database under the accession number SRP095152. The NGS reads were processed using the software program CLC Genomics Workbench 6.5.1 (http://www.clcbio.com) as follows: The NGS data were imported with a 50–1000 nucleotide spacer. After quality control, the reads were trimmed with the parameters quality score limit of 0.05, maximum 2 ambiguous nucleotides, remove 5′ terminal 2 nucleotides, discard reads below a length of 80 nucleotides. Trimmed, paired and broken reads were subsequently mapped as stand-alone read mapping against the sugar beet reference genome sequence RefBeet-1.1 (Dohm et al. 2014; http://bvseq.molgen.mpg.de/index.shtml). Mapping parameters were set to mismatch cost 2, insertion and deletion cost 1, length fraction 0.5 and similarity fraction 0.9. Non-specific matches were ignored. A summary of NGS and read mapping data for each plant and a data set, which combined the reads of all three plants, is provided in Suppl. Table 1. The read mapping file of the combined data set was used for probabilistic variant detection. Thereby, only positions with a minimum coverage of 10, a variant probability of 90 and presence in both forward and revers reads were considered for the detection of sequence polymorphisms.

In total, 140 sequence-based InDel markers with length polymorphisms between 9 and 45 bp were developed that are located across the whole beet genome. InDel flanking primers were designed based on the read mapping files of the parental plants using the software tool OligoCalc (Kibbe 2007). These InDel markers were also used for genotyping F_2 populations B1 and B2. In addition, the *B* locus-specific marker GJ1001c16 was used (Büttner et al. 2010). All primers were obtained from Eurofins Genomics (http://www.eurofinsgenomics.eu). All markers including the primer sequences are listed in Suppl. Table 2. In addition, hypocotyls color was used as phenotypic marker. This trait is encoded by the *R* locus on chromosome 2 and was scored as dominant red versus recessive green (Hatlestad et al. 2012).

Molecular marker analysis

Genomic DNA was extracted from freeze dried leaf samples of F_2 plants by CTAB method and adjusted to 10 ng/µl with 1× low TE buffer. Polymerase chain reaction (PCR) assays were done in a total volume of 15 µl containing 7 ng DNA, 1× PCR buffer, 0.2 mM dNTPs (Invitrogen), 0.2 µM of each primer and 0.02 U Dream Taq DNA polymerase (Thermo Fisher Scientific Inc.). The PCR conditions were 34 cycles of 30 s at 94 °C, 30 s at 56–61 °C depending on the primers and 30 s at 72 °C. PCR products of two InDel markers with different fragment lengths were combined per F_2 plant and separated by gel electrophoresis onto a 3% agarose gel.

Genetic map construction and QTL mapping

Linkage groups were calculated for each F_2 population using JoinMap® version 4.1 (Van Ooijen 2006). Markers were grouped by independent LOD and distances were calculated by regression mapping using the Kosambi mapping function (Kosambi 1943), a LOD threshold value of 3.0 and a maximum recombination frequency of 0.4. The linkage groups were anchored according to the sugar beet reference genome RefBeet-1.1.

For each mapping population, QTL detection was performed by composite interval mapping with PlabMQTL version 0.5 (Utz 2012) assuming a dominant gene model.

Additive and dominance effects were estimated by multiple regression analysis. An experiment wise LOD threshold was determined by 1000 permutations for each QTL analysis (Doerge 2002).

Statistical analysis

For each mapping population, the analysis of variance (ANOVA) was performed for DTB recorded on F_3 families using SAS PROC MIXED (SAS Institute Inc., 2009, Cary, USA, version 9.2), where genotypes (F_3 families) and blocks were considered as fixed and random factors, respectively.

SAS PROC VARCOMP was used to calculate the variance components, which were then used for heritability estimations. Broad sense heritability was estimated according to Hallauer et al. (1988) as $h^2 = \frac{Vg}{Vg + Ve/R}$, where h^2 indicates the broad sense heritability, Vg is the genotypic variance of the F_3 family and Ve is the error variance divided by the total number of replications (R). Normal distribution of DTB was tested with Shapiro–Wilk.

Results

Variation for the year of first reproduction, seasonal bolting time and the number of reproduction cycles

To elucidate genetic mechanisms that control bolting time in beet, we produced three mapping populations. Population A and the sibling populations B1 and B2 were derived by crossing a semelparous sugar beet with iteroparous wild beet genotypes from Ireland and United Kingdom, respectively (Suppl. Figure 1). The semelparous beet is annual and bolts without vernalization, due to the presence of dominant, annual alleles at the bolting locus *B* (El-Mezawy et al. 2002). The iteroparous beets require vernalization and start bolting after winter. Surprisingly, population A segregated after germination for normal and dwarf growth. 140 out of 569 F_2 plants produced normal cotyledons, but afterwards only small, elongated and acuminated leaves. These plants grew extremely slowly and remained very small until the end of the season (Fig. 1a–c). The segregation of normal and dwarf plants corresponded to a Mendelian segregation ratio of 3:1 ($X^2 = 0.047$, non-significant at $\alpha = 0.01$), which suggested a monogenic, dominant-recessive inheritance of dwarf growth. Since most dwarfs failed to bolt and no dwarf developed flowers, all dwarf plants were excluded from bolting time analysis.

Bolting time was determined for F_2 population A under field conditions from 2012 to 2014 and for F_2 populations B1 and B2 under greenhouse conditions from 2012 to 2013. For population A, we recorded the year of first reproduction, seasonal bolting time and the number of reproduction cycles of all normal growing plants. For B1 and B2, we determined seasonal bolting time and number of reproduction cycles only for F_2 plants that bolted without vernalization, because the seasonal bolting time of artificially cold-treated plants was not anymore comparable. All populations segregated for bolting and non-bolting in the year of sowing. The seasonal bolting time ranged from May to August in 2012 and from May to June and August, respectively, in 2013. Furthermore, all populations segregated for single and repeated reproduction (Figs. 1d–h, 2a, b; Tables 1, 2). Field-grown semelparous control plants bolted between May 24 and 31, 2012 and died after reproduction, showing early bolting regarding vernalization requirement and compared to the seasonal bolting time of population A. Field-grown control plants of the Irish accession bolted first after winter, showed an early to moderate seasonal bolting time and repeated reproduction in 2014 (Fig. 2a). Greenhouse-grown controls of the semelparous accession bolted between June 7 and 15, 2012, around the same time as early bolting F_2 plants of B1 and B2. Control plants of the British accession (selfing progenies) segregated under greenhouse conditions in 10 non-bolting, 3 incompletely bolting and 2 regularly bolting plants (June 28 and July 5), indicating a major but not complete vernalization requirement. In contrast, all 33 F_1 plants of the reciprocal cross bolted regularly without vernalization, starting bolting from June 12 to July 9, 2012 within a range of 27 days.

For F_3 phenotyping, 155, 90 and 80 F_3 families of population A, B1 and B2 were analyzed. These families were derived from F_2 plants that bolted without vernalization requirement and produced sufficient seed numbers. In total, 7496, 4320 and 3839 F_3 plants were phenotyped under seminatural conditions in 2015. Variation was observed for vernalization requirement (bolting versus non-bolting in 2015), seasonal bolting time and exclusively in population A for dwarf growth. The dwarf rates ranged from 0 to 0.38. The bolting rates were determined only for normal growing plants and ranged from 0.03 to 1 for population A and B2, and from 0 to 1 for B1. Thereby, 95.5, 92.2 and 93.8% F_3 families of population A, B1 and B2 segregated for bolting and non-bolting without vernalization. All other families showed complete bolting. Variation of seasonal bolting time was observed within and between families. F_3 plants started bolting from June 16 until September 29, 2015 within a range of 105 days (Table 2). The average seasonal bolting time was determined for 147 (A), 78 (B1) and 75 (B2) F_3 families, showing a variation of 38, 58 and 50 days for mean DTB, respectively (Fig. 2c; Table 2). The genotypic effects of F_3 families for DTB were tested

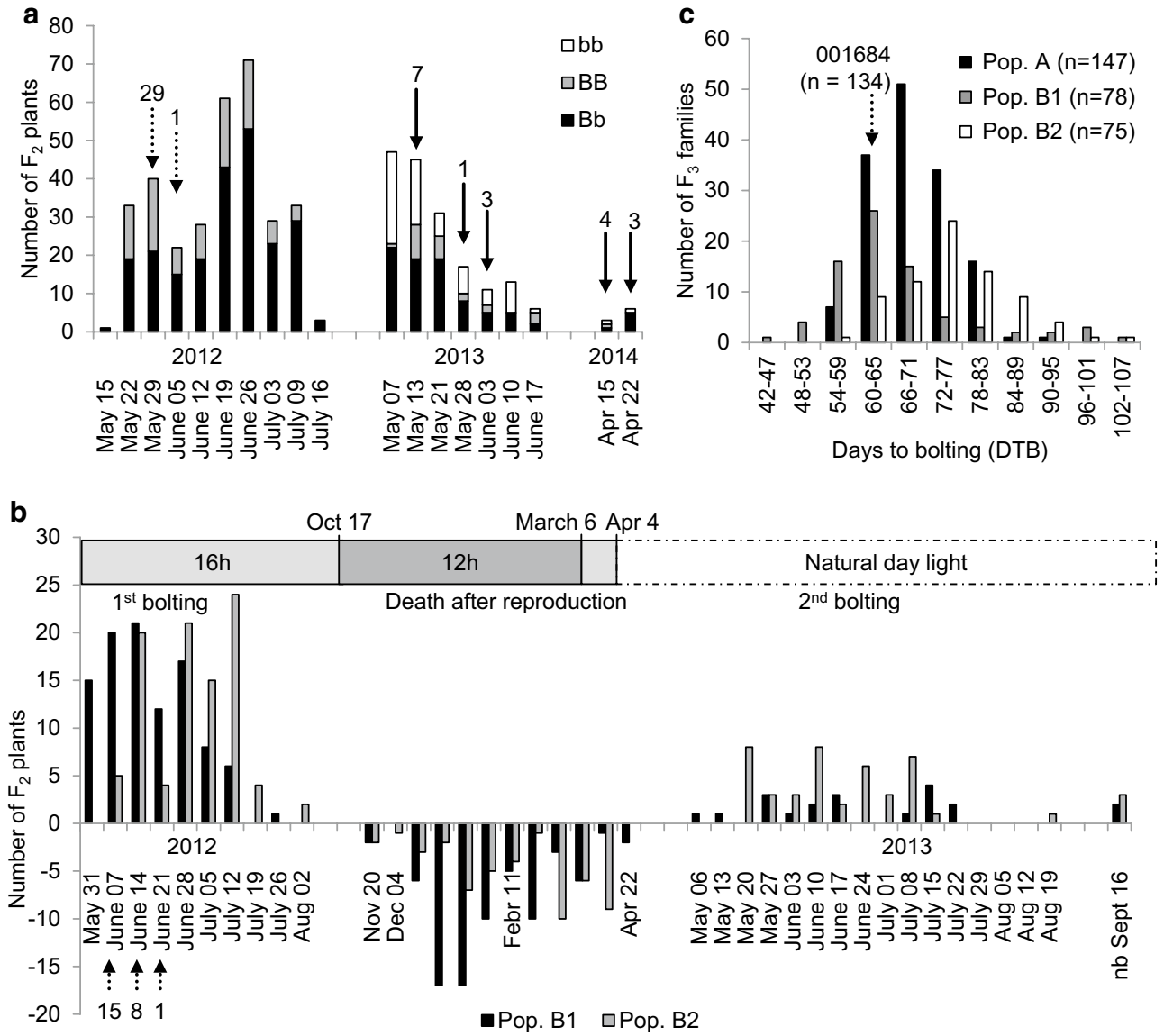

Fig. 2 Bolting time of beet populations A, B1 and B2. **a** All normal F_2 plants of population A, that carried the dominant *B* allele (*BB* or *Bb*) reproduced in 2012, the year of sowing, and partially repeated reproduction in 2013 and 2014. F_2 plants that were homozygous for the recessive *b* allele (*bb*) started first reproduction in 2013 and repeated partially reproduction in 2014. **b** Bolting time and death after reproduction of semel- and iteroparous F_2 plants of the sibling populations B1 and B2, which were grown in the greenhouse under 16-h light to induce reproduction, subsequently under 12-h light to signal the end of the season and finally under natural long-day conditions to induce second reproduction. **c** Bolting time of F_3 families grouped according to the average DTB per family. *Broken arrow* bolting time of the annual parental accession (*BB*), *black arrow* bolting time of the iteroparous Irish parental accession (*bb*)

significant ($p < 0.0001$) by ANOVA. For each population, the distribution of mean DTB of F_3 families was skewed to the left and deviated significantly from normal distribution. Control plants of the semelparous accession started bolting on average after 61.6 ± 9.04 days which is corresponding to early seasonal bolting. Heritability for DTB was estimated as $h^2_A = 0.84$, $h^2_{B1} = 0.96$ and $h^2_{B2} = 0.93$.

The *B* locus determined the year of first reproduction through controlling vernalization requirement

In 2012, the year of sowing, all F_2 populations segregated for bolting versus non-bolting in a ratio of 3:1 ($X^2_A = 0.113$, $X^2_{B1} = 5.977$, $X^2_{B2} = 4.222$, non-significant at $\alpha = 0.01$), emphasizing a monogenic, dominant–recessive inheritance of vernalization requirement. Vernalization requirement

Table 1 Bolting behavior according to the genotype at the *B* locus of F_2 populations A, B1 and B2 that segregate for vernalization requirement and iteroparity

F_2 population	*B* locus genotype	Number of F_2 plants	Bolting behavior in 2012 (year of sowing)		Number of bolting plants in 2013[b]	Number of bolting plants in 2014[b]
			Number of regularly bolting plants	Number of non-bolting plants (with incomplete bolting[a])		
A	*BB*	95	95	0	**23 (24.2%)**	**1 (4.3%)**
	Bb	226	226	0	**80 (35.4%)**	**6 (7.5%)**
	bb	103	0	103	67[c]	**2 (3.0%)**
B1	*BB*	35	35	0	**3 (8.6%)**	n.d.
	Bb	61	60	0 (1)	**15 (25.0%)**	n.d.
	bb	22	5	14 (3)	n.d.	n.d.
B2	*BB*	28	28	0	**10 (35.7%)**	n.d.
	Bb	64	64	0	**32 (50.0%)**	n.d.
	bb	22[c]	3	14 (5)	n.d.	n.d.

The genotype at the *B* locus was determined by marker analysis using marker GJ1001c16. The numbers of plants with repeated reproduction are given in bold

n.d. not determined

[a] Beets with incomplete bolting started bolting, but did not produce flowers

[b] Percentage of plants with repeated reproduction is based on bolting plants out of plants that bolted during the previous season

[c] 36 non-bolting plants were killed by winter 2012/13 resulting in a winter mortality rate of 35.0%

is controlled in most beets by the *B* locus in a dominant–recessive manner (Pin et al. 2012). So we tested whether the *B* locus controls vernalization requirement in our mapping populations by genotyping all F_2 plants with the *B* locus-specific marker GJ1001c16. The results are shown in Table 1. The genotype of the *B* locus explained 100, 94.9 and 97.4% of the bolting phenotype of F_2 populations A, B1 and B2. These results suggest that the *B* locus controls vernalization requirement in our mapping populations. After vernalization, all non-bolting plants bolted regularly.

The reproduction type of beet is independent from the year of first reproduction

All control plants of the semelparous, annual accession bolted in the year of sowing and died after first reproduction combining no vernalization requirement (*BB* genotype) with semelparous growth (Fig. 1f). In contrast, the Irish and British accessions combine vernalization requirement (*bb* genotype) and repeated reproduction/iteroparity. To determine whether the *B* locus also controls the reproduction type, we determined the number of reproduction cycles of F_2 plants according to the genotype at the *B* locus (Table 1). In F_2 population A, 105 out of 424 plants showed repeated reproduction, while all other plants were dead after winter 2012/13 and 2013/14, respectively (Fig. 1d, e). Thereby, repeated reproduction was observed for 24.2% of F_2 plants with homozygous dominant, annual *B* allele (*BB*), 35.4% plants with heterozygous (*Bb*) and 3.0% plants with homozygous recessive *b* allele (*bb*). However, the number of dead plants does not correspond to the number of semelparous plants. Nearly all field-grown F_2 plants were alive after first reproduction and a plant's death after winter could not be clearly assigned to semelparity or insufficient winter-hardiness. Thus, an absolute quantification of iteroparous and semelparous plants was not possible. Nevertheless, 24.2% of the F_2 plants with *BB* genotype (without vernalization requirement) reproduced repeatedly compared to only 3% of beets with *bb* genotype (with vernalization requirement). For the greenhouse-grown populations B1 and B2, the impact of winter on plant survival was excluded. Here, repeated reproduction was observed for 8.6 and 35.7% of B1 and B2 plants being homozygous for the dominant *B* allele and 24.6 and 50.8% of B1 and B2 plants being heterozygous. Repeated reproduction of F_2 plants with homozygous recessive *b* allele was not determined, because bolting time of vernalized and non-vernalized plants was not comparable anymore. The high percentage of F_2 plants that newly combine release from vernalization requirement with iteroparity in all three populations suggests that the *B* locus does not control iteroparity and that, therefore, a F_2 plant's reproduction type is independent from its year of first reproduction.

QTL detection for seasonal bolting time

To identify genetic factors that control bolting time within a season, we performed a QTL analysis based on DTB data

Table 2 Seasonal bolting time data

Population	DTB F_2 plants in 2012	DTB F_2 plants in 2013		DTB F_3 plants in 2015	F_3 family mean DTB
	1st reproduction	2nd reproduction	1st reproduction after winter	1st reproduction	1st reproduction
A (424 normal growing F_2 plants; 155 F_3 families)					
Total number	321	103	67	4809	147
Bolting time	May 15–July 12	May 7–June 17	May 7–June 17	June 16–September 22	June 29–August 6
DTB	27–85	384–425	384–425	43–141	56–94
Variation of DTB	58 days	41 days	41 days	98 days	38 days
H_0: normal distribution	$w = 0.9306$ $p < 0.0001$ H_0 rejected	$w = 0.8736$ $p < 0.0001$ H_0 rejected $w = 0.8605$, $p < 0.0001$ H_0 rejected[a]	$w = 0.8166$ $p < 0.0001$ H_0 rejected	$w = 0.8681$ $p < 0.0001$ H_0 rejected	$w = 0.9640$ $p < 0.0001$ H_0 rejected
B1 (118 F_2 plants; 90 F_3 families)					
Total number	100	18	n.d.	2754	78
Bolting time	May 31–July 24	May 6–July 22	n.d.	June 16–September 29	June 19–August 16
DTB	29–83	369–446		43–148	46–104
Variation of DTB	54 days	77 days	n.d.	105 days	58 days
H_0: normal distribution	$w = 0.9368$ $p > 0.0001$ H_0 accepted	$w = 0.9252$ $p > 0.0001$ H_0 accepted	n.d.	$w = 0.8298$ $p < 0.0001$ H_0 rejected	$w = 0.8758$ $p < 0.0001$ H_0 rejected
B2 (114 F_2 plants; 80 F_3 families)					
Total number	95	42	n.d.	2544	75
Bolting time	June 7–July 30	May 21–August 19	n.d.	June 16–September 29	June 29–August 18
DTB	36–89	384–474	n.d.	43–148	56–106
Variation of DTB	53 days	90 days	n.d.	105 days	50 days
H_0: normal distribution	$w = 0.9452$ $p > 0.0001$ H_0 accepted	$w = 0.9173$ $p > 0.0001$ H_0 accepted	n.d.	$w = 0.9116$ $p < 0.0001$ H_0 rejected	$w = 0.9808$ $p < 0.0001$ H_0 rejected

Seeds of F_2 population A were sown on April 18, 2012, on May 2, 2012 for F_2 populations B1 and B2, and on May 4, 2015 for the F_3 families. Only F_3 families that contained in sum 12 bolting plants in at least 2 replicates with minimum 3 bolting plants per replicate were used for bolting time analysis

DTB days-to-bolting, *n.d.* not determined

[a] Refers to bolting time in 2013 independently of first or second reproduction

of F_2 plants and mean DTB of F_3 families of all three populations. For genetic map construction, we developed 140 InDel markers based on whole-genome sequencing data of the parental plants and F_1 plant of population A. Out of these 140 markers, 116, 86 and 95 markers were polymorphic in population A, B1 and B2, respectively. Subsequently, 191 F_2 plants of population A including 11 dwarfs and 96 F_2 plants of B1 and B2 were genotyped. Genetic maps were constructed for each population, comprising data from 84 to 102 markers (Fig. 3). The total lengths of the genetic maps ranged from 462 to 516 cM covering all nine chromosomes of *B. vulgaris* for each population. The size of the linkage groups ranged from 22.7 to 110.6 cM. Details are given in Suppl. Table 3.

A preliminary QTL mapping was performed using DTB of F_2 plants. For F_2 population A, four significant QTL for seasonal bolting time were detected at experiment alpha rate of $\alpha_E = 0.01$. These QTL mapped to chromosomes 4, 8 and 9 and explained 12.9–33.5% of the phenotypic variation (Table 3). All mapped QTL explained together 53.4% of the phenotypic variation (R^2_{adj}). For F_2 population B1, one QTL was detected on chromosome 4. This QTL co-localizes with a QTL, which was detected in population A, and explained 23.4% of the phenotypic variation. For B2, we mapped a QTL on chromosome 7, which explained 26.4% (Table 3).

Mean DTB data of F_3 families were used for final QTL mapping. The LOD profiles over all chromosomes are shown in Fig. 4 and QTL results are summarized in Table 3. Two significant QTL were mapped for population A on chromosome 4 at position 9 and chromosome 9 at position 25. These QTL were named *SBT-4* and *SBT-9*

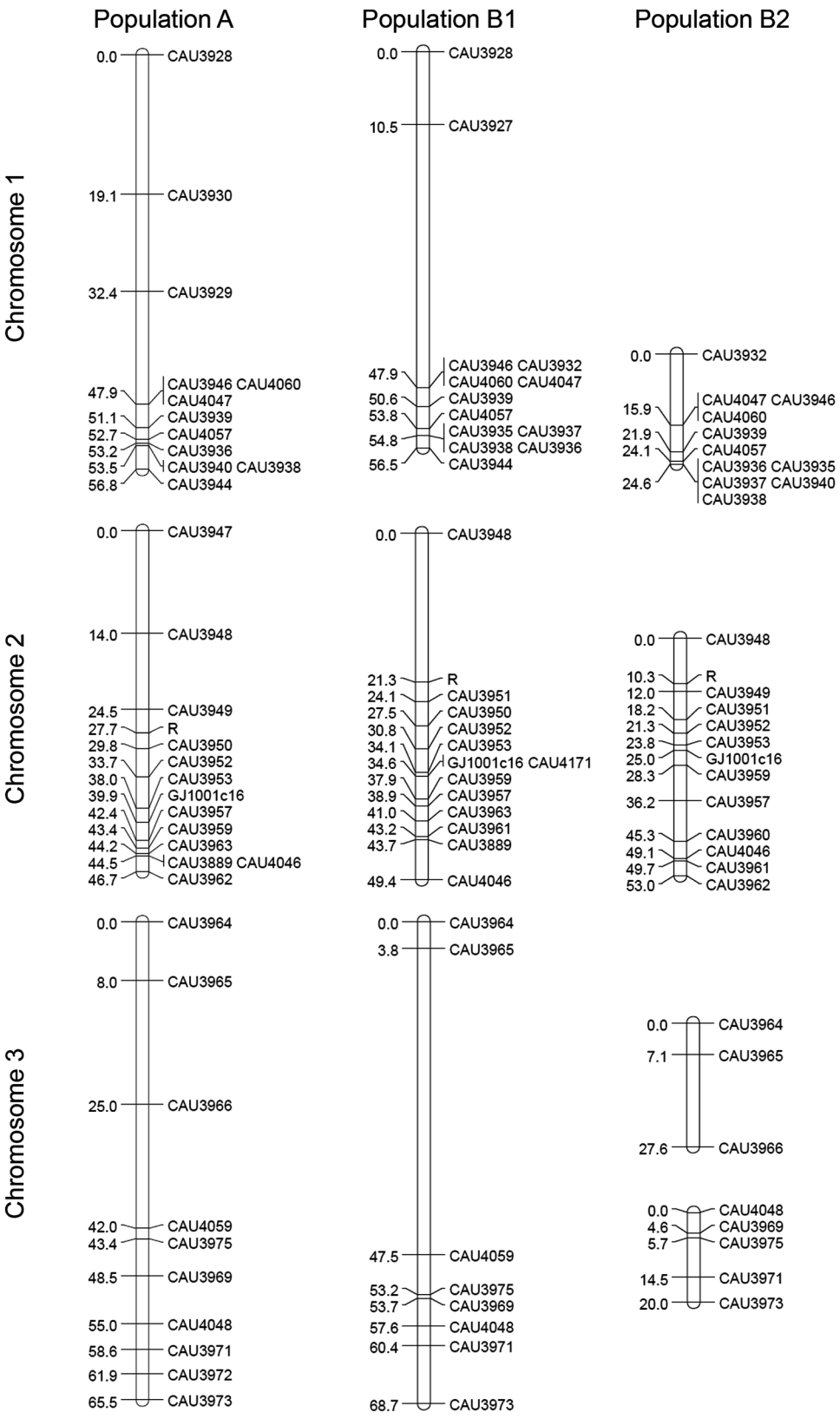

Fig. 3 Genetic maps of F_2 populations A, B1 and B2 covering all 9 chromosomes of *B. vulgaris*. Detected QTL for seasonal bolting time are marked in *grey* (F_2 phenotypic data) and *black* (F_3 phenotypic data). For each QTL, *vertical lines* indicate the QTL supporting interval and *horizontal lines* the QTL position. Two major QTL for seasonal bolting time were detected in different populations based on F_2 and F_3 phenotypic data on chromosome 4 and 9. Additionally, a QTL for dwarf growth was detected in population A, which is marked in *light* and *dark grey* on chromosome 9 for F_2 and F_3 phenotypic data

Fig. 3 continued

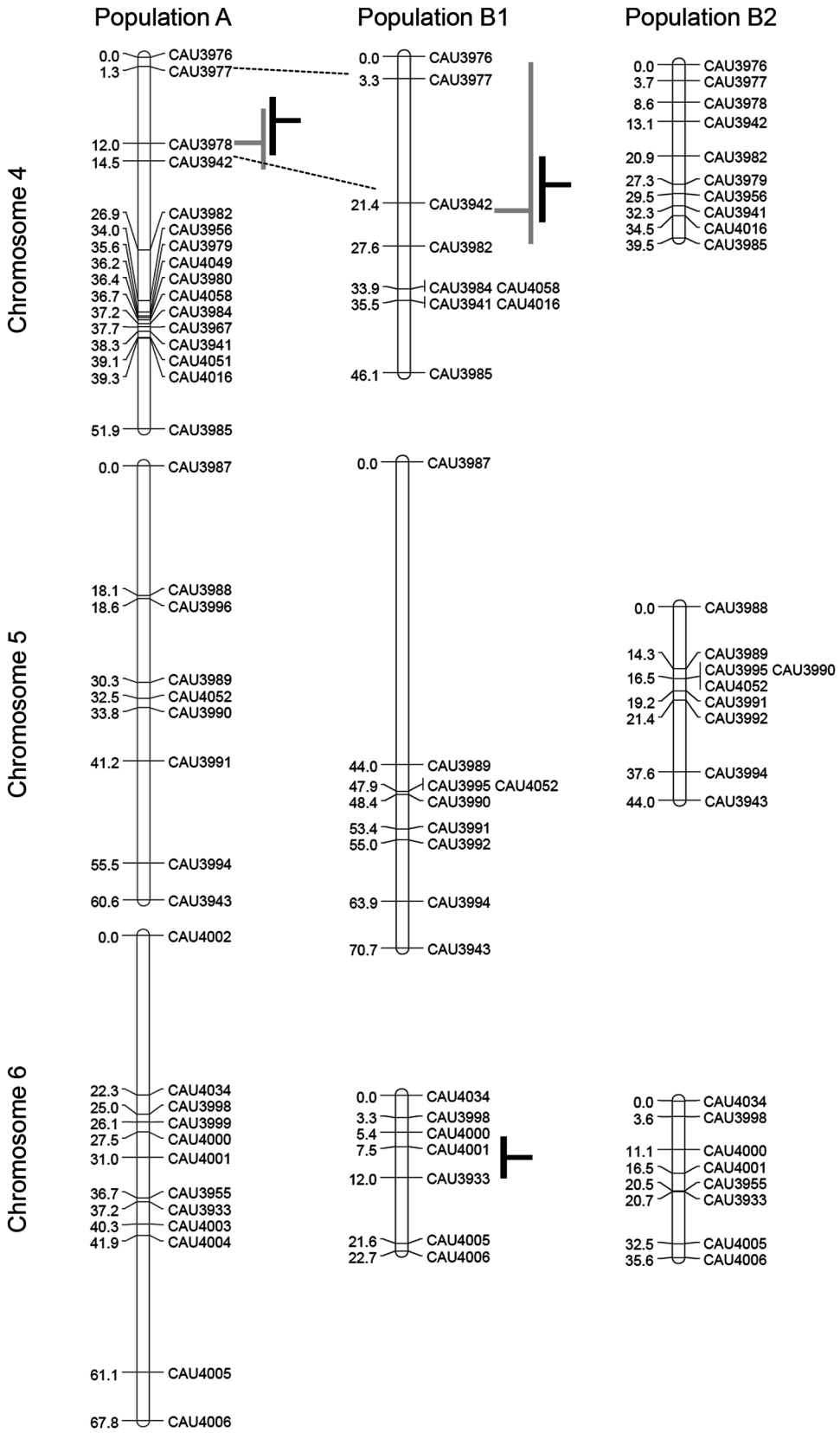

Fig. 3 continued

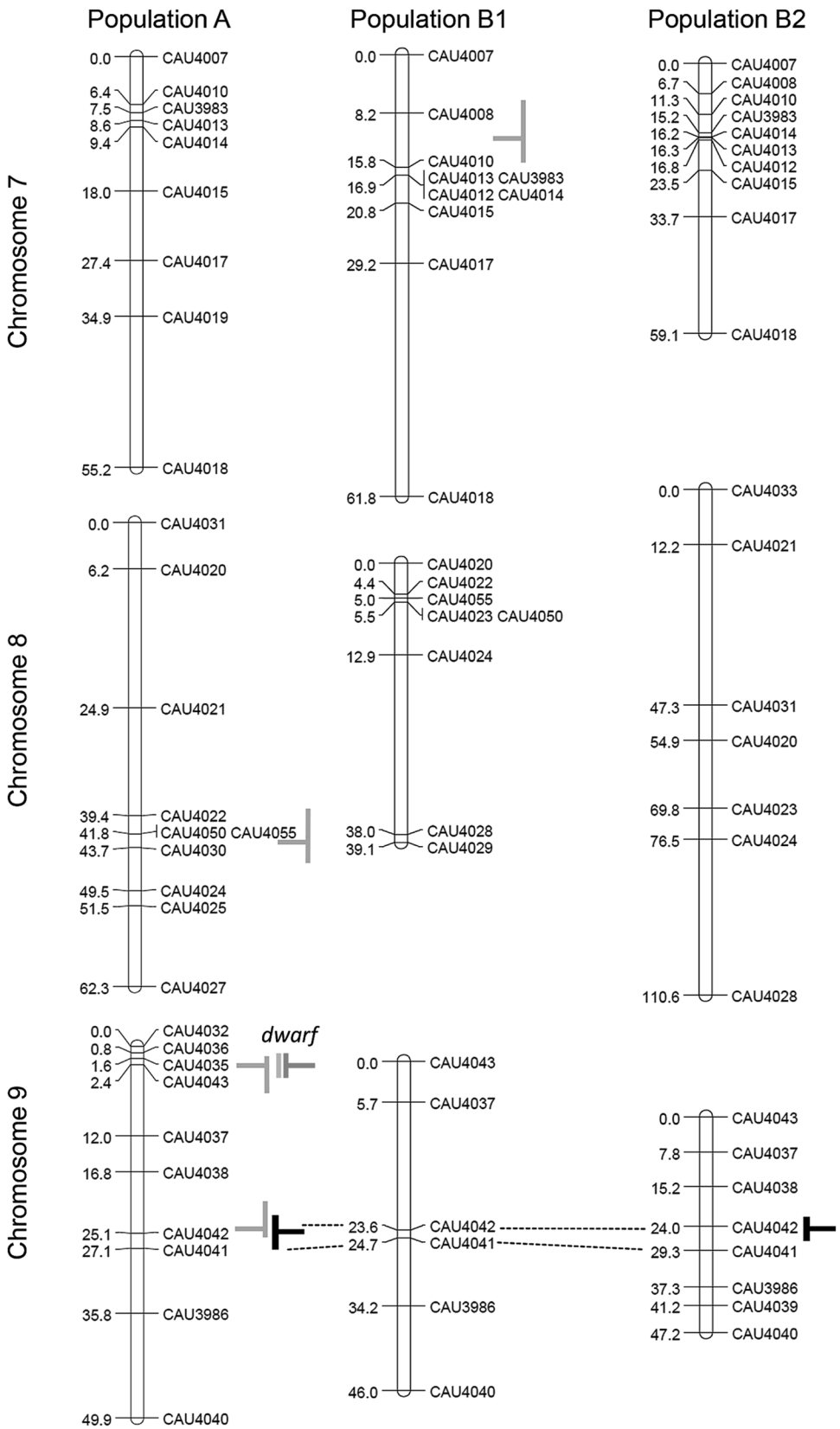

Table 3 QTL results for seasonal bolting time (*SBT*) of mapping populations A, B1 and B2 derived from crosses of the semelparous-annual accession 001684 with a vernalization-requiring, iteroparous Irish (population A) and British beet accession (B1, B2)

Trait	Population	Generation (year) used for phenotyping	QTL name	Chromosome	Position (supporting interval)	Flanking markers	*LOD*	R^2 in %	Additive effect	Dominance effect
Seasonal bolting time	A	F_2 (2012) $n = 191$ plants	$SBT\text{-}4_{AF2}$	4	12 (7–15)	CAU3978/CAU3942	7.07*	12.9	5.9*	−5.0ns
			$SBT\text{-}8_{AF2}$	8	42 (39–44)	CAU4055/CAU4030	4.15*	14.4	6.0*	No
			$SBT\text{-}9a_{AF2}$	9	2 (1–5)	CAU4035/CAU4037	13.47*	33.5	−23.1*	−20.7*
			$SBT\text{-}9b_{AF2}$	9	24 (20–26)	CAU4038/CAU4042	11.32*	32.1	13.8*	No
							$R^2_{adj} = 53.4\%$			
		F_3 (2015) $n = 147$ families	$SBT\text{-}4_A$	4	9 (4–14)	CAU3977/CAU3978	11.50*	21.4	4.7*	No
			$SBT\text{-}9_A$	9	25 (23–28)	CAU4038/CAU4042	14.06*	19.5	5.0*	No
							$R^2_{adj} = 29.5\%$			
	B1	F_2 (2012) $n = 96$ plants	$SBT\text{-}4_{B1F2}$[a]	4	22 (1–27)	CAU3977/CAU3942	6.12*	23.4	7.6*	No
							$R^2_{adj} = 31.8\%$			
		F_3 (2015) $n = 78$ families	$SBT\text{-}4_{B1}$	4	18 (14–23)	CAU3977/CAU3942	17.28*	51.8	12.9*	No
			$SBT\text{-}6_{B1}$	6	9 (6–12)	CAU4001/CAU3933	6.95*	21.9	−5.0ns	−0.7ns
							$R^2_{adj} = 64.5\%$			
	B2	F_2 (2012) $n = 96$ plants	$SBT\text{-}7_{B2F2}$	7	12 (6–16)	CAU4010/CAU3983	6.35*	26.4	9.2*	No
							$R^2_{adj} = 24.8\%$			
		F_3 (2015) $n = 75$ families	$SBT\text{-}9_{B2}$	9	24 (22–26)	CAU4042/CAU4041	14.44*	44.6	7.8*	No
							$R^2_{adj} = 55.5\%$			
Dwarf	A	F_2[b] $n = 191$ plants	$DWARF_{F2}$	9	2 (1–3)	CAU4035/CAU4037	266.29*	99.8	−0.5*	−0.5*
							$R^2_{adj} = 99.9\%$			
		F_3[c] $n = 155$ families	*DWARF*	9	2 (1–3)	CAU4035/CAU4037	47.61*	5.93	−0.8ns	−0.6ns
							$R^2_{adj} = 74.2\%$			

Significant QTL for seasonal bolting time ($\alpha_E = 0.01$) were mapped with seasonal bolting time determined as days-to-bolting (DTB) in the year of sowing

[a] Also supported by seasonal bolting time data of repeated bolting in 2013

[b] Dwarf phenotype recorded as binary code (0 = normal growth, 1 = dwarf)

[c] Dwarf phenotype determined as dwarf rate (number of dwarfs out of the total plant number per F_3 family), R^2 coefficient of determination, R^2_{adj} coefficient of determination for all detected non- and significant QTL

LOD logarithm of the odds, significant at * $\alpha_E = 0.01$, ns non-significant

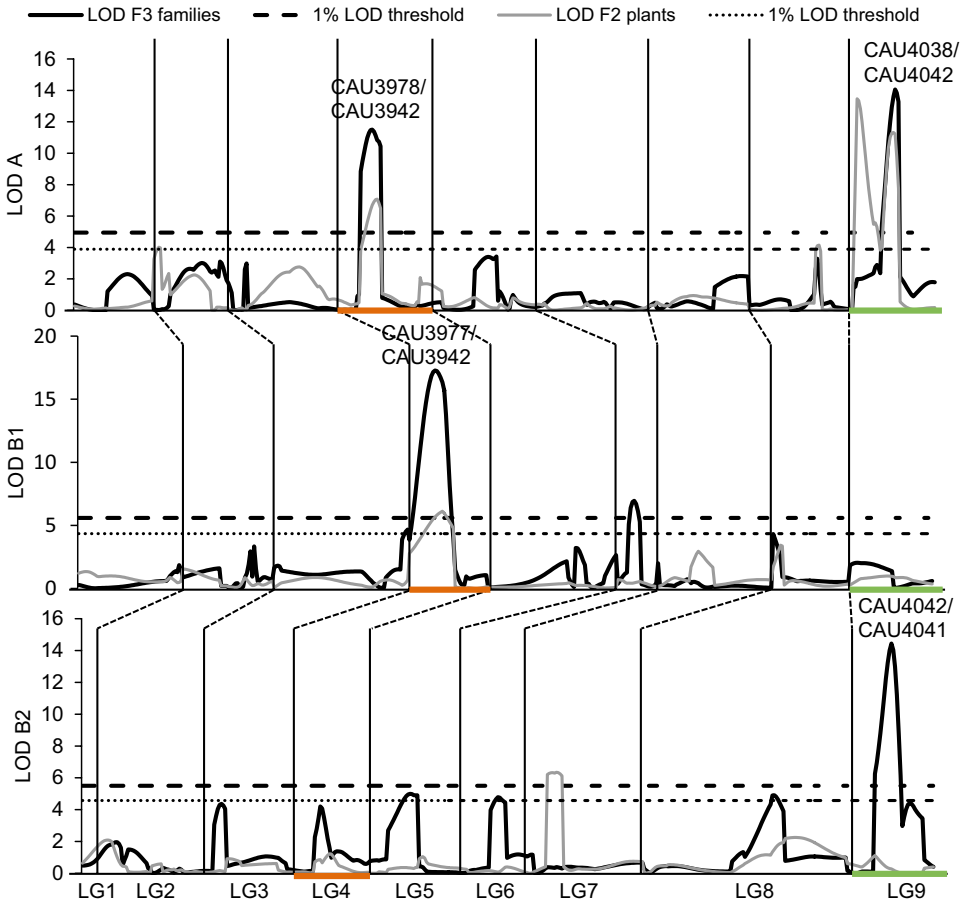

Fig. 4 LOD profiles of F_2 and F_3 phenotypic data for DTB in the year of sowing of population A, B1 and B2 over all chromosomes. The empirical 1% LOD thresholds are based on 1000 permutations. Flanking markers of major QTL on chromosome 4 (*orange*) and 9 (*green*) are mentioned above the LOD peak (color figure online)

and explained 21.4 and 19.5% of the phenotypic variation, which corresponded to 37.5% of the genotypic variation. The additive effects on bolting delay were estimated as 4.7 days for *SBT-4* and 5.0 days for *SBT-9*. The alleles causing bolting delay were derived from the iteroparous parent. F_3 families that were homozygous for late bolting alleles at one or both loci, bolted significantly later than F_3 families that included early bolting alleles at these loci (Fig. 5a, b). An epistatic interaction between *SBT-4* and *SBT-9* was not observed.

Based on F_3 data of population B1, two QTL were mapped: one on chromosome 4 at position 18 and the other one on chromosome 6 at position 9. They were designated as $SBT\text{-}4_{B1}$ and $SBT\text{-}6_{B1}$ (Table 3). Both QTL explained together 64.5% of the phenotypic and 71.0% of the genotypic variation. The allele for bolting delay at $SBT\text{-}4_{B1}$ was derived from the iteroparous parent with an additive effect on bolting delay of 12.9 days. In contrast, the bolting delay allele at $SBT\text{-}6_{B1}$ was derived from the semelparous parent and showed an additive effect on bolting delay of 5.0 days (Table 3; Fig. 5c). F_3 families that were homozygous for late bolting alleles on both loci showed the latest bolting, whereas the earliest bolting was observed for families that carried early bolting alleles in the homozygous stage on both loci, indicating an additive interaction between *SBT-4* and *SBT-6* (Fig. 5d). An epistatic interaction of both QTL was not observed.

For B2, one QTL was detected for seasonal bolting time, which explained 44.6% of the phenotypic and 66.1% of the genotypic variation. This QTL mapped on chromosome 9 at position 24 and was named $SBT\text{-}9_{B2}$ (Table 3). The allele for bolting delay was derived from the iteroparous parent with an additive effect on bolting delay of 7.8 days (Fig. 5e).

SBT-4, *SBT-9* and $SBT\text{-}4_{B1}$ QTL were detected using F_2 and F_3 data (Fig. 4). By comparing the positions of the QTL flanking markers, we found co-localization of *SBT-4* and $SBT\text{-}4_{B1}$ as well as *SBT-9* and $SBT\text{-}9_{B2}$ (Fig. 3). Interestingly, the late bolting alleles were derived from different pollinators. In contrast to $SBT\text{-}6_{B1}$, the *SBT* QTL on chromosome 4 and 9 were mapped using phenotypic data of different generations obtained from different years and under different cultivation conditions. Based on these results, we conclude that seasonal bolting time is predominantly controlled by *SBT-4* and *SBT-9*.

According to RefBeet-1.1, the *SBT-4* flanking markers CAU3977 and CAU3978 are physically located at one

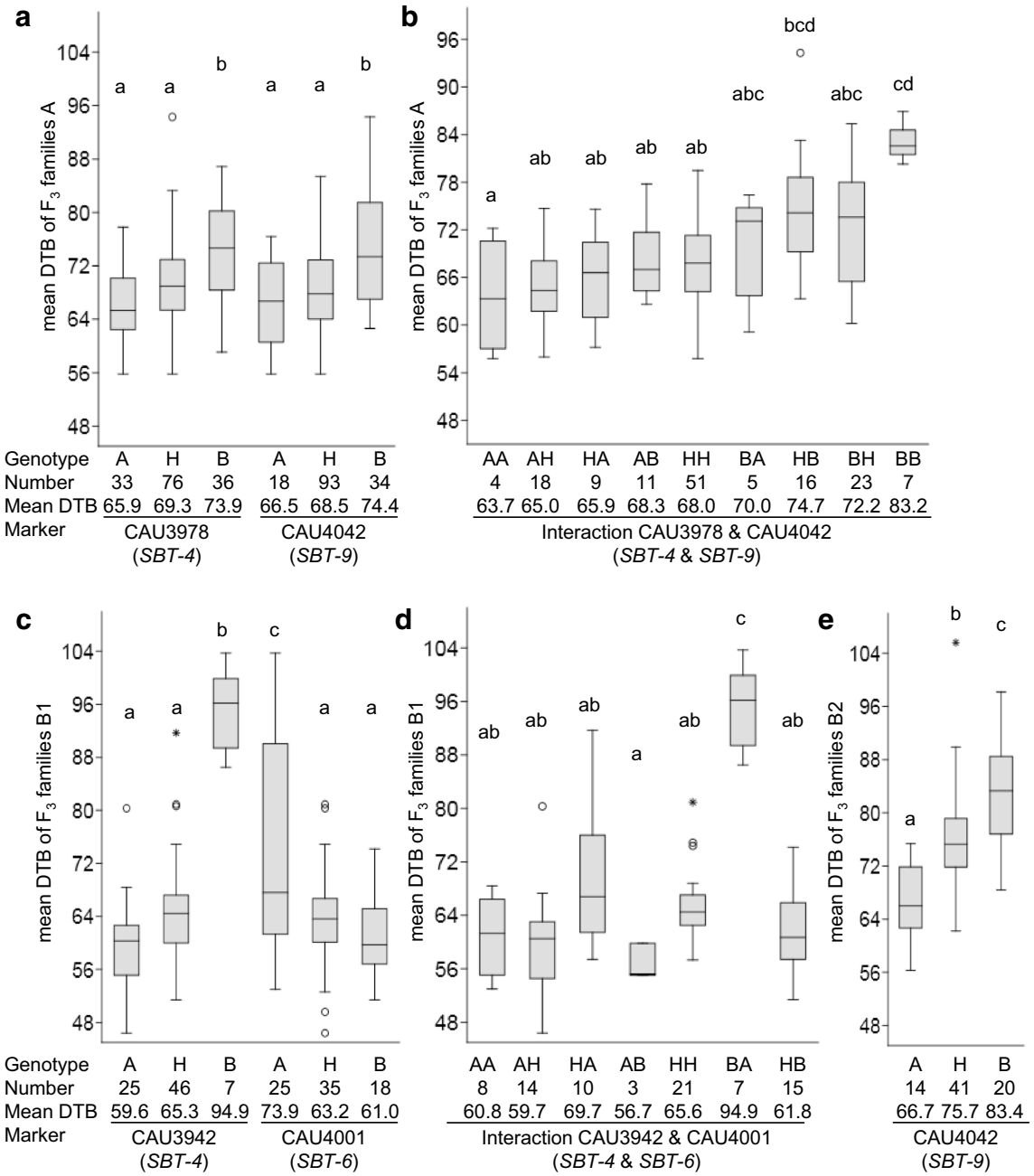

Fig. 5 Box plots of mean DTB of F_3 families depending on the genotype on *SBT* QTL positions. Mean DTB of F_3 families of population A are shown based on **a** the genotype on *SBT-4* and *SBT-9* separately and **b** combining the genotypes of *SBT-4* and *SBT-9*. Mean DTB of B1 F_3 families are presented according to **c** the genotype on *SBT-4* and *SBT-6* separately and **d** combining the genotypes of both QTL. **e** Mean DTB of B2 F_3 families depending on the genotype on *SBT-9*. The genotype is based on marker information of *SBT-4* flanking markers CAU3978 and CAU3942 and *SBT-9* flanking marker CAU4042. The genotype is given as A (homozygous for the allele of the semelparous-annual plant), H (heterozygous), B (homozygous for the allele of the pollinator). *Different letters* indicate significant differences at $\alpha_E = 0.05$ that were determined by Tukey's pairwise post hoc test

scaffold spanning a distance of 561 kb. The marker interval encompasses 61 genes. Only one gene shows homology to flowering genes of *Arabidopsis* and was identified as the floral promoter gene *BvFT2*. Thus, we assume that *BvFT2* is causative for the *SBT-4* effect. The *STB-9* flanking markers are also located at one scaffold with a distance of 715 kb. The marker interval includes more than 100 genes; however, none of them was outstanding as candidate flowering gene. Interestingly, *SBT-9* co-localizes with BR_1, a major QTL for post-winter bolting resistance

in biennial sugar beets (Pfeiffer et al. 2014). Recently, BR_1 was physically mapped to a 103 kb region on chromosome 9 (Tränkner et al. 2016). This region is located in the middle of the *SBT-9* flanking marker interval. This perfect co-localization leads to the hypothesis that the BR_1 gene is causative for the *SBT-9* effect.

Genetic mapping of dwarf growth

Additionally, we mapped a QTL for dwarf growth using F_2 and F_3 phenotypic data. The dwarf QTL mapped on chromosome 9 at position 2 and is flanked by CAU4035 and CAU4037 (Table 3). The allele causing dwarf growth was derived from the semelparous parent and acts recessively. The dwarf QTL is located 23 cM upstream of *SBT-9* (Fig. 3). A candidate gene for dwarfism could not be identified due to the wide marker interval, which includes several RefBeet-1.1 scaffolds.

Discussion

Few major loci enrich the variation of bolting time in beet dramatically

Bolting time is a major trigger in sugar beet breeding to increase beet or seed yield. Therefore, understanding bolting and flowering time regulation in beet is of great importance. Previously, bolting time was mostly measured as bolting before or after vernalization, which distinguished annual and biennial beets. However, we subdivided bolting time into three physiological processes: vernalization requirement, seasonal bolting time, and single versus repeated reproduction. We showed: (1) that each physiological process contributes *per se* to the variation of bolting time, (2) that these processes act independently from each other, (3) that vernalization requirement is controlled by the *B* locus, while seasonal bolting time is predominantly controlled by *SBT-4* and *SBT-9* and (4) that various allele combinations at all three loci account for the immense variation of bolting time in our mapping populations. Thereby, late bolting alleles were identified in the annual as well as the iteroparous parents.

Our results clearly demonstrate the quantitative nature of bolting time and that only few major loci are sufficient to increase the variation of bolting time in beet. This finding is in accordance with flowering time regulation in other plant species. Salome et al. (2011) analyzed the genetic architecture of flowering time in *Arabidopsis* by analyzing 17 F_2 populations derived from 18 distinct accessions. In total 55 QTL were mapped, two to five QTL per population. However, the majority of these 55 QTL clustered in only four genomic regions. In 410 European winter wheat varieties, only one major locus was identified by genome-wide association mapping. This QTL explained 58% of the genotypic variance and corresponded to the photoperiod regulator *Ppd-D1*. Additional QTL and copy number variation at the *Ppd-B1* locus contributed also to flowering time variation, but explained only 0.1–3.2% of the genotypic variance (Langer et al. 2014). In a nested association mapping population derived from 26 barley accessions, eight flowering QTL were identified. The strongest QTL explained 36% of the cross-validated proportion of explained genotypic variance, while the other loci explained only 1–7% (Maurer et al. 2015). In a RIL population of *Brachypodium distachyon* derived from an early and delayed flowering accession with facultative vernalization requirement, two out of six significant flowering time QTL showed major effects on flowering time under various photoperiod and vernalization conditions (Woods et al. 2017). Thus, most of the flowering time variation is obviously controlled by only few major loci, while the adjustment to local conditions seems to be achieved through minor QTL.

BTC1 regulates vernalization requirement, while two other major flowering time loci control seasonal bolting time

Previous studies had identified the *B* locus as major regulator of vernalization requirement (Abegg 1936; Pin et al. 2012). Our results are in accordance with these findings, because the bolting phenotype in all three mapping populations co-segregated with the corresponding genotype at the *B* locus. The *B* locus is located on chromosome 2 and encodes the bolting gene *BTC1* (Pin et al. 2012). While the *B* locus determines the year of first reproduction, *SBT-4* and *SBT-9* majorly control seasonal bolting time. In this study, *SBT-4* and *SBT-9* were repeatedly detected in different populations and generations, different years, under natural as well as artificial greenhouse conditions, explaining 37.5–71.0% of the genotypic variance. Both QTL were detected in population A. However, only one of these QTL was mapped in the sibling populations: *SBT-4* in B1 and *SBT-9* in B2 (Fig. 3). Maybe, the other QTL was not detectable due to the low detection power by analyzing only 96 F_2 plants or 78 and 75 F_3 families, respectively. Otherwise, the annual and the iteroparous crossing parent of the B populations showed 1.7 and 36% heterozygosity, respectively (Suppl. Table 2). Thus, the F_1 parental plants of B1 and B2 were not uniform and the sibling populations differ genetically. Thus, B1 and B2 might have sequence differences on these QTL positions or on additional loci that interact with these QTL, which might explain the detection of only one major *SBT* QTL.

For *SBT-4*, we propose *BvFT2* as candidate gene for following reasons: first, the *SBT-4* marker interval includes

only 61 predicted gene models and only one gene model (Bv4_074770_eewx, *BvFT2*) showed homology to a flowering gene of *Arabidopsis*. Second, the *SBT-4* QTL positions, that were detected based on F_2 and F_3 data of populations A and B1, mapped close to marker CAU3978 (Fig. 3), which is physically located in the 2nd intron of *BvFT2*. Third, *BvFT2* is a known flowering time regulator in beet, which is functionally conserved with the *FT* gene of *Arabidopsis*. It is expressed during reproductive growth in annual and biennial beets. Overexpression of *BvFT2* induces flowering before vernalization, whereas *BvFT2* suppression in annual beets prevents floral phase transition (Pin et al. 2010). Here, we provide the first report that genetic variation in a genomic region that includes *BvFT2* contributes to variation of seasonal bolting time. Whether *BvFT2* is the underlying gene of *SBT-4*, has to be proved in further studies. Furthermore, we detected an additive interaction of *SBT-4* and *SBT-9*, which might indicate a common regulatory mechanism of *BvFT2* and BR_1 as discussed by Tränkner et al. (2016).

No known flowering gene is located in the marker interval of *SBT-9*. *BvBBX19* and *BvFT1* are located also on chromosome 9 close to marker CAU4038 and CAU4037, respectively. According to RefBeet-1.1, *BvBBX19* and *BvFT1* have a distance of about 4.57 Mbp and 7.35 Mbp to *SBT-9*, respectively. Thus, we exclude *BvBBX19* and *BvFT1* as potential candidate genes. However, *SBT-9* co-localizes perfectly with BR_1, a locus that controls bolting after winter in biennial sugar beets (Pfeiffer et al. 2014; Tränkner et al. 2016). The *SBT-9* marker interval with a length of 715 kb covers the 103 kb region of BR_1 completely. Thus, we suggest that the BR_1 gene underlies the *SBT-9* locus. The physically mapped BR_1 locus contains 11 genes and a *CLEAVAGE AND POLYADENYLATION SPECIFITY FACTOR* 73-*I* gene, *BvCPSF73-Ia*, was proposed as BR_1 candidate gene (Tränkner et al. 2016). However, the functional analysis of *BvCPSF73-Ia* is missing, which could confirm its function in bolting time regulation. Even, though the BR_1 and *SBT-9* candidate genes are unknown, this chromosomal region is of major importance for beet breeding, because it controls bolting time from bolting delay before winter up to non-bolting after winter due to genetic variation.

Based on the final QTL mapping, we had detected a third QTL, $SBT\text{-}6_{B1}$. This QTL was detected only in population B1 in one generation and environment. Maybe, this QTL encodes a minor bolting time gene, which is specific for B1 and detectable only under certain growing conditions. Broccanello et al. (2015) reported a single nucleotide polymorphism (SNP) on chromosome 6, which is associated with early and late bolting in beets that carry the biennial *b* alleles in the homozygous state. However, we do not know whether this SNP co-localizes with $SBT\text{-}6_{B1}$, because the SNP is located on a scaffold which is currently not anchored in the sugar beet reference genome.

Another important flowering time gene of beet, *BvBBX19* from the *B2* locus, did not contribute to bolting time variation in our mapping populations, because the crossing parents carried the same *BvBBX19* haplotype. Furthermore, the floral repressor gene *BvFT1*, which is located upstream of *SBT-9*, did not affect bolting time in our mapping populations. *BvFT1* is located close to marker CAU4037, which is not associated with any of the *SBT* QTL mapped on chromosome 9 (Fig. 3). Recently, three QTL for bolting delay after vernalization were identified in a mapping population which were derived from a cross of biennial leaf beet with the same semelparous-annual accession as used in our study. These QTL mapped on chromosome 3, 5 and 9 under natural overwintering conditions (Pfeiffer et al. 2017). However, none of these QTL co-localize with any of our *SBT* QTL. Hébrard et al. (2016) identified 169 differentially expressed genes and 111 differentially methylated regions between bolting-sensitive and resistant vernalized beet genotypes. These genes and regions are distributed across the beet chromosomes and include also *BvFT2*. We propose that only a few major loci massively contribute to the variation of flowering time in beet. Nonetheless, additional minor loci might control the fine-tuning to local conditions in a genotype-specific manner.

No significant QTL were detected for semel- or iteroparous growth using phenotypic data of F_2 populations A, B1 and B2 (data not shown). The evolutionary switch between semelparity and iteroparity occurred several times in plant evolution and might be caused by single gene mutations (Friedman and Rubin 2015). For example, *Arabidopsis* converts from annual to iteroparous growth through mutations in the flowering genes *FRUITFULL* (*FUL*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1*/*SOC1* (Melzer et al. 2008). *Arabis alpina* plants turn from perennial to annual-semelparous growth, when mutated in the *FLC* homologous gene *PERPETUAL FLOWERING 1* (Wang et al. 2009). Thus, we assume that only a few genetic factors control this trait also in beet.

Application of seasonal bolting time QTL for sugar beet breeding

Beside the *B* and *B2* bolting loci, *SBT-4* and $SBT\text{-}9/BR_1$ will be of major importance for sugar beet breeding because these loci control seasonal bolting time before and after winter. We suppose that late bolting alleles of *SBT-4* and *SBT-9* (BR_1) in combination with biennial alleles at *B* will provide beet genotypes with bolting resistance, which is required in beet cultivation to achieve maximum beet

yield. Otherwise, early bolting alleles should be combined to obtain early flowering beet prototypes. These prototypes can be used in breeding programs to shorten generation cycles and hence to accelerate breeding time. This is beneficial, when favorable wild type genes like the rhizomania resistance gene *Rz2* from *B. vulgaris* ssp. *maritima* shall be transferred into elite material of *B. vulgaris* ssp. *vulgaris*. Furthermore, synchronizing of bolting and flowering time for seed production is important, because parents of sugar beet hybrids must bolt and flower at the same time. Thereby, the knowledge of the identified loci and alleles will allow fine-tuning of flowering time. During the breeding process, specific markers such as GJ1001c16 for the *B* locus, CAU3978 for *SBT-4* and CAU4042 for *SBT-9* will support marker-assisted selection of beets with controlled bolting behavior. Due to the importance of these loci, the *Beta* gene pool should be screened for further allelic variations at *SBT-4* and *SBT-9* as already done for the *BTC1* gene of the *B* locus (Pin et al. 2012). Identification of further alleles with minor or larger effects at these loci will support the development of beet genotypes with purpose-oriented bolting time.

Relevance for sea beets (*B. vulgaris* ssp. *maritima*)

The independency of vernalization requirement and repeated reproduction is also relevant for understanding what happens in natural populations. While breeders often only distinguish between annuals and biennials, the situation in sea beet is more complicated. Individuals without vernalization requirement may live and reproduce for several years (Van Dijk 2009) and, therefore, cannot be called annuals. Nevertheless, there obviously is a certain association of reproduction in the year of germination and a short life span. Rather than considering genetic or physiological reasons, observations point to the environmental circumstances as a causal factor. An extreme example is delivered by weed beets (Boudry et al. 1993): the fact that they are eliminated by the farmers after or sometimes even before crop harvest makes that both very early reproduction and lack of investment in survival are selected for. Other forms of sea beet, which grow in natural coastal areas, show a high level of variation. Short-lived plants without vernalization requirement are found in disturbed habitats, while long-lived plants requiring vernalization occur in stable habitats (Hautekèete et al. 2002).

Author contribution statement CT supervised the project, designed and performed experiments, analyzed data and wrote the manuscript, NP supported pheno- and genotyping, MK and HvD provided plant material and information of iteroparous beet accessions, MS generated NGS data, FKO and MH analyzed data, NE performed experiments, analyzed data and wrote the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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