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ORIGINAL PAPER

Genetic analysis of bolting after winter in sugar beet (*Beta vulgaris* L.)

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Abstract
Key message **This study reveals for the first time a major QTL for post-winter bolting resistance in sugar beet (*Beta vulgaris* L.). The knowledge of this QTL is a major contribution towards the development of a winter sugar beet with controlled bolting behavior.**
Abstract In cool temperate climates, sugar beets are currently grown as a spring crop. They are sown in spring and harvested in autumn. Growing sugar beet as a winter crop with an extended vegetation period fails due to bolting after winter. Bolting after winter might be controlled by accumulating genes for post-winter bolting resistance. Previously, we had observed in field experiments a low post-winter bolting rate of 0.5 for sugar beet accession BETA 1773. This accession was crossed with a biennial sugar beet with regular bolting behavior to develop a F_3 mapping population. The population was grown in the greenhouse, exposed to artificial cold treatment for 16 weeks and transplanted to the field. Bolting was recorded twice a week from May until October. Post-winter bolting behavior was assessed by two different factors, bolting delay (determined as days to bolt after cold treatment) and post-winter bolting resistance (bolting rate after winter). For days to bolt, means of F_3 families ranged from 25 to 164 days while for bolting rate F_3 families ranged from 0 to 1. For each factor one QTL explaining about 65 % of the phenotypic variation was mapped to the same region on linkage group 9 with a partially recessive allele increasing bolting delay and post-winter bolting resistance. The results are discussed in relation to the potential use of marker-assisted breeding of winter sugar beets with controlled bolting.

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Introduction

Sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima*) is the only sucrose-storing crop species in temperate climates and accounts second behind sugar cane for about 15 % of the raw equivalents used for worldwide sugar production (FAO 2012). In cold temperate climates, sugar beet is currently grown as a spring sown crop and harvested in autumn of the same year while the crop is still in the vegetative stage. It has a strictly biennial life cycle. To enter the generative stage, sugar beet requires vernalization through prolonged exposure to cold temperatures followed by long day conditions (Biancardi et al. 2005). After transition to the generative stage, elongation of the main shoot occurs which is termed bolting. Interestingly, the requirement for vernalization is not obligate throughout the wild beet *B. vulgaris*

ssp. *maritima*, which is the progenitor of sugar beet. In wild beet, forms with and without vernalization requirement have been described (Van Dijk 2009). This variation is determined mainly by the bolting locus *B* (Abegg 1936; Boudry et al. 1994; El-Mezawy et al. 2002), where annual growth habit (*BB, Bb*) is dominant over vernalization requirement (*bb*). Apart from the *B* locus, a survey of biennial genotypes identified after EMS mutagenesis of an annual accession revealed two additional loci affecting bolting, termed *B2* and *B4* (Abou-Elwafa et al. 2012; Büttner et al. 2010; Hohmann et al. 2005). At each of these loci, the homozygous recessive genotype requires vernalization for bolting.

Although sugar beet cultivars carry the *b* allele (Pin et al. 2012), cold temperatures after sowing could vernalize the plants and lead to bolting before winter (Chiurugwi et al. 2013; Milford et al. 2010), often referred to as early bolting. As early bolting is undesired during crop production, this has always been addressed in breeding and resulted in improved early bolting resistance of modern cultivars (Milford et al. 2010).

In the current sugar beet production system in cold temperate regions, slow leaf formation in spring is regarded as a limiting factor for beet growth and therefore sugar beet yield (Hoffmann and Kluge-Severin 2010; Jaggard et al. 2009). It is expected that yield can be increased when plants develop leaves earlier in spring by sowing already in late summer of the year previous to harvest. These so-called winter beets are expected to have a higher leaf area index in spring (Jaggard et al. 2009). This in return increases light interception resulting in yield increases of theoretically up to 26 % (Hoffmann and Kluge-Severin 2010). Perhaps even more important, winter beets allow an earlier harvest and start of the beet campaign resulting in a higher processing capacity of sugar refineries. Under the different objective to escape drought stress, winter beet production has already been introduced in sugar beet growing areas south of the 40th parallel in the 1950s (Biancardi et al. 2005). This allows harvest in early summer before water gets scarce (Jonsson 1999). Most important production areas include Southern Spain, Morocco and Iran (Esteban Baselga 1999). As winters are mild in these areas, varieties with sufficient early bolting resistance will not start bolting in spring. To grow winter beets under cold temperate conditions, however, two requirements have to be fulfilled: (1) sufficient winter hardiness and (2) control of bolting after winter by bolting suppression during crop production (post-winter bolting resistance) and bolting induction during breeding and seed production. To develop winter sugar beets with controlled bolting behavior, it is necessary to understand the genetics underlying variation of bolting and flowering time (Jung and Müller 2009).

Recently, Pin et al. (2012) cloned the *B*-gene and named it *BOLTING TIME CONTROL 1* (*BTC1*). *BTC1* is a homologue of the *Arabidopsis* circadian clock regulator gene *PSEUDO RESPONSE REGULATOR 7* (*PRR7*). The authors demonstrated the requirement of *BTC1* for flowering through its interaction with two sugar beet paralogs of the *Arabidopsis FLOWERING LOCUS T (FT)* gene termed *BvFT1* and *BvFT2* (Pin et al. 2010). These two genes act antagonistically in beet with *BvFT1* as a floral repressor and *BvFT2* as a floral promoter (Pin et al. 2010; Pin and Nilsson 2012). According to the proposed model, in annuals the dominant allele of *BTC1* suppresses *BvFT1*; whereas, *BvFT2* is upregulated which in return induces flowering without vernalization (Pin et al. 2010, 2012). In contrast, *BvFT1* is expressed in non-vernalized biennial beets that carry the recessive *btc1* allele. Prolonged exposure to cold leads to downregulation of *BvFT1* accompanied by upregulation of *BvFT2* (Pin et al. 2010).

By gene silencing of *btc1* through RNA interference (RNAi), Pin et al. (2012) obtained a transgenic sugar beet that showed complete post-winter bolting resistance after 12 weeks of cold treatment. Post-winter bolting resistance in sugar beet was already reported in the 1930s in Hungary by Bauer (1932). The author claimed the selection of a sugar beet with nearly complete post-winter bolting resistance without further quantifying the level of bolting resistance. Claus (1937) reported a reduction of bolting rate after winter from 0.57 down to 0.15 observed in different autumn sown lines after three generations of selection in Germany. Eleven years later, McFarlane et al. (1948) reported about nine different sugar beet lines that showed high post-winter bolting resistance in the middle of April when sown in August with bolting rates after winter ranging from 0.57 to 0.05. A further report on winter beet breeding efforts was published in 1962 (Eichholz and Röstel 1962). They reported a yield increase in overwintering beets ranging from 13 to 99 % and reduction in bolting rate varying from 0.91 down to 0.05. Wood and Scott (1975) demonstrated the effect of sowing time and application of growth regulators on bolting rate of overwintering beets. A shift in sowing time from late September to the middle of October reduced the bolting rate from 0.54 to 0.23 in mid-June of the following year. Similarly, applying the growth regulator ethephon on overwintered beets in mid-April resulted in a bolting rate reduction by up to 0.3. The first systematic study on the inheritance of post-winter bolting resistance in sugar beet was published by Sadeghian et al. (1993). They analyzed bolting rates after up to 10 weeks of cold treatment in three different sugar beet populations. After 10 weeks of cold treatment, the parents of these populations showed various levels of bolting rates ranging from 0 to 1. The authors could demonstrate that genetic variation in bolting rate after winter is mainly due to additive effects. Dominance effects were also important with dominance towards higher bolting rates, but epistasis seemed to play only a minor role. However, it has to be considered that in this study cold treatments were only

up to 10 weeks. This is on the lower end of vernalization requirements of 10–14 weeks cold treatment as described in the literature (Biancardi et al. 2005).

Induction of bolting depends not only on the length of cold treatment but on the temperature as well. In the literature, optimum temperatures have been reported between 3 and 9 °C (Bachmann et al. 1963; Curth 1962; Smit 1983; Stout 1946). Taking both factors into account, Milford et al. (2010) studied the influence of cold temperatures in spring on early bolting of sugar beet. They expressed the length and intensity of cold treatment as vernalization-weighted hours (vwh) and modeled the bolting rate of sugar beet in response to vwh. This model allows the quantification of bolting sensitivity in terms of vwh-thresholds. The results of this study indicated that more recent sugar beet varieties tend to have a higher vernalization requirement with a threshold of 140 vwh than older varieties with a threshold of 120 vwh, reflecting the improved early bolting resistance due to breeding activities (Milford et al. 2010).

In overwintering field trials in 2008/2009 with 396 *B. vulgaris* accessions described by Kirchhoff et al. (2012), sugar beet accession BETA 1773 showed a high level of post-winter bolting resistance across three locations. Fifty percent of the plants of this accession did not bolt until middle of June of the second year when the experiment was completed (unpublished data). Moreover, the remaining plants from this accession bolted up to 4 weeks later than other sugar beet accessions that were in the experiment. As this bolting phenotype was observed across different environments, there is a strong indication that this is caused by genetic factors.

The purpose of this study was to identify the genetic factors underlying the post-winter bolting behavior of sugar beet accession BETA 1773 and to answer the question if this material can be used for breeding winter sugar beets. Hence we: (1) developed a structured mapping population which segregates for bolting delay and post-winter bolting resistance derived from accession BETA 1773; (2) phenotyped the bolting behavior of this population in the F_2 and F_3 generation; (3) constructed a genetic map for the F_2 population; and (4) mapped quantitative trait loci (QTL) for bolting delay and post-winter bolting resistance.

Materials and methods

Plant material and growth conditions

A F_2 population was developed from hand crossing sugar beet accession BETA 1773 with sugar beet accession 93161P followed by selfing the obtained F_1. BETA 1773 was provided by the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany, and 93161P was provided by Saatzucht Dieckmann, Nienstädt, Germany. BETA 1773 had shown after winter a bolting time delay of about 30 days compared to 93161P and a low post-winter bolting rate of about 0.5 (see above). Both parents carry the biennial allele *b* at the bolting locus *BTC1* (Pin et al. 2012). F_1 plants were grown in the greenhouse and selfed by bag isolation for F_2 seed production. F_2 seeds derived from a single F_1 plant (090757/03) were sown in the greenhouse on November 1, 2010. In total 410 F_2 plants were obtained and grown in 9×9 cm^2 pots (Hermann Meyer KG, Germany) at 20 °C under long day conditions (16 h light, 900 $\mu mol/m^2s^{-1}$, Son-T Agro 400 W, Koninklijke Philips Electronics N.V., Eindhoven, Netherlands). From December 20, 2010 to April 12, 2011 the plants were kept for 16 weeks in a cold chamber at 5 °C under 22 h light (200 $\mu mol/m^2s^{-1}$, Osram Lumilux T8 L 58 W/840, Osram AG, München, Germany). After 1 week of acclimatization at 8 °C and 22 h light, (200 $\mu mol/m^2s^{-1}$, Osram Lumilux T8 L 58 W/840, Osram AG, München, Germany), all F_2 plants were planted to a field nursery in Kiel, Germany, on April 19, 2011. Bolting F_2 plants were bag isolated for production of F_3 seed. F_3 seeds were obtained from 276 out of 384 bolting plants. Seed production of 108 plants was not successful due to lack of seed set of 89 plants and incomplete bolting of 19 plants. Plants with incomplete bolting showed only stem elongation without flower development. In addition, 26 plants did not bolt at all until September 15, 2011 and F_3 seed could not be produced from these plants either.

Phenotypic analysis and bolting experiments

During F_3 seed production, the F_2 population was already phenotyped for a preliminary genetic analysis on bolting behavior. The population was grown under conditions described above and the onset of bolting of F_2 plants was recorded from May 20 until August 31, 2011. Post-winter bolting behavior of F_2 plants was assessed by two different factors, bolting delay (determined as days to bolt after cold treatment, DTB) and post-winter bolting resistance (as a binary bolting code). DTB is defined as the number of days until the elongation of the main shoot started [BBCH 51 (Meier 2001)] from the day that the plants had left the cold chamber. The binary bolting code was based on the F_2 phenotype on August 31, 2011. All bolting plants were scored with a bolting code (BC) of 1 and all plants without visible shoot elongation (bolting-resistant plants) were scored with a BC of 0.

The F_3 generation was tested in replicated bolting tests based on F_3 seed obtained from selfing the F_2 plants (see above). As seed was rare and a harsh winter could kill parts of the experiment, F_3 plants were artificially cold treated and transplanted into the field in spring. For this,

seeds of 254 F_3 families with sufficient numbers of seed were sown in the greenhouse on December 9, 2011 into quickPot-plates96T (Hermann Meyer KG, Germany) and kept for 4 weeks at 20 °C under LD conditions (16 h light, 900 µmol/m^2s^{-1}, Son-T Agro 400 W, Koninklijke Philips Electronics N.V., Eindhoven, Netherlands). Cold treatment was done for 16 weeks under LD conditions (5 °C, 16 h light, 200 µmol/m^2s^{-1}, Osram Lumilux T8 L 58 W/840, Osram AG, München, Germany). On May 02, 2012 248 F_3 families and the parental accessions were planted to a field nursery in Kiel, Germany as a randomized complete block design with two replications and single rows as experimental units. In each row, up to eight plants were planted if available with a between row distance of 45 cm and a within row distance of 20 cm. Two further replicates were planted to the field on May 07, 2012. Due to lack of original seed we used a selfing progeny of BETA 1773 which was generated by bag isolation of a plant that had bolted after overwintering in the field in 2009/2010. Six out of 254 F_3 families did not germinate. Onset of bolting was recorded twice a week from the end of May until middle of October 2012 as described above. Post-winter bolting behavior of F_3 families was also assessed by bolting delay and post-winter bolting resistance. Different from F_2 phenotyping, bolting delay was determined by the average DTB of a F_3 family. For plants without visible shoot elongation by the end of the experiment, a DTB of 166 for replicate 1 and 2, or 161 for replicate 3 and 4 was recorded corresponding to the number of DTB on the last day of the experiment (October 15, 2012). Post-winter bolting resistance was determined at the end of the experiment as the number of bolting plants divided by the total number of plants per F_3 family. Incomplete bolting plants (see above) were treated as bolting plants. For an overview on the work flow of population development and phenotyping see Supplementary Fig. 1.

Molecular marker analysis

Genomic DNA was isolated from freeze-dried leaf samples of the sugar beet F_2-population. This was done following a slightly modified CTAB protocol (Saghai-Maroof et al. 1984). DNA concentration was adjusted to 10 ng/µl.

Amplified fragment length polymorphism (AFLP) markers were used as described by El-Mezawy et al. (2002), except that *Pst*I was used instead of *Eco*RI. Pre-amplification was done with primers M01 and P01, and the main-amplification was done with primers M31–M46 in combination with primers P31–P46 (Vos et al. 1995; http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html). Polymorphic fragments were named according to the primer combination used for amplification, followed by fragment length and an abbreviation of the parent which carried the fragment (nb = BETA 1773, b = 93161P). AFLP fragment sizes were determined by comparison with the 50–700 bp sizing standard (LI-COR®, Bad Homburg, Germany) on a LI-COR 4300 DNA analyzer using a 6.5 % KB^{Plus} gel matrix (LI-COR®, Bad Homburg, Germany). For data analysis the GelBuddy Tilling Gel Analysis Tool v.1.4.2_08 from the Howard Hughes Medical Institute (Seattle, USA) was used. The marker strategy was to genotype only a subpopulation of 124 F_2 plants with AFLP and to enrich QTL regions with sequence derived co-dominant markers applied to the whole population that was tested in the F_3 generation.

To anchor the genetic map, previously mapped simple sequence repeats (SSRs), expressed sequence tags (ESTs) and genes (Laurent et al. 2007; McGrath et al. 2007; Pin et al. 2010; Schneider et al. 2007; Viard et al. 2002) were tested for polymorphism. Polymerase chain reaction (PCR) products that did not differ after agarose gel electrophoresis were Sanger sequenced (Institute for Clinical Molecular Biology (IKMB), University Kiel, Germany) in order to detect single nucleotide polymorphisms (SNPs) or short insertions or deletions (InDels) which could later be converted into cleaved amplified polymorphic sequence (CAPS) markers. Moreover, pooled DNA from F_2 plants was used for Illumina Hiseq 2000 sequencing. The obtained 2 × 100 bp reads were aligned to the draft sugar beet reference genome RefBeet-0.9 (http://bvseq.molgen.mpg.de) and used for InDel marker development.

Primers were designed using the OligoCalc software tool (http://www.basic.northwestern.edu/biotools/oligocalc.html) thereby favoring primers with a length of 18–24 bp, a GC content between 40 and 60 %, and a basic melting temperature of 52–65 °C, without tendency for self-annealing or hairpin formation. All primers used in this study were obtained from MWG Biotech AG (Ebersberg, Germany). Primer sequences will be available from the authors on request.

Statistical analysis

An analysis of variance (ANOVA) was performed for DTB and bolting rate recorded on F_3 families. The ANOVA was done with SAS PROC MIXED (SAS Institute Inc., 2009, Cary, USA, Version 9.2) where the genotype (F_3 family) was treated as a fixed factor while blocks were treated as random effects. For both traits, least square means were estimated as well as variance components for all families with three or more plants over all four replicates. Broad-sense heritability was estimated according to Hallauer et al. (1988) as $\hat{h}^2 = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_G^2 + \hat{\sigma}_e^2/R}$ where $\hat{\sigma}_G^2$ and $\hat{\sigma}_e^2$ are the variance components estimated from the ANOVA for the genotypic and error variance, respectively, with R as the number of replications. Deviation from normal distribution of F_2 data

as well as means for F_3 families were tested with a Shapiro–Wilk test using the software R (R Development Core Team 2010).

QTL analysis

The linkage map was calculated with JoinMap® version 4.1 Package (Van Ooijen 2006) using the Kosambi mapping function (Kosambi 1943), the regression mapping algorithm, a LOD threshold value of 3.0 and a maximum recombination frequency of $R = 0.4$. Linkage groups were anchored with SSRs, ESTs and sequence based markers with known map positions.

QTL analysis was performed by composite interval mapping with the PlabQTL version 1.2 (Utz and Melchinger 2006) assuming a dominant gene model. An experiment wise LOD threshold for the QTL analysis was determined by 1,000 permutations (Doerge 2002).

Results

Phenotypic analysis

The F_2 population showed a wide variation for bolting behavior giving a range in bolting delay of DTB = 103 days (Supplementary Fig. 2). The distribution of DTB between F_2 plants was skewed to the right and deviated significantly from normal distribution as tested by Shapiro–Wilk ($w = 0.7688$; $p = <0.0001$). At the end of the bolting experiment with the F_2 population (October 17, 2011), 384 out of 410 F_2 plants had started bolting (BC = 1). The remaining 26 plants did not bolt by October 17 and were recorded as post-winter bolting resistant (BC = 0). Bolting phenotypes are documented in Supplementary Fig. 3.

In the F_3 generation, we observed variation within as well as between families. Single plants started bolting from May 29, 2012 until October 2, 2012 giving a range in bolting delay of DTB = 126 days. Due to low germination, data from only 186 F_3 families were included in the analysis. In the ANOVA genotypic effects of F_3 families were tested significant ($p < 0.0001$) for DTB with family means ranging from 25 to 164 days (Fig. 1). Normal distribution was rejected by the Shapiro–Wilk normality test ($w = 0.9196$; $p < 0.0001$). Heritability for DTB was estimated as $h^2 = 0.85$. As expected, the parental accessions 93161P and BETA 1773 (selfing progeny) differed strongly with a DTB of 28 and 151, respectively.

For post-winter bolting resistance determined as bolting rate, genotypic effects of F_3 families were also tested significant ($p < 0.0001$) and family means ranged for bolting rate from 0 to 1 (see histogram Fig. 1). Normal distribution was rejected by the Shapiro–Wilk normality test ($w = 0.8516$; $p < 0.0001$). Heritability for bolting rate was estimated as $h^2 = 0.84$. By October 15, 2012 three families showed complete post-winter bolting resistance in comparison to 39 families that bolted completely. The remaining 144 families were segregating in their bolting behavior (see Fig. 1). The bolting rate estimated for population parent BETA 1773 (selfing progeny) was 0.12 compared to population parent 93161P which bolted completely (bolting rate = 1). Bolting phenotypes of F_3 families are documented in Supplementary Fig. 4. Days to bolt and bolting rate showed a highly negative correlation of $r = -0.986$ (see Supplementary Fig. 5).

Molecular marker analysis

A linkage map was calculated with 119 markers (76 AFLP, 1 SSR, 37 InDel and 5 SNP-based CAPS markers). The total length of the linkage map is 737 cM covering all nine *B. vulgaris* chromosomes. The size of the linkage groups ranges from 69.9 to 100.1 cM (Supplementary Table 1).

A preliminary QTL mapping was performed with the phenotypic data of the F_2 population. For bolting delay, two QTL could be mapped on linkage group 9 at position 48 and 68. The QTL were named $DTB_{\_F2\_1}$ and $DTB_{\_F2\_2}$ and explain together 87.1 % of the phenotypic variation (Table 1). Mapping with binary bolting data (BC) resulted also in two QTL on linkage group 9 at position 49 and 69. These QTL were named BC_1 and BC_2 and explain together 64.1 % of the phenotypic variation. Detailed information of LOD, R^2, additive and dominant effects are listed in Table 1.

In the final QTL mapping with the F_3 data, one QTL for the trait bolting delay was detected at position 56 of chromosome 9 with a LOD of 38.12 (Fig. 2; Supplementary Fig. 6). This QTL is named DTB_1 and explains 66 % of the phenotypic variation and 76.8 % of the genotypic variation for DTB (Table 1). DTB_1 is flanked by the markers CAU3841 on map position 53.7 cM and CAU3846 on map position 57.8 cM. The allele causing bolting delay was derived from BETA 1773. The additive effect on bolting delay was estimated as 41 days with a partial dominance effect towards early bolting of 19 days (Table 1). Further, with the F_3 data one QTL could be mapped for the trait post-winter bolting resistance, designated as BR_1. BR_1 is flanked by the markers CAU3839 on map position 43.0 cM and CAU3841 on map position 53.7 cM. The allele causing a reduced bolting rate is derived from BETA 1773 with an additive effect in bolting rate reduction of 0.35 and partial dominance effect of 0.23 coming from the allele of the regular bolting parent 93161P. The partially recessive inheritance of post-winter bolting resistance is shown in Fig. 3 by boxplotting the bolting rates against genotypes of the markers that are flanking BR_1.

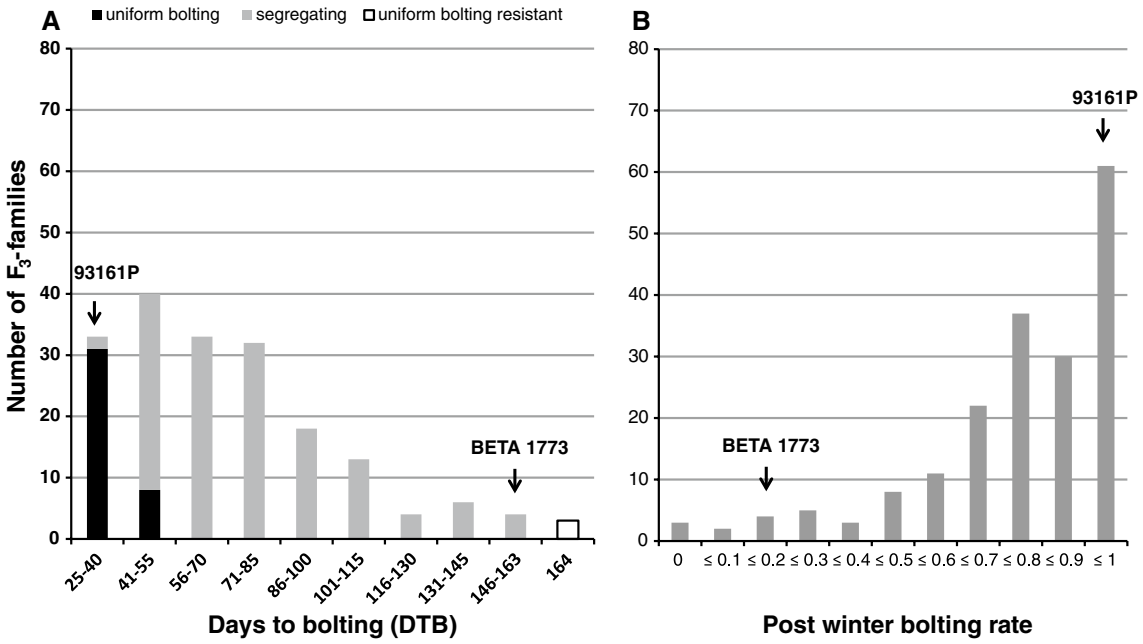

Fig. 1 **a** Phenotypic segregation for bolting delay determined as days to bolt (DTB) in 186 sugar beet F_3 families derived from a cross of sugar beet accessions BETA 1773 and 93161P. DTB are defined as days to bolt after the end of artificial cold treatment, which was May 2 (replications 1 and 2) and May 7, 2012 (replications 3 and 4), respectively. In case of plants which did not bolt during the experiment, a DTB of 166 (replications 1 and 2) or 161 (replications 3 and 4) was recorded considering to the number of DTB until the end of the experiment (October 15, 2012). Parental accessions were carried along the experiments as controls and their DTB mean is indicated by the *black arrows*. It is also indicated, whether families were completely or partially bolting resistant at the end of the experiment by color of bars. **b** Phenotypic variation for post-winter bolting resistance in the same set of 186 sugar beet F_3 families. Post-winter bolting resistance was determined as bolting rate. Bolting rate was recorded at October 15, 2012 and ranges from 0 to 1 (0, all plants of the family showed a bolting-resistant phenotype; 1, all plants of the family bolted). Parental accessions were carried along the experiments as controls and their bolting rate is indicated by the *black arrows*

Table 1 QTL results for post-winter bolting behavior of 410 F_2 plants and 186 F_3 families derived from crossing sugar beet accessions BETA 1173 and 93161P

Generation used for phenotyping	QTL name	Linkage group	Position	Flanking markers	Confidence interval	LOD	R^2	Additive effects	Dominant effects
F_2	DTB_{F2_1}	9	48	CAU3839 and CAU3841	45–51	34.57	52.2	−39.6	−33.8
F_2	DTB_{F2_2}	9	68	CAU3844 and CAU3838	65–71	19.96	34.9	−30.0	−21.8
							87.1		
F_2	BC_{F2_1}	9	49	CAU3839 and CAU3841	45–53	22.79	38.5	0.26	0.25
F_2	BC_{F2_2}	9	69	CAU3844 and CAU3838	65–72	13.75	25.6	0.19	0.18
							64.1		
F_3	DTB_1	9	56	CAU3841 and CAU3846	53–58	38.12	65.9	−41.2	−18.6
F_3	BR_1	9	50	CAU3839 and CAU3841	48–52	36.85	65.0	0.35	0.23

For F_2 plants QTLs were mapped for bolting delay determined as days to bolt after cold treatment (DTB) and post-winter bolting resistance (recorded as a binary bolting code, BC). In the F_3 families, QTLs were mapped for bolting delay (as DTB) and post-winter bolting resistance (as bolting rate, BR). For experimental details see "Materials and methods"

R^2 coefficient of determination, *LOD* logarithm of the odds

Discussion

Post-winter bolting resistance is a crucial trait for the development of a winter beet with controlled bolting behavior in cold temperate regions. This is the first report of a major QTL for post-winter bolting behavior explaining a bolting delay of 82 days and post-winter bolting resistance as a bolting rate reduction by 0.7. The identification of this QTL is a first step towards developing a winter sugar beet by exploiting natural variation in the sugar beet gene pool.

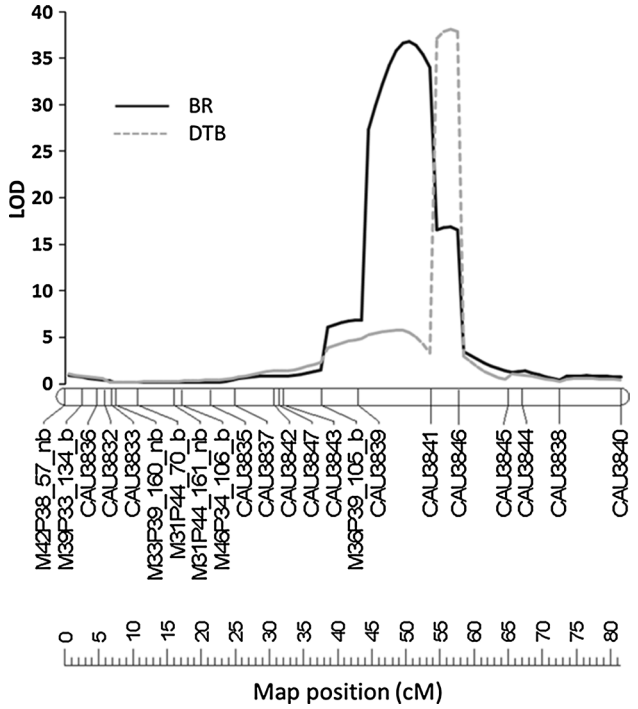

Fig. 2 Detailed QTL mapping results for bolting delay and post-winter bolting resistance on linkage group 9 of sugar beet based on 186 sugar beet F_3 families derived from a cross of sugar beet accessions BETA 1773 and 93161P. The linkage map in cM is indicated by the *horizontal bar* on which marker names are also located. Names of AFLP markers end on nb if the dominant marker allele is derived from the parent BETA 1773 or on b if derived from the parent 93161P. The LOD curve for days to bolt (DTB) is plotted in *dashed grey*, for bolting rate (BR) it is indicated in *solid black*

Combining this QTL with other genetic factors could result in a winter sugar beet with complete post-winter bolting resistance.

Genetic model for post-winter bolting resistance

In our study, we phenotyped post-winter bolting behavior of the F_2 population by two descriptors, bolting delay (determined as DTB) and post-winter bolting resistance (determined as bolting rate). Interestingly, for each individual trait, we detected an almost identical major QTL regarding size, genetic effects and position on the genetic map. Although the two QTL are separated by 6 cM and confidence intervals are not overlapping, we cannot exclude that the genetic factor(s) underlying the QTLs DTB_1 and BR_1 are identical. First, confidence intervals reported by PlabQTL are only an approximation and have to be taken with care as they rather provide a lower boundary for the true supporting interval (Utz and Melchinger 2006). Second, one common QTL makes sense because a delay in bolting time may well have a dosage effect on bolting rate. If bolting is delayed towards decreasing day length at the end of season, light conditions might not be favorable to induce the transition into the generative stage. This putative interaction between bolting delay and day length might also explain in part the incomplete bolting phenotype that we observed in the F_2 and F_3 generations which had been observed before by Driessen (2003) and Schneider (1960). The effect of day length can be clearly seen

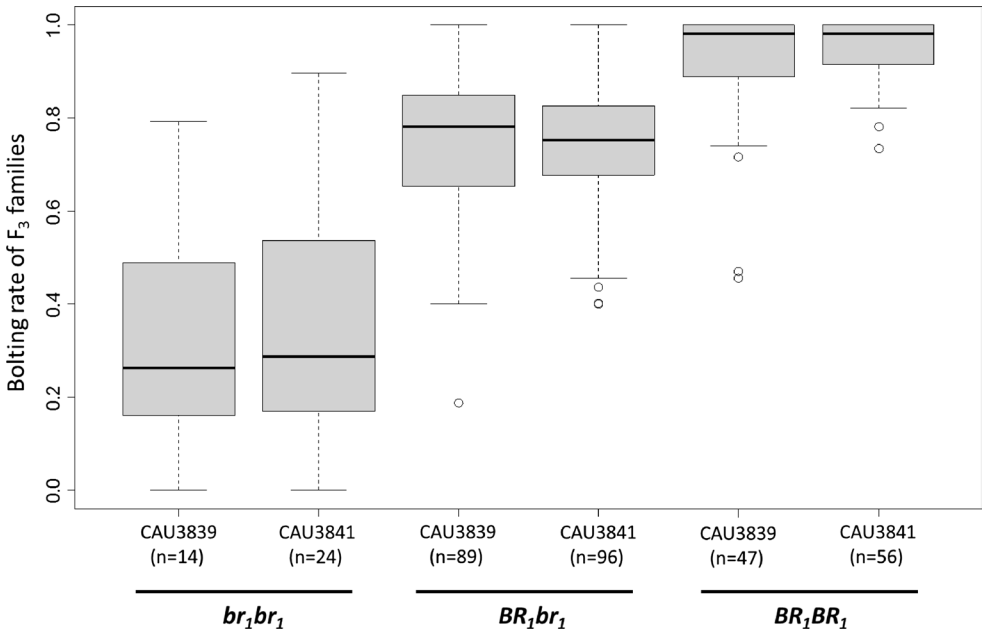

Fig. 3 Boxplot of post-winter bolting resistance of sugar beet F_3 families depending on the genotype at the QTL BR_1. F_3 families were derived from a cross of sugar beet accessions BETA 1773 and 93161P. The genotype of BR_1 is predicted by either of the QTL flanking markers CAU3839 and CAU3841. Bolting rate is ranging from 0 (no plant of the family bolted) until 1 (all plants of the family bolted). The sample size (n) of F_3 families representing each marker class is provided

when comparing F_3 plants that had started bolting until June 21 (increasing day length) and those that had started bolting afterwards (decreasing day length). Only 16 % of the plants that had started bolting before June 21 did not develop inflorescences. In contrast, 80 % of the plants that had started bolting after June 21 did not develop inflorescences (data not shown). For these plants, light conditions might have been sufficient to induce bolting but with further decrease of day length flower development failed.

The lacking co-localization of DTB_1 and BR_1 might be due to the phenotyping approach of each trait. Bolting rate was clearly defined as the number of bolting plants divided by the total number of plants per row at the end of the experiment. In contrast, quantification of bolting delay by DTB was to some extent ambiguous for F_3 plants that had not started bolting until the end of the experiment on October 15, 2012. We decided to record a DTB of 166 and 161 for these plants, which corresponds to the last day of recording in replications 1 and 2, and 3 and 4, respectively. Although it seems reasonable to set a higher DTB to account for these plants, the chosen value would be rather speculative in nature and bias the DTB family means towards a distribution that is similar to bolting rate. This was the case when we recorded larger DTBs for bolting-resistant F_3 plants assuming they require additional time for bolting. With increasing DTB for non bolting plants, the resulting QTL was identical with the QTL BR_1 for bolting rate (data not shown). Due to the uncertainties of defining DTB we put more confidence in the position of the QTL BR_1.

A preliminary QTL mapping in the F_2 population for bolting delay (as DTB) and post-winter bolting resistance (as BC) resulted in two QTL for each trait. For each trait, the larger QTL is comparable to the QTL mapped in the F_3 population: DTB_{F2_1} with DTB_1 and BC_{F2_1} with BR_1. A reason why the second QTL was not detected in the F_3 population could be that not all F_2 plants are represented in the F_3 population. This is due to lack of seed caused by late bolting, incomplete bolting and bolting-resistant plants or due to low seed germination of some F_3 families. In case of post-winter bolting-resistant F_2 plants that are not represented in the F_3 generation, selection might have changed the population structure. In addition, the F_3 population size shrank drastically to 186 F_3 families by poor germination. This smaller population size resulted in a lower statistical power and precision in QTL mapping (Beavis 1998). On the other hand, the precision of phenotypic data based on F_3 family means should be higher compared with F_2 data collected on single plants and are likely the reason for more distinct QTL peaks in the F_3 population. Moreover, a population size of 186 is similar to other QTL studies in sugar beet (Barzen et al. 1992; Lein et al. 2008) and should be sufficiently large. Therefore, while in the F_3 population we cannot exclude that a minor QTL might have been missed, the major QTL should have been mapped with higher precision due to better phenotypic data.

Due to the limited amount of obtained F_3 seed it was not possible to test the material in different environments. In addition, we decided to use artificial cold treatment instead of overwintering in the field to avoid destruction of the experiment due to unpredictable harsh winter conditions. Although in the literature it is reported that 10–14 weeks of cold treatment is sufficient for the plants to enter the generative phase (Biancardi et al. 2005; Lexander 1980, 1987), we decided to use 16 weeks to reach similar conditions to a field experiment, where low temperatures from 10 °C or less over a period of 4 months are not unusual. The bolting rates of the parents indicate that 16 weeks of cold treatment was sufficient to vernalize the accession 93161P (bolting rate = 1). However, we could not reproduce the previously observed bolting rate of 0.50 for parent BETA 1773 (bolting rate = 0.12). Milford et al. (2010) showed that not only the duration of cold treatment but also temperature is affecting the vernalization response of sugar beet. Therefore, the higher bolting rate of BETA 1773 observed in our initial field experiment might be explained by a lower average temperature of 2.5 °C from November through February (data not shown) compared to a temperature of 5 °C in the cold chamber. Apart from that, as pointed out by Gusta and Wisniewski (2013), artificial cold treatment in a climate chamber does not produce the complex environmental conditions as present in nature. Differences in the environmental influences on field overwintering plants versus artificially cold treated plants are the exposition to greater light intensities, the varying light spectrum from autumn to spring, varying diurnal temperatures and the influences of wind as well as no space limitation to root growth (Gusta and Wisniewski 2013; Robertson et al. 1994; Wisniewski et al. 2006). Taken this together could hint at insufficient vernalization conditions in our experiment to reproduce the bolting rates of BETA 1773 previously observed under field conditions. Also, it leads to an overestimation of the post-winter bolting resistance associated with BR_1. Therefore, the effect of this QTL has to be validated under overwintering conditions in the field.

Our results indicate that post-winter bolting resistance in our population is inherited by one major QTL on linkage group 9 and possibly a number of minor QTL which we could not identify. Given the complexity of physiological factors that influence bolting (Lexander 1980), it is not surprising that apart from major genes bolting is also affected by minor genes. This is supported by studies on post-winter bolting resistance by Sadeghian et al. (1993) who reported different genetic models for different genetic backgrounds.

The accession BETA 1773 was chosen as a crossing parent because of a low bolting rate of 0.5 observed after

overwintering in the field. BETA 1773 is listed in the genebank under the name 'Kaweaa' (IPK 2006). Kaweaa had been already tested for post-winter bolting resistance in Spain in the 1970s by Lasa and Medina (1978). The authors reported a large variation for bolting rate of this accession under different field environments and sowing dates without further quantifying their observations. In the International Database for Beta (JKI 2012) potential duplicates of Kaweaa are named "Klein Aa" and "KWS Aa". Wood and Scott (1975) reported for the sugar beet cultivar "Kleinwanzleben AA" bolting rates of 0.54 after overwintering in a field in England, which is almost identical with our observations. This bolting rate could be interpreted as a 1:1 segregation between bolting and bolting-resistant genotypes in a heterogeneous accession. If that is the case, selection for bolting resistance should result in a line with a decreased bolting rate or even complete post-winter bolting resistance. However, Wood and Scott (1975) did not observe any reduction in bolting rate after one generation of selection of plants that bolted only after a second winter. Therefore, it is more likely that the accession BETA 1773 is not segregating for bolting genes. Instead, the data suggest that BETA 1773 is uniform for genes causing a reduced bolting rate.

In this study, we mapped a QTL for post-winter bolting resistance on linkage group 9. Therefore, the floral repressor gene *BvFT1* on linkage group (Pin et al. 2010) comes first to mind as a potential candidate gene. However, the *BvFT1* gene specific marker CAU3835 maps on position 21.4 cM on the other arm of linkage group 9 28.6 cM apart from QTL BR_1 and 34.6 cM apart from QTL DTB_1. A more promising candidate gene is the *Beta vulgaris GIBBERELLIN 3-OXYGENASE-LIKE 1* gene *BvGA3ox1* (NCBI: DQ864511.1) a homologue to the *GIBBERELLIN 3 BETA-DIOXYGENASE 1* in *Arabidopsis thaliana*. This candidate gene mapped 2.3 cM upstream of DTB_1 and 3.7 cM downstream of BR_1 (marker CAU3841). However, before addressing possible candidate genes, fine mapping is required to further narrow down the QTL region. This is currently done in an effort to clone the underlying gene. Further, for cloning it would be helpful to validate the QTL in a different genetic background.

Implementation of BR_1 in winter beet development

The knowledge of the detected QTL is helpful for developing a winter sugar beet with accession BETA 1773. Marker-assisted selection of the QTL can be done using the markers CAU3893 and CAU3841 which are flanking the QTL (BR_1) region. Due to the partially recessive inheritance, the QTL has to be transferred into both hybrid components in order to obtain homozygous recessive hybrids. Even in the homozygous state, the identified QTL does not confer sufficient post-winter bolting resistance for a winter beet cropping system. However, combining this QTL with other QTL for bolting delay or reduced bolting rate might well result in a sugar beet with complete post-winter bolting resistance. Such QTL are currently being mapped in leaf beet (unpublished data). However, the development of post-winter bolting-resistant sugar beets is only suitable if there is a mechanism to induce bolting for seed production.

If the gene underlying the QTL turns out to be involved in the phytohormone metabolism as assumed for the above mentioned candidate gene *BvGA3ox1* (Mutasa-Goettgens et al. 2009), bolting might be induced by the application of phytohormones.

Another approach for bolting induction in post-winter bolting-resistant beets is the virus-induced flowering (VIF) technique. Once the underlying gene has been cloned, this method allows transferring floral inducing signals into bolting-resistant plants in order to initiate flowering at a specific time. The efficiency of this technique was recently demonstrated in cotton (McGarry and Ayre 2012), apple (Yamagishi et al. 2011) and soybean (Yamagishi and Yoshikawa 2011). In each case, plants were treated with transgenic viruses carrying a copy of the *FT* gene from *Arabidopsis thaliana*. After infection *FT* was expressed in the host plant resulting in early bolting phenotypes. In our case, provided that the gene is cloned, this technique can be used to express the functional copy of the gene underlying the QTL. Co-expressing the gene together with a dominant *BTC1* copy would further increase the efficiency of VIF since then the actually bolting-resistant host plants could be induced to bolt even without cold treatment. Although the VIF system is working with transgenic viruses, the transgenes will not be passed into the seeds that are produced on the VIF-treated plant. As such seeds are non-transgenic, applying VIF might not be subject to regulation.

Conclusions

With the prospect of overcoming bolting resistance for seed production by VIF or the application of hormones, the development of a non-transgenic winter beet seems to be feasible. This, however, requires the identification of further genes for bolting resistance, which in combination with BR_1 results in a winter beet with complete post-winter bolting resistance during crop production in cold temperate regions.

Author contributions NP designed and performed the experiments, analyzed the data and wrote the manuscript, CT, IL and IG provided sequence information, AEM conceived the study and provided sequence and marker information, CJ conceived the study, supervised the project and revised the manuscript. FK conceived the study, supervised

the design and analysis of the experiments and helped to draft the manuscript. All authors read and approved the final manuscript.

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Conflict of interest None of the authors have any conflicts of interest associated with this study.

References

Abegg F (1936) A genetic factor for the annual habit in beets and linkage relationships. J Agric Res 53:493–511

Abou-Elwafa SF, Buttner B, Kopisch-Obuch FJ, Jung C, Muller AE (2012) Genetic identification of a novel bolting locus in Beta vulgaris which promotes annuality independently of the bolting gene B. Mol Breed 29:989–998

Bachmann L, Curth P, Röstel HJ (1963) Untersuchungen über Gibberellin-Verabreichung in verschiedenen Wachstumsphasen der *Beta*-Rüben. Theor Appl Genet 33:50–57

Barzen E, Mechelke W, Ritter E, Seitzer JF, Salamini F (1992) RFLP markers for sugar beet breeding: chromosomal linkage maps and location of major genes for rhizomania resistance, monogermy and hypocotyl colour. Plant J 2:601–611

Bauer AB (1932) *Beta Hivernalis*. Beiträge zu den Versuchen des Prof. Németh mit winterbeständigen Rüben in Ungarn. Fortschritte der Landwirtschaft 7:1–4

Beavis WD (1998) QTL analyses: power, precision and accuracy. In: Paterson AH (ed) Molecular analysis of complex traits. CRC Press, Boca raton, pp 145–161

Biancardi E, Campell LG, Skaracis GN, De Biaggi M (eds) (2005) Genetics and breeding of sugar beet. Science Publishers Inc, Enfield

Boudry P, Wieber R, Saumitou-Laprade P, Pillen K, Dijk H, Jung C (1994) Identification of RFLP markers closely linked to the bolting gene *B* and their significance for the study of the annual habit in beets (*Beta vulgaris* L.). Theor Appl Genet 88:852–858

Büttner B, Abou-Elwafa S, Zhang W, Jung C, Müller A (2010) A survey of EMS-induced biennial *Beta vulgaris* mutants reveals a novel bolting locus which is unlinked to the bolting gene *B*. Theor Appl Genet 121:1117–1131

Chiurugwi T, Holmes HF, Qi A, Chia TYP, Hedden P, Mutasa-Gottgens ES (2013) Development of new quantitative physiological and molecular breeding parameters based on the sugar-beet vernalization intensity model. J Agr Sci 151:492–505

Claus E (1937) Die Züchtung einer schosswiderstandsfähigen Zuckerrübe. Die deutsche Zuckerindustrie 62(243–244):263–264

Curth P (1962) Special Investigations on Vernalisation and Photoperiodicity of Sugar Beet. Zeitschrift fur Pflanzenzuechtung-Journal of Plant Breeding 47:254

Doerge RW (2002) Mapping and analysis of quantitative trait loci in experimental populations. Nat Rev Genet 3:43–52

Driessen S (2003) *Beta vulgaris* subsp. *maritima* an Deutschlands Ostseeküste. Fakultät für Mathematik, Informatik und Naturwissenschaften. Rheinisch-Westfälische Technische Hochschule, Aachen

Eichholz W, Röstel HJ (1962) Züchtung der Winterzuckerrübe. Theor Appl Genet 32:281–290

El-Mezawy A, Dreyer F, Jacobs G, Jung C (2002) High resolution mapping of the bolting gene *B* of sugar beet. Theor Appl Genet 105:100–105

Esteban Baselga JA (1999) Charactérisation de la betterave de semis d'automne. Proc. IIRB, Sevilla, pp 1–8

FAO (2012) FAOSTAT. http://faostat.fao.org. Accessed 10 Jan 2014

Gusta LV, Wisniewski M (2013) Understanding plant cold hardiness: an opinion. Physiol Plant 147:4–14

Hallauer AR, Carena MJ, Filho JBM (1988) Quantitative genetics in maize breeding, Vol. 6. In: Handbook of plant breeding. Iowa State University Press, Oxford

Hoffmann CM, Kluge-Severin S (2010) Light absorption and radiation use efficiency of autumn and spring sown sugar beets. Field Crops Res 119:238–244

Hohmann U, Jacobs G, Jung C (2005) An EMS mutagenesis protocol for sugar beet and isolation of non-bolting mutants. Plant Breed 124:317–321

IPK (2006) GBIS/I—Genebank Information System of the Institute of Plant Genetics and Crop Plant Research Gatersleben. http://gbis.ipk-gatersleben.de. Accessed 17 Jan 2014

Jaggard KW, Qi A, Ober ES (2009) Capture and use of solar radiation, water, and nitrogen by sugar beet (*Beta vulgaris* L.). J Exp Bot 60:1919–1925

JKI (2012) International Database for Beta—(IDBB) (Online Database). http://idbb.jki.bund.de. Accessed 22 Jan 2014

Jonsson BO (1999) Development of a winter beet alternative for South Europe. In: Proceedings of IIRB, Sevilla, pp 69–76

Jung C, Müller A (2009) Flowering time control and applications in plant breeding. Trends Plant Sci 14:563–573

Kirchhoff M, Svirshchevskaya A, Hoffmann C, Schechert A, Jung C, Kopisch-Obuch FJ (2012) High degree of genetic variation of winter hardiness in a panel of *Beta vulgaris* L. Crop Sci 52:179–188

Kosambi DD (1943) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lasa JM, Medina B (1978) Multiplication of bolting resistant sugarbeet (*Beta vulgaris* L.). Anales de la Estacion Experimental de Aula Dei 14:163–172

Laurent V, Devaux P, Thiel T, Viard F, Mielordt S, Touzet P, Quillet M (2007) Comparative effectiveness of sugar beet microsatellite markers isolated from genomic libraries and GenBank ESTs to map the sugar beet genome. Theor Appl Genet 115:793–805

Lein JC, Sagstetter CM, Schulte D, Thurau T, Varrelmann M, Saal B, Koch G, Borchardt DC, Jung C (2008) Mapping of rhizoctonia root rot resistance genes in sugar beet using pathogen response-related sequences as molecular markers. Plant Breed 127:602–611

Lexander K (1980) Present knowledge of sugar beet bolting mechanisms. In: 43th Winter Congress of the Institut International de Recherches Betteravieres, Brussels, pp 245–258

Lexander K (1987) Characters related to the vernalization requirement of sugar beet. In: Manipulation of flowering. Butterworths, London, pp 147–158

McFarlane JS, Price C, Owen FV (1948) Strains of sugar beets extremely resistant to bolting. Proc Am Soc Sugar Beet Technol 5:151–153

McGarry RC, Ayre BG (2012) Geminivirus-mediated delivery of florigen promotes determinate growth in aerial organs and uncouples flowering from photoperiod in cotton. PLoS One 7:e36746

McGrath JM, Trebbi D, Fenwick A, Panella L, Schulz B, Laurent V, Barnes S, Murray SC (2007) An open-source first-generation molecular genetic map from a sugarbeet × table beet cross and its extension to physical mapping. Crop Sci 47:S-27

Meier U (2001) Phenological growth stages and BBCH-identification keys of beet. In: Growth stages of mono- and dicotyledonous plants. BBCH Monograph, Federal Biological Research Center for Agriculture and Forestry, Germany

Milford GFJ, Jarvis PJ, Walters C (2010) A vernalization-intensity model to predict bolting in sugar beet. J Agric Sci 148:127–137

Mutasa-Goettgens E, Qi AM, Mathews A, Thomas S, Phillips A, Hedden P (2009) Modification of gibberellin signalling (metabolism & signal transduction) in sugar beet: analysis of potential targets for crop improvement. Transgenic Res 18:301–308

Pin PA, Nilsson O (2012) The multifaceted roles of FLOWERING LOCUS T in plant development. Plant Cell Environ 35:1742–1755

Pin PA, Benlloch R, Bonnet D, Wremerth-Weich E, Kraft T, Gielen JJL, Nilsson O (2010) An antagonistic pair of FT homologs mediates the control of flowering time in sugar beet. Science 330:1397–1400

Pin PA, Zhang W, Vogt SH, Dally N, Buettner B, Schulze-Buxloh G, Jelly NS, Chia TYP, Mutasa-Goettgens ES, Dohm JC, Himmelbauer H, Weisshaar B, Kraus J, Gielen JJL, Lommel M, Weyens G, Wahl B, Schechert A, Nilsson O, Jung C, Kraft T, Mueller AE (2012) The role of a pseudo-response regulator gene in life cycle adaptation and domestication of beet. Curr Biol 22:1095–1101

Robertson AJ, Weninger A, Wilen RW, Fu P, Gusta LV (1994) Comparison of dehydrin gene-expression and freezing tolerance in bromus-inermis and secale-cereale grown in controlled environments, hydroponics, and the field. Plant Physiol 106:1213–1216

R Development Core Team (2010) R: a language and environment for statistical computing, 2.12 edn. In: R Foundation for Statistical Computing, Vienna

Sadeghian SY, Becker HC, Johansson E (1993) Inheritance of bolting in three sugar-beet crosses after different periods of vernalization. Plant Breed 110:328–333

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci 81:8014–8018

Schneider G (1960) Biochemische und entwicklungsphysiologische Untersuchungen zur Frage des Schossens und der Blütenbildung der Zuckerrübe. Planta 55:669–686

Schneider K, Kulosa D, Soerensen TR, Mohring S, Heine M, Durstewitz G, Polley A, Weber E, Jamsari Lein J, Hohmann U, Tahiro E, Weisshaar B, Schulz B, Koch G, Jung C, Ganal M (2007) Analysis of DNA polymorphisms in sugar beet (*Beta vulgaris* L.) and development of an SNP-based map of expressed genes. Theor Appl Genet 115:601–615

Smit AL (1983) Influence of external factors on growth and development of sugar-beet (*Beta vulgaris* L.)—dissertation. Centre for Agricultural Publishing and Documentation

Stout M (1946) Relation of temperature to reproduction in sugar beets. J Agric Res Wash DC 72:49–68

Utz HF, Melchinger AE (2006) PLABQTL—a computer program to map QTL. Version 1.2. User manual. Institute of Plant Breeding, Seed Science and Population Genetics, University Hohenheim, Germany

Van Dijk H (2009) Evolutionary change in flowering phenology in the iteroparous herb *Beta vulgaris* ssp. *maritima*: a search for the underlying mechanisms. J Exp Bot 60:3143–3155

Van Ooijen JW (2006) Join Map® 4—software for the calculation of genetic linkage maps in experimental populations. User manual. Wageningen, The Netherlands

Viard F, Bernard J, Desplanque B (2002) Crop-weed interactions in the Beta vulgaris complex at a local scale: allelic diversity and gene flow within sugar beet fields. Theor Appl Genet 104:688–697

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M et al (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23(21):4407–4414

Wisniewski ME, Bassett CL, Renaut J, Farrell R Jr, Tworkoski T, Artlip TS (2006) Differential regulation of two dehydrin genes from peach (*Prunus persica*) by photoperiod, low temperature and water deficit. Tree Physiol 26:575–584

Wood DW, Scott RK (1975) Sowing sugar beet in autumn in England. J Agric Sci 84:97–108

Yamagishi N, Yoshikawa N (2011) Expression of *FLOWERING LOCUS T* from *Arabidopsis thaliana* induces precocious flowering in soybean irrespective of maturity group and stem growth habit. Planta 233:561–568

Yamagishi N, Sasaki S, Yamagata K, Komori S, Nagase M, Wada M, Yamamoto T, Yoshikawa N (2011) Promotion of flowering and reduction of a generation time in apple seedlings by ectopical expression of the *Arabidopsis thaliana FT* gene using the *Apple latent spherical virus* vector. Plant Mol Biol 75:193–204