

Aus dem Institut für Pflanzenbau und Pflanzenzüchtung
der Christian-Albrechts-Universität zu Kiel

**Genetic mapping of QTL for bolting time after winter
in sugar beet (*Beta vulgaris* L.)**

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List of Abbreviations

%	Per cent
°C	Degree Celsius
µl	Microlitre
µmol/m ² s ⁻¹	Micromoles of photons per square meter and second
A	Adenine
A.D.	<i>Anno Domini</i>
AFLP	Amplified fragment length polymorphism
<i>AGL20</i>	<i>AGAMOUS-LIKE 20</i>
ANOVA	Analysis of variance
<i>B</i>	Bolting locus
<i>B2</i>	Second bolting locus
<i>B3</i>	Third bolting locus
BAC	Bacterial artificial chromosome
BBCH	Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie
BC	Bolting code
BES	BAC-end sequences
<i>BFT</i>	<i>BROTHER OF FT</i>
BNYVV	Beet necrotic yellow vein virus
bp	Base pair
BR	Bolting rate
BSA	Bulked segregant analysis
BSBV	Beet soil borne virus
<i>BTC1</i>	<i>BOLTING TIME CONTROL 1</i>
<i>Bv</i>	<i>Beta vulgaris</i>
<i>BvBBX19</i>	<i>B-BOX DOMAIN PROTEIN 19</i>
C	Cytosine
CaMV	Cauliflower mosaic virus
CAPS	Cleaved amplified polymorphic sequence
<i>CDF2</i>	<i>CYCLING DOF FACTOR 2</i>
cDNA	Complementary DNA
cM	Centimorgan
CMS	Cytoplasmic male sterility
<i>CO</i>	<i>CONSTANS</i>
<i>COL1</i>	<i>CONSTANS-LIKE 1</i>
CRISPR/Cas	Clustered regularly interspaced short palindromic repeats/ CRISPR associated
CTAB	Cetyl trimethylammonium bromide
dbEST	database for "expressed sequence tags"
DNA	Deoxyribonucleic acid
DSB	Double strand breaks
DTB	Days to bolt after cold treatment
e.g.	<i>Exempli gratia</i>
EMS	Ethyl methanesulfonate
EST	Expressed sequence tag
et al.	<i>Et alii</i>

F ₁	First generation
F ₂	Second generation
F ₃	Third generation
FAO	Food and Agriculture Organization of the United Nations
FISH	Fluorescence <i>in situ</i> hybridization
<i>FL1</i>	<i>FLC-LIKE 1</i>
<i>FLC</i>	<i>FLOWERING LOCUS C</i>
<i>FLK</i>	<i>FLOWERING LOCUS KH DOMAINS</i>
<i>FPA</i>	<i>FLOWERING LOCUS PA</i>
<i>FT</i>	<i>FLOWERING LOCUS T</i>
<i>FT1</i>	<i>FLOWERING LOCUS T 1</i>
<i>FT2</i>	<i>FLOWERING LOCUS T 2</i>
<i>FVE1</i>	<i>FLOWERINF LOCUS VE 1</i>
G	Guanine
GA	Gibberellic acid
<i>Ga20ox1</i>	<i>GIBBERELLIN 2-OXIDASE 1</i>
<i>GA3ox1</i>	<i>GIBBERELLIN 3-OXYGENASE-LIKE 1</i>
GABI	Genomanalyse im Biologischen System Pflanze
GBS	Genotyping-by-sequencing
GI	Gene Index
<i>GI</i>	<i>GIGANTEA</i>
h	Hour
h ²	Heritability
ha	Hectar
i.e.	<i>Id est</i>
InDel	Insertion or deletion marker
KASP	Competitive allele-specific PCR
kbp	Kilo base pairs =1000 bp
LD	Linkage disequilibrium
<i>LD</i>	<i>LUMINIDEPENDENS</i>
<i>LDL1</i>	<i>LSD1-LIKE 1</i>
<i>LHP1</i>	<i>LIKE HETEROCHROMATIN 1</i>
LOD	Logarithm of odds
<i>LSD1</i>	<i>LYSINE SPECIFIC HISTON DEMETHYLASE 1</i>
LTR	Long terminal repeats
Mbp	Mega base pairs = 1,000,000 bp
<i>MFT1</i>	<i>MOTHER OF FT 1</i>
miRNA	MicroRNA
mRNA	Messenger RNA
MYB	Myeloblastosis
na	not available
NCBI	National Center for Biotechnology Information
ng	Nanogram
p	probability value
PAR	Photosynthetically active radiation

PCR	Polymerase chain reaction
<i>PEP1</i>	<i>PERPETUAL FLOWERING 1</i>
<i>PRR7</i>	<i>PSEUDO RESPONSE REGULATOR 7</i>
pwbr	Post winter bolting resistant
QTL	Quantitative trait locus
R	Recombination frequency
R ²	Coefficient of determination
RAPD	Random amplified polymorphic DNA
RCBD	Randomized complete block design
RefBeet	Reference sequence of the beet genome
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RNAi	RNA interference
RNA-Seq	RNA-Sequencing
RTq	quantitative Realtime
SHOREmap	Short read mapping
SNP	Single nucleotide polymorphism
ssp.	Subspecies
SSR	Simple sequence repeat
<i>SWP1</i>	<i>SWIRM DOMAIN PAO PROTEIN/LSD-LIKE 1</i>
t	Ton
<i>TFL1</i>	<i>TERMINAL FLOWER 1</i>
<i>TOL1</i>	<i>TONNEAU-LIKE 1</i>
T	Thymine
USDA/ARS	United States Department of Agriculture/Agricultural Research Service
var.	Variation
VIF	Virus induced flowering
vwh	Vernalization-weighted hours
W	Watt

1 General introduction

1.1 Breeding winter beets

Sugar beet (*Beta vulgaris* ssp. *vulgaris* L. var. *altissima*) cultivation is of high agronomical importance because sugar beet is the only sucrose storing crop plant used for sugar production in Europe. It accounts besides sugar cane (*Saccharum officinarum*) for about 11.7 % of the worldwide sugar production (FAOSTAT, 2013). In 2013, sugar beets were cultivated in Germany on 357,400 ha yielding on average 63.9 t/ha (FAOSTAT, 2013). In addition to white sugar production, about 26,000 ha were cultivated for biogas (about 3,800 ha) and bioethanol (about 22,200 ha) production in 2011 (Kleffmann, 2011). Sugar beets are currently grown as spring sown crop in cool temperate climates, whereby the seeds are sown in spring and the beet is harvested in autumn while the plant is still in the vegetative state.

In the current production system an important factor limiting beet growth and thereby sugar beet yield is the slow seedling development and an insufficient canopy of young sugar beet plants at the beginning of the vegetation period (Hoffmann and Kluge-Severin, 2011; Kenter et al., 2006; Scott and Jaggard, 1993). It was demonstrated that spring sown sugar beets do not reach full canopy closure before mid of June (Kenter, 2003). Thus, from emergence until June, only a proportion and not the optimum amount of photosynthetically active radiation (PAR) can be captured by the young sugar beet plants (Röver, 1995). However, the yearly radiation maximum has already passed at this time point (Hoffmann and Kluge-Severin, 2011).

One strategy to overcome this problem might be the cultivation of overwintering sugar beets. By sowing already in autumn of the year previous to harvest, these so called winter beets are expected to have a higher leaf area index in spring compared to spring sown sugar beets (Jaggard et al., 2009). This enables an increased light absorption, particularly in April and May (Hoffmann and Kluge-Severin, 2010). An increased capture of PAR in spring leads to a raise in photosynthesis resulting in an improved yield (Röver, 1995). Based on this, Hoffmann and Kluge-Severin (2010) estimated a theoretical yield increase of up to 26 %. Moreover, winter beets allow earlier harvesting dates and consequently, an earlier start of the beet campaign which results in a higher processing capacity of sugar refineries. However, the expected benefit of autumn sown sugar beets due to additional light capture can only be exploited when the cultivated plants do not bolt in spring and summer (Hoffmann and Kluge-Severin, 2010).

For the cultivation of winter beets under cold temperate climate conditions two important requirements have to be fulfilled. On the one hand, winter survival of the plants must be ensured through sufficient winter hardiness. On the other hand, bolting behavior of the plants must be controlled, as bolting is not desired for winter beet cultivation because it reduces sugar content during crop production. However, for breeding and seed production bolting must be enabled. Therefore, a thorough understanding of the genetics of bolting and flowering is essential for the development of winter sugar beets. Both, the genetic basics of winter hardiness as well as bolting control are currently investigated.

1.2 Cultivated species of the genus *Beta*

1.2.1 Systematics of *Beta* species

Plants of the genus *Beta* are dicotyledonous and belong to the order of Caryophyllales (family *Amaranthaceae*, subfamily: *Chenopodiaceae*) (TheAngiospermPhylogenyGroup, 2009).

Cultivated beets (*Beta vulgaris* ssp. *vulgaris*) are divided into four agronomically important groups: sugar beet (*B. vulgaris* ssp. *vulgaris* Sugar Beet Group), leaf beet (*B. vulgaris* ssp. *vulgaris* Leaf Beet Group), garden beet (*B. vulgaris* ssp. *vulgaris* Garden Beet Group) and fodder beet (*B. vulgaris* ssp. *vulgaris* Fodder Beet Group) (Lothar Frese, pers. communication; USDA/ARS, 2013). Cultivated forms of the genus *Beta* show high morphological variation in leaf and root development. While garden beet and fodder beet form a thickened root and hypocotyl, which differ strongly in form and color, leaf beets form lush leaves with large and strong leaf stalks. These leaf stalks serve as storage organs of the plant and contain the principal nutrients, which is the reason for its long tradition as leaf vegetable. Sugar beets form a thickened taproot. Interestingly, only 90 % of the root is actually root-derived. The remaining 10 % is derived from the hypocotyl (Elzebroek and Wind, 2008; Hancock, 2012). All forms store sucrose in their roots or leaves, whereby the amount of sucrose is highly divergent. Sugar beet stores the highest amount of sucrose and today the sucrose content ranges between 15 and 18 % of the total dry weight (Märländer et al., 2011).

Via molecular analysis like RFLP based DNA fingerprinting (Jung et al., 1993; Letschert et al., 1994), it was possible to confirm that the wild sea beet *B. vulgaris* ssp. *maritima* is the progenitor of all domesticated beet species. In 2006, a new taxonomy model was proposed by Kadereit (2006), and since 2009, this taxonomy is officially accepted (Germplasm Resources Information Network, USDA). All cultivated forms of beet are included in the section *Beta*, and form the primary gene pool. They are easily crossable with each other and especially the wild progenitor *B. vulgaris* ssp. *maritima* has been frequently used as a major source to obtain tolerance and resistance to abiotic and biotic stress factors (Frese et al., 2001; Panella and Lewellen, 2007). The secondary gene pool of the genus *Beta* comprises the section *Corollinae* (Kadereit et al., 2006).

1.2.2 Domestication and breeding

All cultivated forms are derived from sea beet (*B. vulgaris* ssp. *maritima*), which was domesticated in the eastern Mediterranean area about 12,000 years ago (Biancardi et al., 2005; Biancardi et al., 2010; Simmonds, 1976). Domestication of beet probably began with the usage of leaves as pot herbs and animal feed (Ford-Lloyd et al., 1975; Hancock, 2012). The earliest references to beets and its usage as leaf vegetables date back to the Greeks Aristophanes, Aristotle, Theophrastus and Euripides (Biancardi et al., 2005; Hancock, 2012). Romans used the beet rather extensively and during the climax of the Roman Empire the beets spread with the ‘barbarian’ invaders from Greece and Italy all over Europe (Hancock, 2012). In 77 A.D., Pliny the Elder was the first who described botanical characteristics, growing techniques, nutritional and medical properties and the possibility of autumn and spring sowing (Biancardi et al., 2005). In the medieval Europe, beets were used as livestock fodder, especially in France and Spain (Biancardi et al., 2010).

Nowadays, the most important use of sugar beets is based on Andreas Sigismund Marggraf’s discovery in 1747, that sugar crystals from beet juice are identical to those from sugar cane (Elzebroek and Wind, 2008). Based on this finding the ancestor of all modern sugar beet cultivars, the “White Silesian”, was developed by Marggraf’s student Franz Carl Achard, through applying mass selection in fodder beet (Biancardi et al., 2005; Fischer, 1989). The first beet root processing sugar factory was built in 1801 in Cunern, Silesia (Hancock, 2012). The main source of sugar at this time was still sugarcane, but beet sugar became more and more important as an alternative for sugar production in Europe. The decision to substitute imported cane sugar by beet sugar was made in 1811 during the Napoleonic war (Cooke, 1993). The sugar beet industry became firmly established on a world-wide scale during the past 200 years while the crop itself was substantially improved by breeding (Biancardi et al., 2005; Draycott, 2006).

Sugar beet breeding techniques, like using particular methods of recurrent selection and combining abilities, have obtained a number of significant results in enhancing yield traits and genetic resistances against several diseases (Biancardi et al., 2010). Today all released sugar beet varieties are hybrids (<http://www.bundessortenamt.de>). This fact resulted from two major advances in sugar beet breeding: monogermity and cytoplasmatic male sterility (CMS). Monogermity, which means the presence of only a single flower at each inflorescence node of the plant, facilitates single seed production. This fact allows pelletizing of the single seeds and results in high precision drilling techniques which ensure a regular stand of 80,000 to 100,000 equally spaced plants per hectare without the formerly necessary step of manual thinning after emergence (Biancardi et al., 2005; Biancardi et al., 2010). The second major advance was the discovery of CMS by Owen in 1945 (Biancardi et al., 2010). CMS allows the production of male sterile seed parents carrying pollen-less anthers, and has replaced expensive hand emasculation procedures, an important step due to the fact that sugar beet is not completely self-incompatible (Owen, 1945). Nowadays, the production of monogerm hybrid sugar beet seed is performed by crossing genetically monogerm seed parents and multigerm pollen-producing pollinators.

During sugar beet breeding history many differential objectives became important to focus on for optimizing the beet crop. The most relevant aim in sugar beet breeding is to improve the sugar yield by increasing the amount of extractable sugar. Although sugar yield and sugar quality traits are mainly influenced by environmental factors, the selection of improved varieties is still continued. One decisive factor for stable root yield has been a strong selection against premature (early) bolting, because bolting drastically reduces yield and quality (Jung and Müller, 2009) and can cause difficulties during the mechanical harvest process (Smyth et al., 2014). Premature bolting can be promoted by an early sowing in spring and thus an exposure of the young plants to low temperatures, meeting the beets' vernalization (process, in which prolonged exposure to cold temperatures promotes flowering) requirements. Moreover, besides yield and quality breeding, resistance breeding is of great importance, because next to abiotic environmental factors, the beet growth is also affected by biotic environmental factors, i.e. pathogens like fungi (e.g. *Rhizoctonia solani* and *Ramularia beticola*), viruses (e.g. beet necrotic yellow vein virus (BNYVV) and beet soil borne virus (BSBV)), insects, and the beet cyst nematode *Heterodera schachtii* (Biancardi et al., 2005; Dohm et al., 2012).

1.3 Genome analysis of sugar beet

In 1991, the haploid genome size of sugar beet was estimated to be 758 million base pairs ($n = x = 9$) (Arumuganathan and Earle, 1991). All nine chromosomes are morphologically similar in the mitotic metaphase and have an average length of 2.5 μm (Arumuganathan and Earle, 1991; Bennett and Smith, 1976; Bosemark, 1969; Bosemark and Bormotov, 1971). In 2012, the sugar beet genome sequence was published by Dohm et al. (2012). The genome sequence comprises 567 Mbp, of which 85 % could be assigned to all nine chromosomes. Flavell et al. (1974) estimated the amount of highly repetitive sequence in the sugar beet genome to comprise at least 63 %, mainly consisting of numerous different retrotransposons and satellite DNA families (Zakrzewski et al., 2010).

1.3.1 Genetic mapping

An older, but still very helpful tool for genetic analysis is the use of molecular markers, which serve as fundament for numerous applications ranging from single gene localization to the improvement of plant material in breeding programs via marker-assisted selection. In sugar beet several genetic linkage maps have been constructed, e.g. by Barzen et al., 1992; Barzen et al., 1995; Hallden et al., 1996; Laurent et al., 2007; McGrath et al., 2007; Nilsson et al.,

1997; Pillen et al., 1993; Pillen et al., 1992; Rae et al., 2000; Schondelmaier et al., 1996; Uphoff and Wricke, 1995; Viard et al., 2002. Various molecular marker techniques have been applied for the construction of these genetic linkage maps such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), cleaved amplified polymorphic sequences (CAPS), and single nucleotide polymorphism (SNP) with the aim of gene localization, mapping of quantitative trait loci (QTL), and for a more detailed understanding of the genome organization.

Populations used for linkage mapping are ideally derived from F₁ plants which are obtained by crossing homozygous parents. They are characterized by large conserved DNA regions with high linkage disequilibrium (LD), since the bi-parental populations have undergone only few recombination events. Thus, linkage mapping can be used for identification of major QTL by a limited number of markers. One disadvantage of linkage mapping is that QTL mapping can result in large confidence intervals of several centiMorgan (cM) that span numerous candidate genes due to large LD blocks. This makes the identification of potential candidate genes much more difficult (Darvasi et al., 1993; Ingvarsson and Street, 2011; Mackay et al., 2009; Zhu et al., 2008). Moreover, only QTL based on allelic diversity of the two parents can be detected.

In 2007, a dense genetic map was published by Schneider et al. (2007). This map comprises 524 loci covering 664.3 cM of the beet genome. Three different mapping populations (K1, K2 and D1) served as basis for this joined map. The latest high resolution and most dense map was recently published by Dohm et al. (2012). It is based on the K1 mapping population and comprises in total 983 SNP markers derived from 283 EST- and 700 BAC-end sequences (BES). The total length of the map is 866 cM.

In recent years, association studies, also referred as LD mapping, have become an increasingly valuable option in genetics for studying the correlation between genetic variants and LD-based trait differences (Guo et al., 2016; Hall et al., 2010; Lu et al., 2011; Rafalski, 2010; Zhang et al., 2013). Compared to linkage mapping, association mapping does not require specific mapping populations. It is a population based method for the identification of trait-marker relationships based on LD (Bac-Molenaar et al., 2015; Flint-Garcia et al., 2003; Zanke et al., 2015). Thus, association mapping is suited for mapping in breeding and gene bank material. Compared to linkage mapping in a bi-parental population, in which recombination occurred only in a rather limited number of generations after crossing, association mapping makes use of historical recombination events that occurred during many generations (Bac-Molenaar et al., 2015; Flint-Garcia et al., 2003; Hall et al., 2010; Mackay et al., 2009). This results in a higher mapping resolution due to smaller and more numerous LD blocks. In contrast to linkage mapping, a sufficient power to detect QTL requires a higher number of individuals in the mapping population. A complexity of association mapping might be the effect of disregarded population structure which can inflate the number of false positive associations (Ingvarsson and Street, 2011). In order to minimize the risk of detection of false positives, population structure can be controlled by markers which are inherited independently of the QTL. In sugar beet, association mapping was successfully used to identify highly quantitative traits (Abbasi et al., 2015; Broccanello et al., 2015; Reif et al., 2010; Stich et al., 2008a; Stich et al., 2008b; Würschum, 2012; Würschum et al., 2011).

1.3.2 Structural analysis of the *Beta vulgaris* genome

BAC libraries represent major resources for marker development, physical mapping, DNA sequencing and cloning of genes. For *B. vulgaris*, several large insert BAC libraries were established with the purpose of cloning bolting and flowering time genes, CMS restorer genes and resistance genes for instance. These established libraries cover the sugar beet genome between 5.6-fold and 8.8-fold (Fang et al., 2004; Gindullis et al., 2001; Hagihara et al., 2005;

Hohmann et al., 2003; Jacobs et al., 2009; McGrath et al., 2004; Schulte et al., 2006). More than 56,000 BES are publicly available (McGrath et al., 2004; Zakrzewski et al., 2010). Published BAC libraries were used by Dohm et al. (2012) for hybridization of gene-derived oligomer probes against the libraries to construct a genome-wide gene-based physical map. In the final map, which encompasses 535 publically available, chromosomally anchored contigs (<http://bvseq.molgen.de/PhysMap/start.physmap.shtml>), in total 22,815 BAC clones and 8,361 gene-derived oligomer probes were successfully integrated.

Further valuable tools for studying the beet genome are transcriptomics or expression profiling. During the last 15 years several cDNA libraries from different beet tissues were generated with the objective to clone candidate genes for the beet cyst nematode resistance gene *HsI^{pro-1}*, candidate genes related to root development and carbohydrate metabolism or candidate genes for *Rhizoctonia solani* resistance (Bellin et al., 2007; Bellin et al., 2002; Cai et al., 1997; Herwig et al., 2002; Nagendran and McGrath, 2006; Pestsova et al., 2008). At present 30,340 *B. vulgaris* expressed sequence tags (ESTs) (May 2014) are publically available (NCBI dbEST, <http://www.ncbi.nlm.nih.gov>). Moreover, during a Gene Index (GI) Project (<http://compbio.dfci.harvard.edu/tgi>) a collection of putative genes and their functional roles was established and 17,186 unique entries are listed in the *B. vulgaris* GI (BvGI) (May 2014).

The mitochondrial genome of sugar beet has also been sequenced. The results were published in 2000 (Kubo et al., 2000). The genome size was estimated to 368,799 bp with a GC content of 43.9 %. The 149,696 bp of the *B. vulgaris* chloroplast sequence were submitted to the GenBank in 2007 (EF534108).

In 2004, the sugar beet genome mapping and sequencing consortium started its work with the goal of generating a *de novo* assembly of the sugar beet genome sequence. Exactly ten years later, sequencing and annotation of the genome from the doubled haploid sugar beet line KWS2320 was completed and the results are available for download at <http://bvseq.molgen.mpg.de/Genome/start.genome.shtml>. Dohm et al. (2014) published the sugar beet reference genome sequence as the first non-rosid, non-asterid eudicot genome. For the genome sequencing approach plant material from roots and leaves was sequenced on the Roche/454 FLX, Illumina HiSeq2000 and ABI3730 XL sequencing platforms and several sequence resources were used for the assembly. The so generated 'RefBeet' sequence assembly comprised in total 569 Mbp in 43,721 sequences (N50 = 1.7 Mbp). Subsequently, the authors incorporated Illumina sequencing data generated from PCR-free libraries and analyzed genotyping-by-sequencing (GBS) data, leading to an optimized assembly of 566.6 Mbp in 2,171 scaffolds and 38,337 unscaffolded contigs (N50 = 2.01 Mbp). Eighty-five per cent of the generated sequence could be assigned to the nine sugar beet chromosomes. The quality of the sequence was proven by assigning 94 % of publically available ESTs in the RefBeet sequence, indicating that gene-containing regions are comprehensively covered. In addition, the authors compared BAC clones to their corresponding regions in the reference genome sequence and they found them to be correctly assembled within one scaffold. In total, 252 Mbp of RefBeet was identified as repetitive sequence, of which the largest group was built by long terminal repeat (LTR) retrotransposons. Furthermore, a total of 27,421 protein-coding genes have been predicted in RefBeet, supported by mRNA evidence. Moreover, phylogenetic analyses indicated evidence for the separation of Caryophyllales before the split of rosids and asterids, and revealed lineage-specific gene family expansions and complete losses (Dohm et al., 2014).

The work of the sugar beet genome mapping and sequencing consortium was also supported by the results of Peasold et al. (2012), who published a reference *B. vulgaris* fluorescence *in situ* hybridization (FISH) karyotype. This enabled marker localization, anchoring of contigs

and scaffolds for the reference sequence annotation and the analysis of chromosomal distribution patterns of major repetitive sequence families.

Another recent development facilitating the identification of genes of interest are next generation sequencing techniques, which were applied for sequencing the beet transcriptome via RNA-Sequencing (RNA-Seq) and enable a rapid gene discovery (Mutasa-Göttgens et al., 2012). The RNA-Seq approach was successfully applied for the identification of the *R* locus gene *CYP76AD1*, a gene which encodes a novel highly expressed phytochrome P450 required for red betalain production (Hatlestad et al., 2012).

1.4 Bolting and flowering time regulation

1.4.1 Regulation of flowering time in *Arabidopsis thaliana*

In general, a major transition in the life cycle of all plant species is the switch from vegetative growth to the development of reproductive organs. This complex biological control of bolting and flowering time is best understood in the model species *Arabidopsis thaliana*. Genetic studies have defined four major pathways: (1) the autonomous pathway, (2) the photoperiodic pathway, (3) the vernalization pathway and (4) the gibberellic acid (GA) pathway (Andres and Coupland, 2012). All these pathways are tightly connected and intersect in a small number of floral integrator genes, like *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS* (*SOC1*), *FLOWERING LOCUS T* (*FT*) and *AGAMOUS-LIKE 24* (*AGL24*) (Blümel et al., 2015). The transition from vegetative to reproductive growth is tightly controlled by environmental cues, that are regulating the vernalization pathway and the photoperiod pathway, as well as endogenous signals, which mediate the autonomous and the gibberellin pathway (Celesnik et al., 2013). The autonomous and vernalization pathway are interacting to regulate the expression of *CONSTANS* (*CO*) and *FLOWERING LOCUS C* (*FLC*), two major genes regulating flowering through control of the floral integrator genes, such as *FT*, in an antagonistic way (Song et al., 2013). While *FLC* negatively regulates *FT* expression, *CO* promotes *FT* expression under long day conditions (Lee and Lee, 2010). *FLC* itself is downregulated during the vernalization process due to vernalization-induced histone methylation (Zhu et al., 2015), and *CO* is regulated by photoreceptors, day length and genes of a central circular feedback system, also referred to as circadian clock (Johansson and Staiger, 2014). It was shown that the clock is besides light, the most important stimulus influencing the clock components, which are also entrained by endogenous factors, such as sugar signals, stresses and hormones (Bolouri Moghaddam and Van den Ende, 2013). On the other hand, the clock itself is involved in regulation of gibberellin biosynthesis (Blazquez et al., 2002). Gibberellins play important roles in key physiological processes, such as bolting and flowering under unfavorable conditions (Galvão et al., 2015) and are acting through DELLA proteins (Schwechheimer, 2012).

1.4.2 Life cycle of perennial species

Arabidopsis is an annual species which completes its life cycle in a couple of weeks. Besides annuals, there are biennial and perennial species, which complete their life cycle within two or more years, respectively.

Plants with perennial growth habit are separated into two different botanical categories. The first category describes semelparous (or monocarpic) perennials. Plant species belonging to this group are flowering only once in their life subsequent to an often very long vegetative growth period (Zhou et al., 2013). The second category of perennials comprises species with an iteroparous (or polycarpic) life cycle. Plant species in this category are able to perform several cycles of reproduction during their lifespan (Zhou et al., 2013), e.g. grapevine, kiwifruit (Albani and Coupland, 2010) and citrus (Tan and Swain, 2006). They can either show seasonal flowering, whereby flowering occurs for a short period between vegetative

growth phases, or they can flower continuously through the year (Albani and Coupland, 2010).

An overview about the characteristics and growth habit of semelparous and iteroparous life cycles is shown in **Table 1**.

Table 1: Overview about characteristics, growth habit and botanical category of semelparous and iteroparous life cycles.

Life cycle	Growth habit	Characteristics
semelparous (monocarpic)	annual	one fast cycling growing season for flowering and seed set followed by senescence and dieback
	biennial	two, by cold or drought separated, vegetation periods for flowering and seed set, followed by senescence and dieback
	perennial	more than two, by cold or drought separated, vegetation periods for flowering and seed set, followed by senescence and dieback
iteroparous (polycarpic)	perennial	several cycles of reproduction during their whole life span

Currently, it seems that the developmental transition from juvenile to adult and flowering in perennials is probably more complex than in annual plant species. Moreover, the molecular mechanisms in general are less well understood due to numerous technical limitations associated with tree crops, usually related to their size in combination with long juvenile vegetative phases and limited genetic knowledge (Tan and Swain, 2006). Juvenile vegetative phases are varying strongly in their duration among species. They can last from a few weeks to several years (Albani and Coupland, 2010) and can thus make breeding a very time-consuming process (Tan and Swain, 2006). Since the genetic characterization of perennial trees or shrubs would therefore need a long time, it is natural to investigate perennial plants with shorter cycles as for example the perennial relative of *Arabidopsis thaliana*, *Arabis alpina*.

Arabis alpina is an arctic-alpine polycarpic perennial member of the family *Brassicaceae* (Ansell et al., 2008; Tedder et al., 2015). Due to favorable characteristics, such as being diploid and self-fertile, having a relatively small genome, and being susceptible to transformation by *Agrobacterium tumefaciens*, *A. alpina* is suitable as a model species for studying the molecular base of perenniality and local adaptation (Tedder et al., 2015). Wang et al. (2009) demonstrated that the *A. alpina* gene *PERPETUAL FLOWERING 1 (PEP1)* encodes a MADS-box transcription factor that mechanistically links seasonal flowering and polycarpic growth. *PEP1* is described as the ortholog of the *A. thaliana FLC* gene and its regulation differed in *A. alpina*, allowing the repeated response to seasonal cycles observed in perennials (Wang et al., 2009). Bergonzi and Albani (2011) demonstrated in their experiments that *pep1* mutants commit more axillary branches to flowering but remain polycarpic, a fact indicating that *PEP1* is not the only factor influencing polycarpy in *A. alpina*. Moreover, *PEP1* plays a redundant role with the *A. alpina TFL1 (TERMINAL FLOWER 1)* ortholog to regulate flowering in axillary shoot branches (Bergonzi and Albani, 2011; Wang et al., 2011). In *Arabidopsis thaliana*, *TFL1* is described as a gene encoding a floral repressor (Hanano and Goto, 2011). Results of different expression experiments led Wang et al. (2011) to the suggestion that *A. alpina TFL1* blocks flowering in young plants exposed to cold by setting a threshold for a flowering pathway whose activity increases as the plant ages, thus contributing

to several perennial traits. Albani et al. (2012) demonstrated that *A. alpina* PEP1 is encoded by two overlapping genes that contribute to genetic variation in perennial flowering.

Although several groups are investigating the perennial model *A. alpina*, or herbaceous and woody perennials with longer life cycles, the general determinants of this multifaceted variation in life history remain poorly understood until today (Roberto et al., 2016).

1.4.3 Life cycle in *B. vulgaris*

To ensure their reproduction, *Beta* species have evolved annual, biennial as well as perennial life cycles. Sea beets (*B. vulgaris* L. ssp. *maritima*) from Mediterranean regions show an annual growth habit. Under long day conditions, these beets are bolting and flowering rapidly within the first year. In order to reproduce, they do not feature a vernalization requirement (facultative requirement; Lexander 1980). In contrast, sea beets growing in northern latitudes behave as biennials and need to be vernalized to start bolting and flowering in the second year under increasing day length in order to complete their life cycle. Besides annuals and biennials, also long-lived iteroparous perennial beets occur, which are typically flowering in successive cycles (Hautekeete et al., 2002). Hautekeete et al. (2001) performed an experiment under controlled conditions, involving six different life cycle types of the *Beta* species complex (from strictly semelparous to long-living iteroparous plants), whereby, surprisingly for the authors, no significant difference among life cycles within semelparous and iteroparous beets under the point of reproductive effort could be detected. Perennial post-winter bolting resistant sugar beets have already been reported in history since the 1930s (Bauer, 1932; Claus, 1937; Eichholz and Röstel, 1962; McFarlane et al., 1948; Wood and Scott, 1975).

All cultivated beets are biennials. In the first year the beet plants grow vegetative, forming a large taproot (instead of leaf beet) and a leaf rosette. In the second year the plants switch from the vegetative to the generative growth to develop reproductive organs. This switch is induced by exposure to low, non-freezing temperatures over winter and increasing long day conditions in spring. Thus, cultivated beets have an obligate requirement for vernalization to enter the reproductive stage (Boudry et al., 2002; Lexander, 1987; Milford et al., 2010).

During the breeding process of high yielding sugar beet varieties breeders strongly selected against premature (early) bolting. It is known since the 1930s that the tendency for bolting without vernalization is under the control of the major dominant Mendelian factor *B* (Abegg, 1936; Munerati, 1931). This factor was recently identified as the pseudo-response regulator (PRR) gene *BOLTING TIME CONTROL 1* (*BTC1*) (Pin et al., 2012). The authors showed that plants carrying the dominant *BTC1* allele can overcome the vernalization requirement and show an annual growth habit. In contrast, plants with a biennial growth habit harbor the recessive *btc1* allele. It was also shown that the *BTC1* gene shares homology with the *A. thaliana* circadian clock gene *PRR7* and moreover, sequencing of the coding region in several sea beet and cultivated accessions revealed the presence of eleven haplotypes (“a”-“k”). Out of these, three different haplotypes for biennials (“a”-“c”) and eight for annuals (“d”-“k”) have been identified. The haplotype “a” was derived from a biennial reference accession, whereas the haplotype “d” originated from an annual reference accession. All tested cultivated beet accessions were found to carry the haplotype “a”. In most of the tested sea beet accessions *BTC1* alleles similar to the haplotype “d” were found. In total, the alleles of the reference accessions differ by eleven SNPs and a ~28 kbp insertion in the promoter region, which is only present in accessions showing a biennial growth habit (Pin et al., 2012). Moreover, the authors demonstrated by downregulation of *BTC1/btc1* expression via RNA interfering (RNAi) in annual and biennial beets, that *BTC1* is a key regulator controlling the life cycle of beet.

In addition to the *BTC1* gene, Pin et al (2010) described two paralogous copies of the *A. thaliana* *FT* gene, referred to as *BvFT1* and *BvFT2*, and further showed that both genes play a major role in floral transition in beet. While *BvFT1* acts as a floral repressor, *BvFT2* retained the *FT* function and acts as an inducer of flowering. Pin et al. (2010) demonstrated successfully, that plants failed to bolt for more than 400 days when *BvFT2* is downregulated through RNAi in annual plants. Moreover, overexpression of *BvFT2* in biennials resulted in early bolting plants that started to bolt already in tissue culture 18 weeks after transformation. By contrast, annual plants overexpressing the floral repressor gene *BvFT1* remain vegetative for more than six months. Expression analysis revealed that the continued vegetative growth was associated with reduced *BvFT2* expression (Pin et al., 2010). Accordingly, Pin et al. (2012) proposed a model for the life cycle control in beets with *BTC1* acting upstream of *BvFT1* and *BvFT2*. According to this model, a dominant *BTC1* allele represses *BvFT1* and activates *BvFT2* to promote bolting and flowering under inductive light conditions. Vice versa, beets carrying the recessive *btc1* allele do not respond to long day photoperiods and remain in a vegetative stage before winter, since *BvFT1* is active and represses *BvFT2*. When these biennial beets are exposed to cold temperatures, the *btc1* expression increases. Accordingly, the expression of *BvFT1* gradually decreases and enables the activation of *BvFT2* to initiate bolting and flowering as days become longer (Pin et al., 2012). In recent years, further additional bolting loci (*B2* and *B3*) have been identified in segregating populations derived from crosses between an annual *B. maritima* accession and biennial accessions which were isolated after EMS mutagenesis (Hohmann et al., 2005). While *B3* has not been mapped yet, the locus *B2* was mapped on chromosome 9 (Büttner et al., 2010). Recently, Dally et al. (2014) demonstrated that the *B2* flowering time locus of beet encodes a zinc finger transcription factor which is diurnally regulated and acts like *BTC1* upstream of *BvFT1* and *BvFT2*. According to its closest homolog from Arabidopsis, encoding a B-BOX DOMAIN PROTEIN 19 (BBX19), this gene was termed *BvBBX19*. Based on their results, the authors proposed a new model for flowering induction in beet in which *BTC1* and *BvBBX19* complement each other and together acquire a CO function to regulate their downstream targets *BvFT1* and *BvFT2* (Dally et al., 2014).

Comparable to Arabidopsis, in beet, a complex interaction between photoperiod and vernalization under inductive long day conditions is known (Chiurugwi et al., 2013). In 2007, a homolog of *FLC* was cloned in beet by Reeves et al. (2007) and found to be functionally related to the *FLC* gene from *Arabidopsis thaliana*. However, Vogt et al. (2014) demonstrated that this *FLC* homologue (*BvFLI*) does not play a major role in the control of vernalization response in beet.

For post-winter bolting resistance, a first systematic study was performed by Sadeghian et al. (1993), in which the induction of bolting was determined in three populations derived from crossing parental lines differing in their levels of post-winter bolting resistance. The bolting tendency was recorded after different periods of vernalization and the authors demonstrated a mainly additive genetic variation contributing to the observed post-winter bolting rate. A first major QTL for post-winter bolting resistance was identified on chromosome 9 by Pfeiffer et al. (2014) (**Chapter 2**). Recently, Broccanello (2015) identified a SNP located in the intronic region of a putative Matrix Metalloproteinase gene on chromosome 6 that shows association with the bolting tendency in beet.

1.5 Aims and objectives

To achieve a breeding system for winter sugar beet varieties with controlled bolting behavior, it is necessary to know the genetic factors underlying the traits bolting delay and bolting resistance after winter, as their genetic basis and their mode of inheritance is undetermined until now.

The aim of this investigation was the identification and genetic mapping of the quantitative genetic factors affecting bolting delay and bolting resistance after winter in *B. vulgaris*. Due to this, the initial objectives of this study were:

- (1) to map QTL for post-winter bolting delay and post-winter bolting resistance in segregating populations, derived from beet accessions with noticeable bolting behavior
- (2) to create selectable markers for the post-winter bolting resistance locus
- (3) *in silico* cloning of post-winter bolting resistance gene candidates
- (4) haplotyping *BvFTI* in post-winter bolting resistant beets
- (5) to perform bolting induction experiments with plant hormones

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

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2.1 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.

2.2 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 F₃ 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 F₃ families ranged from 25 to 164 days while for bolting rate F₃ 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.

2.3 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 F₂ and F₃ generation; (3) constructed a genetic map for the F₂ population; and (4) mapped quantitative trait loci (QTL) for bolting delay and post-winter bolting resistance.

2.4 Material and methods

2.4.1 Plant material and growth conditions

A F₂ population was developed from hand crossing sugar beet accession BETA 1773 with sugar beet accession 93161P followed by selfing the obtained F₁. 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). F₁ plants were grown in the greenhouse and selfed by bag isolation for F₂ seed production. F₂ seeds derived from a single F₁ plant (090757/03) were sown in the greenhouse on November 1, 2010. In total 410 F₂ plants were obtained and grown

in $9 \times 9 \text{ cm}^2$ pots (Hermann Meyer KG, Germany) at $20 \text{ }^\circ\text{C}$ under long day conditions (16 h light, $900 \mu\text{mol/m}^2\text{s}^{-1}$, 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 $5 \text{ }^\circ\text{C}$ under 22 h light ($200 \mu\text{mol/m}^2\text{s}^{-1}$, Osram Lumilux T8 L 58 W/840, Osram AG, München, Germany). After 1 week of acclimatization at $8 \text{ }^\circ\text{C}$ and 22 h light, ($200 \mu\text{mol/m}^2\text{s}^{-1}$, 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.

2.4.2 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 $20 \text{ }^\circ\text{C}$ under LD conditions (16 h light, $900 \mu\text{mol/m}^2\text{s}^{-1}$, Son-T Agro 400 W, Koninklijke Philips Electronics N.V., Eindhoven, Netherlands). Cold treatment was done for 16 weeks under LD conditions ($5 \text{ }^\circ\text{C}$, 16 h light, $200 \mu\text{mol/m}^2\text{s}^{-1}$, 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 Figure 1**.

2.4.3 Molecular marker analysis

Genomic DNA was isolated from freeze-dried leaf samples of the sugar beet F₂-population. This was done following a slightly modified CTAB protocol (Saghai-Marooft 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 F₂ plants with AFLP and to enrich QTL regions with sequence derived co-dominant markers applied to the whole population that was tested in the F₃ 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 F₂ 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.

2.4.4 Statistical analysis

An analysis of variance (ANOVA) was performed for DTB and bolting rate recorded on F₃ families. The ANAVO was done with SAS PROC MIXED (SAS Institute Inc., 2009, Cary, USA, Version 9.2) where the genotype (F₃ 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 F₂ data as well as means for F₃ families were tested with a Shapiro–Wilk test using the software R (R Development Core Team 2010).

2.4.5 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).

2.5 Results

2.5.1 Phenotypic analysis

The F_2 population showed a wide variation for bolting behavior giving a range in bolting delay of DTB = 103 days (**Supplementary Figure 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 Figure 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 (**Figure 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 **Figure 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 **Figure 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 Figure 4**. Days to bolt and bolting rate showed a highly negative correlation of $r = -0.986$ (see **Supplementary Figure 5**).

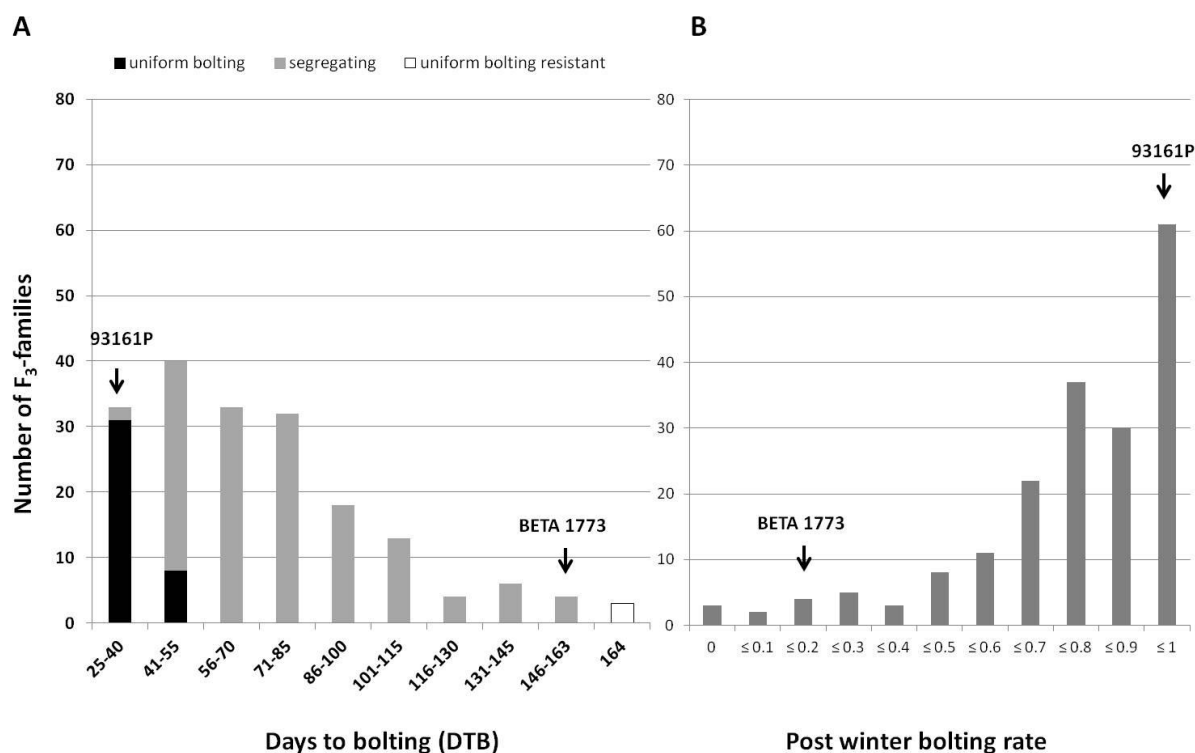


Figure 1: a) Phenotypic segregation for bolting delay determined as days to bolt (DTB) in 186 sugar beet F₃ families derived from a cross of sugar beet accessions BETA 1773 and 93161P. DTB are defined as days to bolt after the end of artificial cold treatment, which was May 2 (replications 1 and 2) and 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 F₃ 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

2.5.2 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 F₂ 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 *BC₁* and *BC₂* and explain together 64.1 % of the phenotypic variation. Detailed information of LOD, R², additive and dominant effects are listed in **Table 1**.

In the final QTL mapping with the F₃ data, one QTL for the trait bolting delay was detected at position 56 of chromosome 9 with a LOD of 38.12 (**Figure 2, Supplementary Figure 6**). This QTL is named *DTB₁* and explains 66 % of the phenotypic variation and 76.8 % of the genotypic variation for DTB (**Table 1**). *DTB₁* 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 **Figure 3** by boxplotting the bolting rates against genotypes of the markers that are flanking BR_1 .

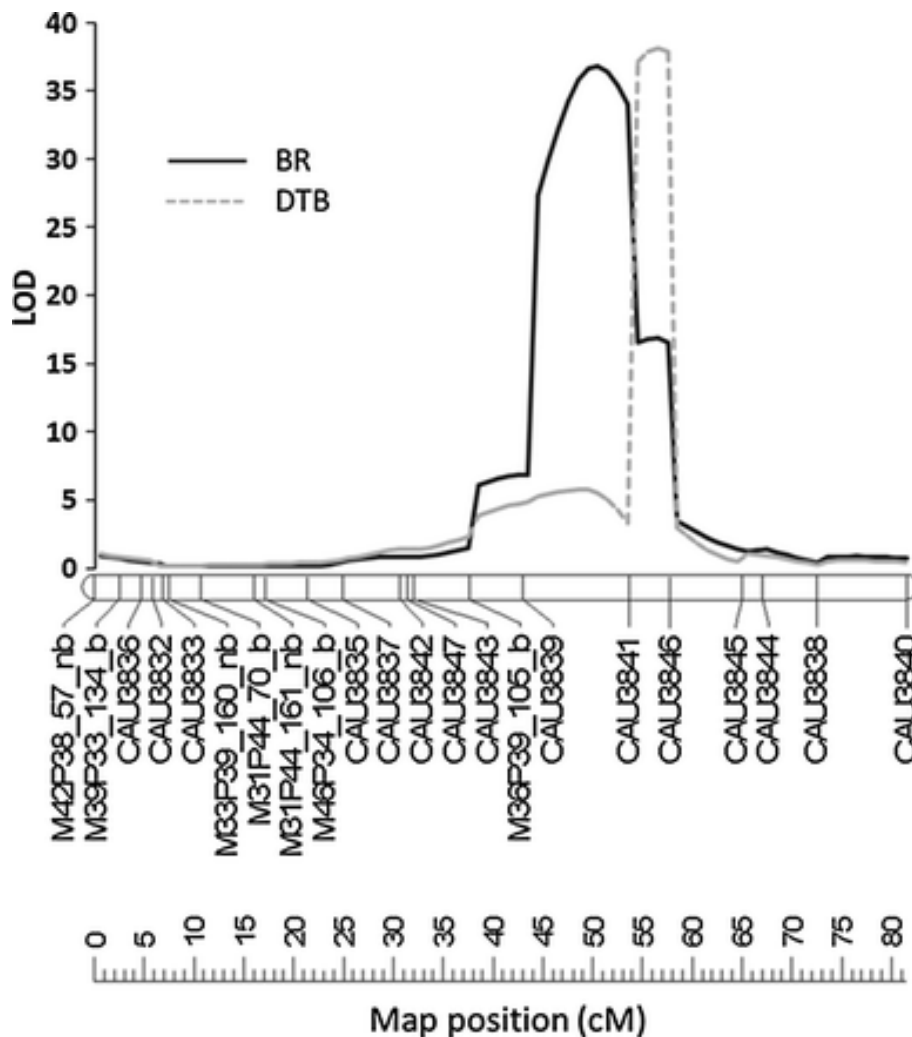


Figure 2: Detailed QTL mapping results for bolting delay and post-winter bolting resistance on linkage group 9 of sugar beet based on 186 sugar beet F_3 families derived from a cross of sugar beet accessions BETA 1773 and 93161P. The linkage map in cM is indicated by the horizontal bar on which marker 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

Table 2: QTL results for post-winter bolting behavior of 410 F₂ plants and 186 F₃ families derived from crossing sugar beet accessions BETA 1173 and 93161P. For F₂ 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 F₃ families, QTLs were mapped for bolting delay (as DTB) and post-winter bolting resistance (as bolting rate, BR). For experimental details see “**Material and methods**”

<i>Generation used for phenotyping</i>	<i>QTL name</i>	<i>Linkage group</i>	<i>Position</i>	<i>Flanking markers</i>	<i>Confidence interval</i>	<i>LOD</i>	<i>R²</i>	<i>Additive effects</i>	<i>Dominant effects</i>
F ₂	<i>DTB_{F2_1}</i>	9	48	CAU3839 and CAU3841	45 - 51	34.57	52.2	-39.6	-33.8
F ₂	<i>DTB_{F2_2}</i>	9	68	CAU3844 and CAU3838	65 - 71	19.96	34.9	-30.0	-21.8
							87.1		
F ₂	<i>BC_{F2_1}</i>	9	49	CAU3839 and CAU3841	45 - 53	22.79	38.5	0.26	0.25
F ₂	<i>BC_{F2_2}</i>	9	69	CAU3844 and CAU3838	65 - 72	13.75	25.6	0.19	0.18
							64.1		
F ₃	<i>DTB₁</i>	9	56	CAU3841 and CAU3846	53 - 58	38.12	65.9	-41.2	-18.6
F ₃	<i>BR₁</i>	9	50	CAU3839 and CAU3841	48 - 52	36.85	65.0	0.35	0.23

R²: coefficient of determination

LOD: logarithm of the odds

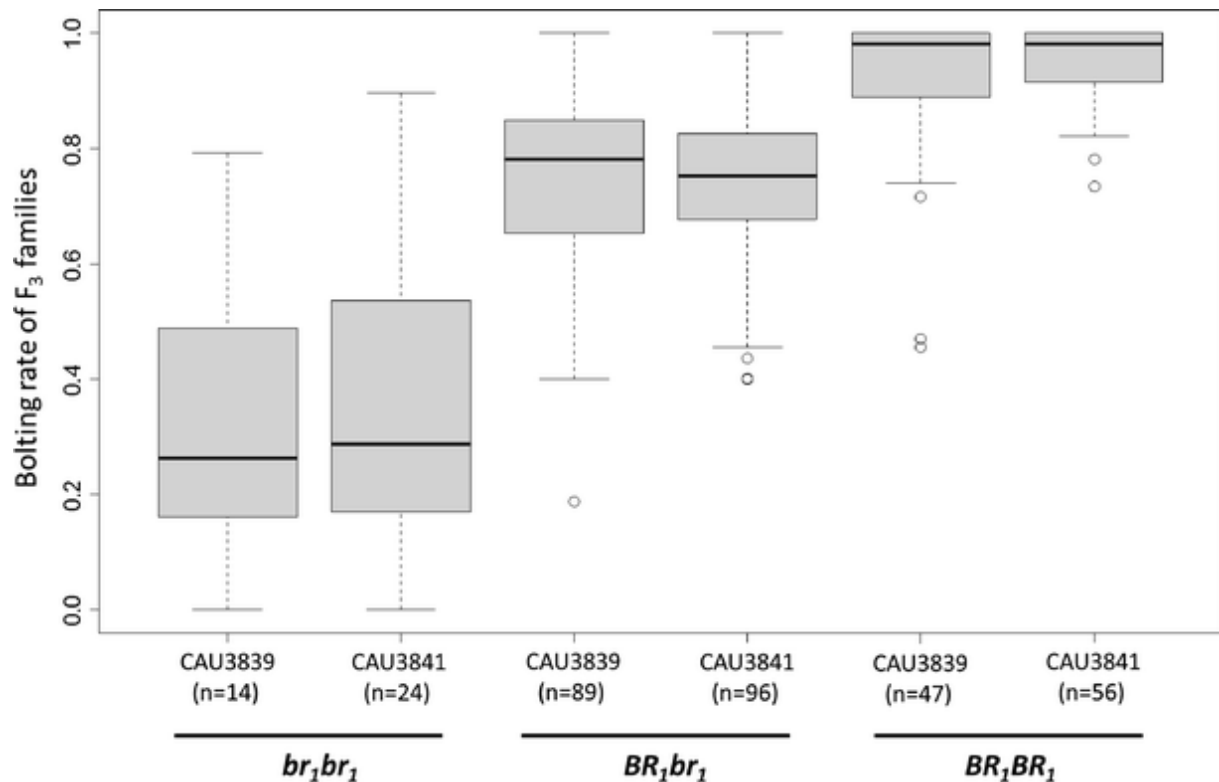


Figure 3: Boxplot of post-winter bolting resistance of sugar beet F_3 families depending on the genotype at the QTL BR_1 . F_3 families were derived from a cross of sugar beet accessions BETA 1773 and 93161P. The genotype of BR_1 is predicted by either of the QTL flanking markers CAU3839 and CAU3841. 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.

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

2.6.1 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 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: $DTB_{F_2_1}$ with DTB_1 and $BC_{F_2_1}$ 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 *BvFTI* on linkage group 9 (Pin et al. 2010) comes first to mind as a potential candidate gene. However, the *BvFTI* 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.

2.6.2 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 CAU3839 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-Göttgens 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.

2.7 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 *BR₁* results in a winter beet with complete post-winter bolting resistance during crop production in cold temperate regions.

2.8 Acknowledgments

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3 Genetic analysis of delayed bolting after winter in leaf beet (*Beta vulgaris* L.)

Under review, Plant Breeding

3.1 Abstract

In the biennial sugar beet (*Beta vulgaris* L. ssp. *vulgaris*), prolonged exposure to cold during winter followed by increasing day length in spring (vernalization) induces bolting and flowering. This drastically decreases the beet yield and quality when sugar beet is grown as an overwintering crop. Therefore, in order to convert the spring crop to a winter crop, floral transition has to be controlled. This could be achieved by accumulation of genes for bolting delay after winter. To identify such genes, a QTL mapping was performed in a segregating population derived from crossing an annual sugar beet and a biennial leaf beet with delayed bolting after winter. Up to 281 F₃ families of this population were tested for bolting delay after winter in two different experiments with natural or artificial vernalization. Three QTL for bolting delay were mapped on linkage groups 3, 5, and 9 explaining up to 54,6 % of the phenotypic variation.

3.2 Introduction

Growing sugar beet is important for the production of crystal sugar, biogas and bioethanol. Currently, sugar beets are sown in spring and are harvested in autumn of the same year. In order to extend the growth period of sugar beets sowing could be performed in autumn of the year prior to harvest. This will lead to a yield increase that is estimated to be up to 26 % (Hoffmann and Kluge-Severin 2010) due to better light interception by a higher leaf area index in spring (Jaggard et al. 2009). A second benefit of these so-called winter beets is an earlier harvest and consequently an earlier start of the beet campaign resulting in a better use of processing capacities of sugar refineries.

While the concept of winter beets is straightforward, lacking control of bolting after winter is a major obstacle. Bolting is the elongation of the main shoot as the consequence of the transition to the reproductive stage, which is triggered by a process called vernalization, a prolonged exposure to cold temperatures followed by long day conditions (Biancardi et al. 2005; Milford et al. 2010). While the vernalization requirement keeps spring sown beets from bolting, overwintering beets are bolting in spring. During bolting resources are put in the elongation of the main shoot at the expense of beet yield. Therefore, to fully exploit the potential of a winter beet, bolting after winter has to be completely controlled. At the same time bolting is required for seed production. This requires a good understanding of the genetics underlying bolting and flowering in sugar beet (Jung and Müller 2009).

In recent years key genes regulating bolting in sugar beet have been identified. These include two *FLOWERING LOCUS T* genes (*BvFT1* and *BvFT2*) and the *B* locus gene *BOLTING TIME CONTROL 1* (*BTC1*). *BTC1* was identified by Pin et al. (2012) as a homologue of the circadian clock regulator gene *PSEUDO RESPONSE REGULATOR 7* (*PRR7*) from *Arabidopsis thaliana*. Plants with biennial growth habit harbor the recessive *btc1* allele at the *B* locus. Contrastingly, plants carrying the dominant *BTC1* allele in the homozygous (*BB*) or heterozygous (*Bb*) state do not require vernalization and show an annual growth habit. Sequencing of the coding region in multiple sea beet and cultivated accessions revealed the presence of eleven haplotypes, including three haplotypes for biennials and eight for annuals. Moreover, the authors demonstrated that *BTC1* is an upstream regulator of two sugar beet paralogs of the *Arabidopsis* *FLOWERING LOCUS T* (*FT*). It was shown that these two paralogs act antagonistically, with *BvFT1* being a floral repressor and *BvFT2* a floral

promoter (Pin et al. 2010; Pin and Nilsson 2012). According to the proposed model, in annual beets the dominant allele of *BTC1* represses *BvFT1* whereas *BvFT2* is upregulated, thus resulting in flowering without vernalization (Pin et al. 2010, Pin et al. 2012; Pin and Nilsson 2012). By contrast, the floral repressor *BvFT1* is expressed in non-cold treated biennials carrying the recessive *btc1* allele. Prolonged exposure to cold temperatures leads to downregulation of *BvFT1* followed by upregulation of *BvFT2* (Pin et al. 2010). Recently Dally et al. (2014) demonstrated that a second bolting locus in beet (*B2*) encodes a zinc finger transcription factor that is diurnally regulated and like *BTC1* also acts upstream of *BvFT1* and *BvFT2*. This gene was termed *BvBBX19*, according to its closest homolog from *Arabidopsis*. Dally et al. (2014) proposed a model for flowering induction in which *BTC1* and *BvBBX19* complement each other in beet and thus acquire a function similar to that of *CONSTANS* (*CO*) in *Arabidopsis* to regulate their downstream targets *BvFT1* and *BvFT2*.

In addition to bolting time variation before winter, there is also genetic variation for bolting after winter in the *Beta vulgaris* gene pool (Sadeghian et al. 1993). This variation includes natural accessions with a high level of post-winter bolting resistance. This was observed for sugar beet accession BETA 1773 (seed code: 080299) and leaf beet accession 82.5523 (seed code: 080550) in overwintering field trials described by (Kirchhoff et al. 2012). After overwintering, fifty percent of plants in either of these accessions did not bolt until the middle of June when the experiment was completed (unpublished data). The remaining plants bolted, but bolting was delayed by four weeks. In a mapping population derived from BETA 1773 Pfeiffer et al. (2014) mapped a major QTL for post-winter bolting resistance which was identical with a QTL for bolting delay after winter. However, this QTL explained only 65 % of the phenotypic variation and is not sufficient for developing a winter beet with complete post-winter bolting resistance, i.e. complete absence of bolting in all plants. In order to gain full control of bolting after winter the identification of more QTL for post-winter bolting resistance is required. A source for that could be leaf beet accession 82.5523, provided that the genetics underlying the observed bolting behavior is different from BETA 1773.

Therefore, the objective of this study was to identify the genetic factors underlying the post-winter bolting behavior of leaf beet accession 82.5523. Hence we: (1) developed a structured mapping population from accession 82.5523; (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 behavior after winter.

3.3 Material and methods

3.3.1 Plant material and growth conditions

A structured F_2 mapping population was developed from hand crossing leaf beet accession 82.5523 with sugar beet accession 93167P followed by selfing the obtained F_1 . Leaf beet 82.5523 was provided by Agroscope Changins Wädenswil, Wädenswil, Switzerland, and sugar beet 93167P was provided by Saatzucht Dieckmann, Nienstädt, Germany. Leaf beet 82.5523 had shown in overwintering field trials a bolting time delay of 37 days compared to other beet accessions grown by Kirchhoff et al. (2012) and a low post-winter bolting rate of about 0.5. Sugar beet 93167P has annual bolting behavior and was chosen as pollinator to speed up population development by obtaining an F_1 genotype that does not require vernalization for bolting induction. 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 (090972/08) were sown in the greenhouse on May 21, 2010 and in total 1.166 F_2 plants were grown in 9 x 9 cm² pots (Hermann Meyer KG, Germany) at 20°C under long day conditions (16 h light, 900 $\mu\text{mol}/\text{m}^2\text{s}^{-1}$, Son-T Agro 400 W, Koninklijke Philips Electronics N.V., Eindhoven, Netherlands). 875 F_2 plants bolted successively within 32 days after sowing (until June 22,

2010) and were expected to carry the *B* allele in the homozygous (*BB*) or heterozygous (*Bb*) state. After confirmation by marker analysis these plants were discarded from the experiment. The remaining 291 did not bolt until July 27, 2010. These plants were transplanted into 13 x 13 cm² pots (Hermann Meyer KG, Germany). Twenty-six days after transplanting (August 23, 2010) day length was extended to 22 h (1200 $\mu\text{mol}/\text{m}^2\text{s}^{-1}$, Son-T Agro 600 W, Koninklijke Philips Electronics N.V., Eindhoven, Netherlands) to verify the biennial phenotype of the selected plants in addition to marker analysis (see below). From September 14, 2010 to January 5, 2011 all 291 remaining plants were kept for 16 weeks in a cold chamber at 5°C under 22 h light (200 $\mu\text{mol}/\text{m}^2\text{s}^{-1}$, Osram Lumilux T8 L 58W/840, Osram AG, München, Germany). After 1 week of acclimatization at 8 °C and 22 h light (200 $\mu\text{mol}/\text{m}^2\text{s}^{-1}$, Osram Lumilux T8 L 58W/840, Osram AG, München, Germany), all plants were transferred from the cold chamber back to the greenhouse and kept under long day conditions at 20 °C under 22 h light (1200 $\mu\text{mol}/\text{m}^2\text{s}^{-1}$, Son-T Agro 600 W, Koninklijke Philips Electronics N.V., Eindhoven, Netherlands). Bolting *F*₂ plants were bag isolated for production of *F*₃ seed. *F*₃ seeds were obtained from 274 out of 281 bolting plants. Ten plants did not bolt and were cold treated again for a second time between March 31 and September 30, 2011 (26 weeks) under the same conditions as outlined above. After acclimatization those plants were planted into new soil in 13 x 13 cm² pots and kept in the greenhouse under long day conditions (20 °C, 22 h light, 1200 $\mu\text{mol}/\text{m}^2\text{s}^{-1}$, Son-T Agro 600 W, Koninklijke Philips Electronics N.V., Eindhoven, Netherlands).

3.3.2 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 biennial *F*₂ plants was recorded from January 17 until March 7, 2011. Bolting was defined as first visible elongation of the main stem [BBCH 51 (Meier 2001)]. 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 bolting starts counted from the day that the plants had left the cold chamber. The binary bolting code was based on the bolting phenotype on March 7, 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). Bolting tests were performed under natural overwintering conditions in the field as well as under artificial cold conditions in the cold chamber followed by transplanting to the field. For the experiment under natural conditions seeds of 269 *F*₃ families with sufficient numbers of seed, which could be produced after first cold treatment (see above), were used. The experiment was conducted on a field located in Hohenschulen, Northern Germany by direct sowing on August 4, 2011 as a randomized complete block design (RCBD) with two replications and 2.1 m long single rows with a between row distance of 45 cm as experimental units. In each row, 30 seeds were sown and after emergence thinned to 20 plants per row. Onset of bolting was recorded twice a week from the beginning of April until the end of June 2012 as described above. DTB was recorded as DTB after May 1, 2012. Different from *F*₂ phenotyping, bolting delay was determined by the average of DTB of a *F*₃ family.

For the experiment performed under artificial cold treatment *F*₃ plants were artificially cold treated and transplanted into the field in spring. For this, seeds of 281 *F*₃ families (including 5 families from *F*₂ plants that had been cold treated twice) were sown in the greenhouse into quickPot-plates96T (Hermann Meyer KG, Germany) on December 9, 2011 and kept for 4 weeks at 20 °C under long day conditions (16 h light, 900 $\mu\text{mol}/\text{m}^2\text{s}^{-1}$, Son-T Agro 400 W,

Koninklijke Philips Electronics N.V., Eindhoven, Netherlands). Cold treatment was done for 16 weeks under long day conditions (5 °C, 16 h light, 200 $\mu\text{mol}/\text{m}^2\text{s}^{-1}$, Osram Lumilux T8 L 58W/840, Osram AG, München, Germany). On May 2, 2012, the F₃ families were planted to a field nursery in Kiel, Germany as a RCDB with two replications and single rows as experimental units. In each row, four to eight plants were planted with a between row distance of 45 cm and a within row distance of 20 cm. Onset of bolting was recorded twice a week from the end of May until end of June 2012 as described above. Post-winter bolting behavior of F₃ families was also assessed by post-winter bolting delay, where DTB was scored in relation to the day that plants left the cold chamber.

3.3.3 Molecular marker analysis

Genomic DNA was isolated from freeze dried leaf samples from all 1,166 single plants of the F₂-population. This was done following a slightly modified CTAB protocol (Saghai-Maroo et al. 1984). DNA concentration was adjusted to 10ng/ μl .

To confirm the genotype of the obtained F₁ plants they were tested by using the co-dominant PCR (polymerase chain reaction) marker GJ1001c16 (Büttner et al. 2010) localized within the *B*-locus. This marker shows a 28 bp deletion in the sequence of the seed parent (82.5523) in comparison to the pollinator (93167P). In addition to the F₁ plants this marker was also used to confirm the genotype of all 291 biennial F₂ plants and moreover, of 575 annual F₂ plants.

For genotyping of the F₂ population, 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 = 82.5523, b = 93167P). AFLP fragment sizes were determined by comparison with the 50-700bp sizing standard (LI-COR, LI-COR Biosences, Lincoln, USA) 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 Version 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 F₂ plants with AFLP and after a first QTL analysis to enrich QTL regions with sequence derived co-dominant markers applied to all biennial F₂ plants whose F₃ progenies were tested in the F₃ generations.

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. 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.

Primers were designed using the software OligoCalc (<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.

3.3.4 Statistical analysis

An analysis of variance (ANOVA) was performed for DTB recorded on F₃ families in the bolting experiments. The ANOVA was done with SAS PROC MIXED (SAS Institute Inc., 2009, Cary, USA, Version 9.2), where the genotype (F₃ family) was treated as a fixed factor while blocks were treated as random effects. For DTB, least square means were estimated as well as variance components for all families with at least three plants in each replication.

Broad-sense heritability was estimated according to Hallauer et al. (1988) as
$$\hat{h}^2 = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_G^2 + \frac{\hat{\sigma}_e^2}{R}}$$

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 F₂ data as well as means for F₃ families were tested with a Shapiro-Wilk test using the software R (R Development Core Team 2010).

3.3.5 QTL analysis

The linkage map was calculated with the JoinMap[®] 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).

3.4 Results

3.4.1 Phenotypic analysis

The F₂ population grown in the greenhouse showed a wide variation for bolting behavior before cold treatment with a segregation of 875:291 for bolting versus non bolting plants. The observed segregation ratio did not deviate significantly ($\chi^2 = 0,001$, $P < 0.0001$) from a 3:1 segregation ratio as expected for monogenic dominant recessive inheritance which is the case for the *B*-locus.

After cold treating the non-bolting plants for 16 weeks 281 of 291 plants started bolting from 12 (January 17, 2011) to 61 (March 7, 2011) days after leaving the cold chamber (**Supplementary Figure 8**). This gave a range in DTB of 49 days. The distribution of DTB was skewed to the right and deviated significantly from normal distribution as tested by Shapiro Wilk ($w=0.8011$; $p < 0.0001$). Ten plants of the population did not bolt until March 31, 2011 and were recorded as post-winter bolting resistant (BC=0). Bolting phenotypes are documented in **Supplementary Figure 9**.

In the F₃ population grown under natural overwintering conditions bolting started on May 04, 2012 and the last F₃ plant started bolting on June 22, 2012. In contrast to the experiment with the F₂ population, no non bolting phenotypes could be observed. Means for DTB of 265 F₃ families ranged from 7 to 29 days (**Figure 4**). Genotypic effects for DTB were tested significant in the ANOVA ($p = < 0.0001$). Normal distribution was rejected by Shapiro Wilk normality test ($w=0.9827$; $p\text{-value} = 0.0026$) and the heritability for DTB was estimated as $h^2 = 0.91$.

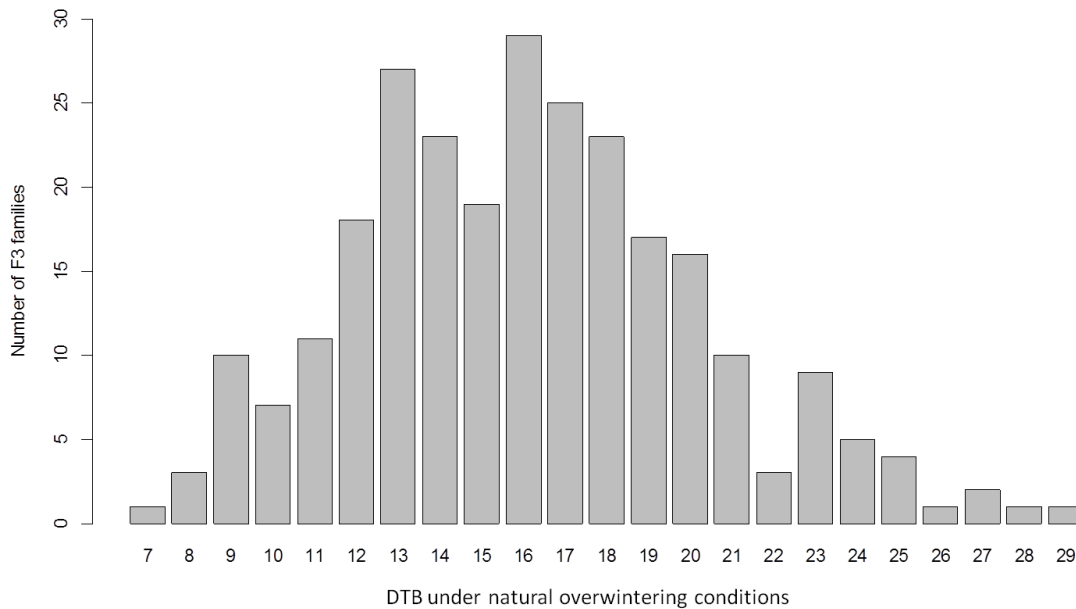


Figure 4: Phenotypic segregation for bolting delay determined as days to bolt (DTB) in 265 F₃ families derived from a cross of leaf beet 82.5523 and sugar beet accession 93167P in the experiment performed under natural overwintering conditions. DTB are defined as days to bolt after May 1, 2012.

In the F₃ population cold treated under artificial conditions single plants started bolting on May 29, 2012 and the last plant started bolting on July 9, 2012. In contrast to the experiment with the F₂ population, no non bolting phenotypes could be observed, even in the progenies of F₂ plants that had bolted only after a second cold treatment. Means for DTB of 253 F₃ families ranged from 27 to 43 days (**Figure 5**). Genotypic effects for DTB were tested significant in the ANOVA ($p = <0.0001$). Normal distribution was rejected by shapiro-wilk normality test ($w=0.7274$; $p\text{-value} = <2.2e-16$). Heritability for DTB was estimated as $h^2 = 0.66$.

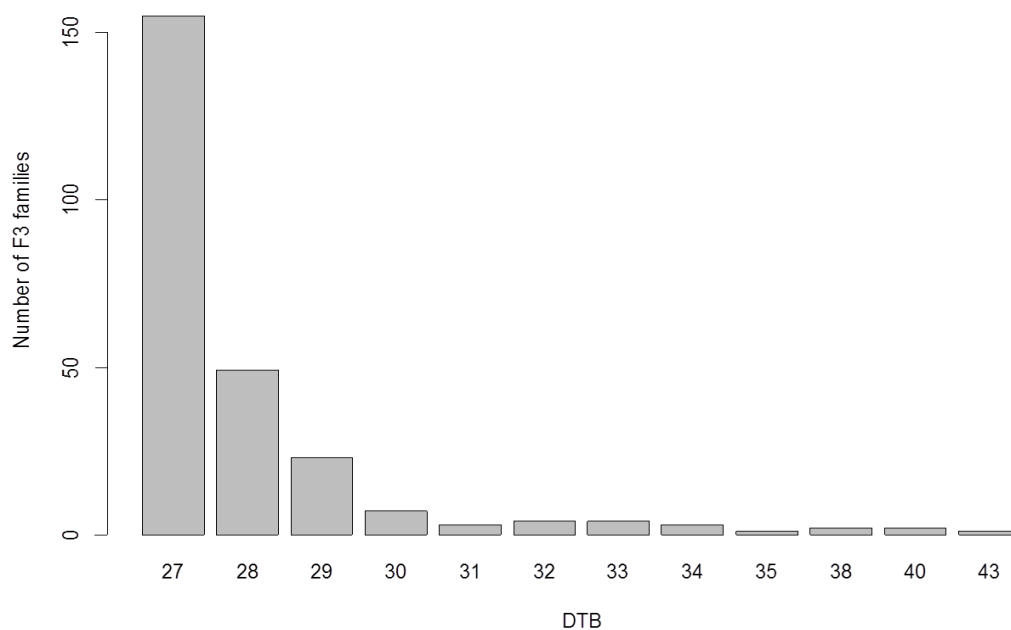


Figure 5: Phenotypic segregation for bolting delay determined as days to bolt (DTB) in 253 F₃ families derived from a cross of leaf beet 82.5523 and sugar beet accession 93167P in the experiment performed under artificial overwintering conditions. DTB are defined as days to bolt after the end of artificial cold treatment (May 2, 2012).

As all plants in the experiments had started to bolt by July 9, only the trait DTB was considered for further analysis.

Plotting of DTB under natural overwintering conditions against DTB under artificial overwintering conditions is displayed in **Figure 6**. The Bi-plot shows the correlation ($r=0.568$) between days to bolt (DTB) under natural overwintering and artificial vernalization of 246 F_3 families. Although the correlation was not that high as expected the results show that families bolted in a similar manner regardless of their overwintering conditions (artificial vs. natural), and families that bolted early or late when artificially cold treated also bolted early or late as if they had been overwintered under natural conditions, respectively.

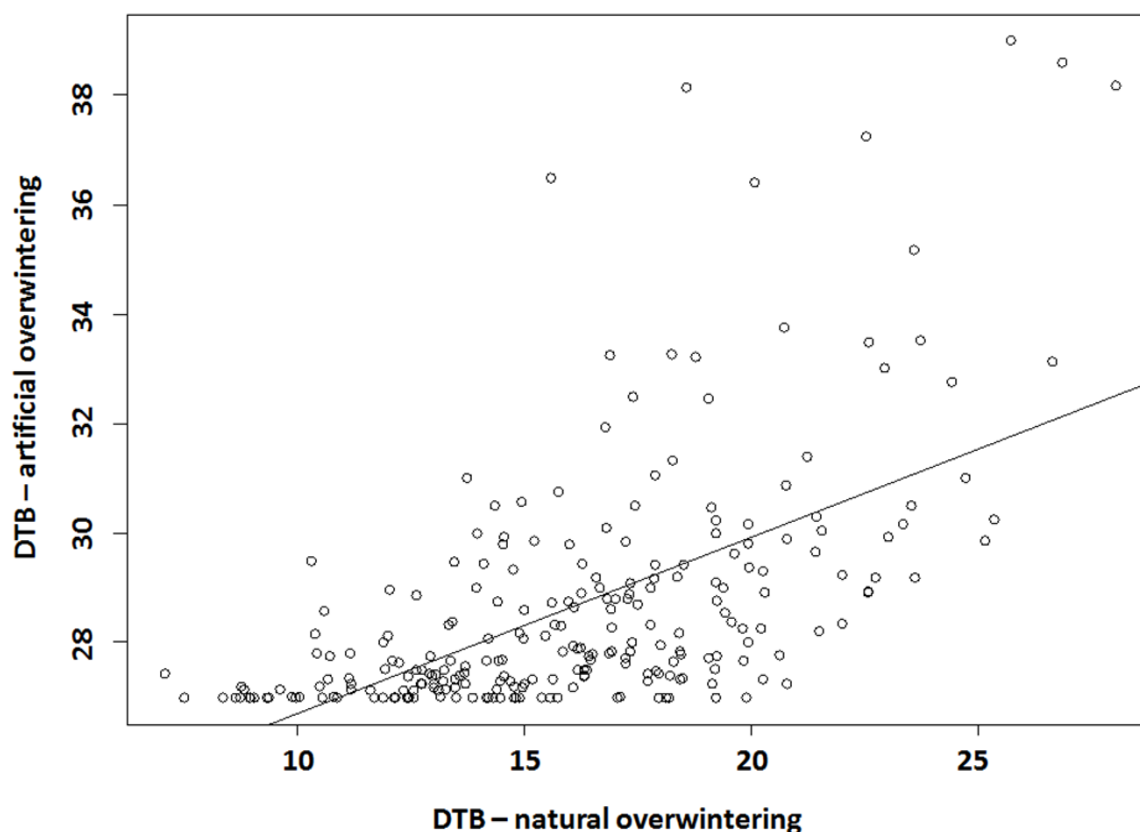


Figure 6: Least square means for days to bolting (DTB) from F_3 families tested in both experiments plotted against each other ($n=246$) ($r=0.568$).

3.4.2 Linkage map and QTL analysis

The heterozygous (Bb) F_1 genotype was confirmed by using the co-dominant B locus marker GJ1001c16. The same marker was used for confirmation of all 291 biennial F_2 plants (homozygous bb) and 565 annual plants (homozygous BB or heterozygous Bb).

A linkage map was calculated with 139 markers (119 AFLP, 4 SSR, 5 InDel, 9 SNP based CAPS markers and 2 SNP-Assays) on 287 F_2 plants. The total length of the linkage map is 690.7 cM covering all nine *B. vulgaris* chromosomes. The size of linkage groups ranges from 67.7 to 86.5 cM (**Supplementary Table 6, Supplementary Figure 10**).

QTL mapping with the F_3 data generated under natural overwintering conditions resulted in three QTL on chromosome 3 (LOD = 7.38), chromosome 5 (LOD = 26.02) and chromosome 9 (LOD = 7.03). The QTL $DTBnat_1$ on chromosome 3 was mapped to position 43 cM and explains 29.8 % of the observed phenotypic variation (**Table 3, Figure 7**). The second QTL $DTBnat_2$ was mapped to chromosome 5 explaining 46.3 % of the phenotypic variation

(position 63 cM). *DTBnat₃* was mapped to position 16 cM on chromosome 9 explaining 31.1 % of the phenotypic variation. Based on the generated linkage map it is located close to the flowering repressor gene *BvFTI* (mapped on position 26.2 cM and position 27.3 cM with two sequenced based markers (CAU3851, CAU3850) on chromosome 9). All three QTL jointly explain and adjusted $R^2 = 54.6$ % of the observed phenotypic variation. For all three QTL the allele causing bolting delay was derived from leaf beet parent 82.5523 cumulating to a total bolting delay of 19 days in the homozygous state.

QTL mapping with the F_3 data generated under artificial vernalization conditions resulted in two QTL on chromosome 3 (LOD = 3.09) and chromosome 5 (LOD = 11.65). The QTL on chromosome 3 (*DTBart₁*) was mapped to position 38 cM and explains 14.1% of the observed phenotypic variation. The second QTL (*DTBart₂*) was mapped to chromosome 5 and explains 24.6 % of the variation. In total both QTL explain 20.7 % of the observed phenotypic variation (adjusted R). For both QTL the allele causing bolting delay was derived from leaf beet parent 82.5523 cumulating to a total bolting delay of 9 days in the homozygous state.

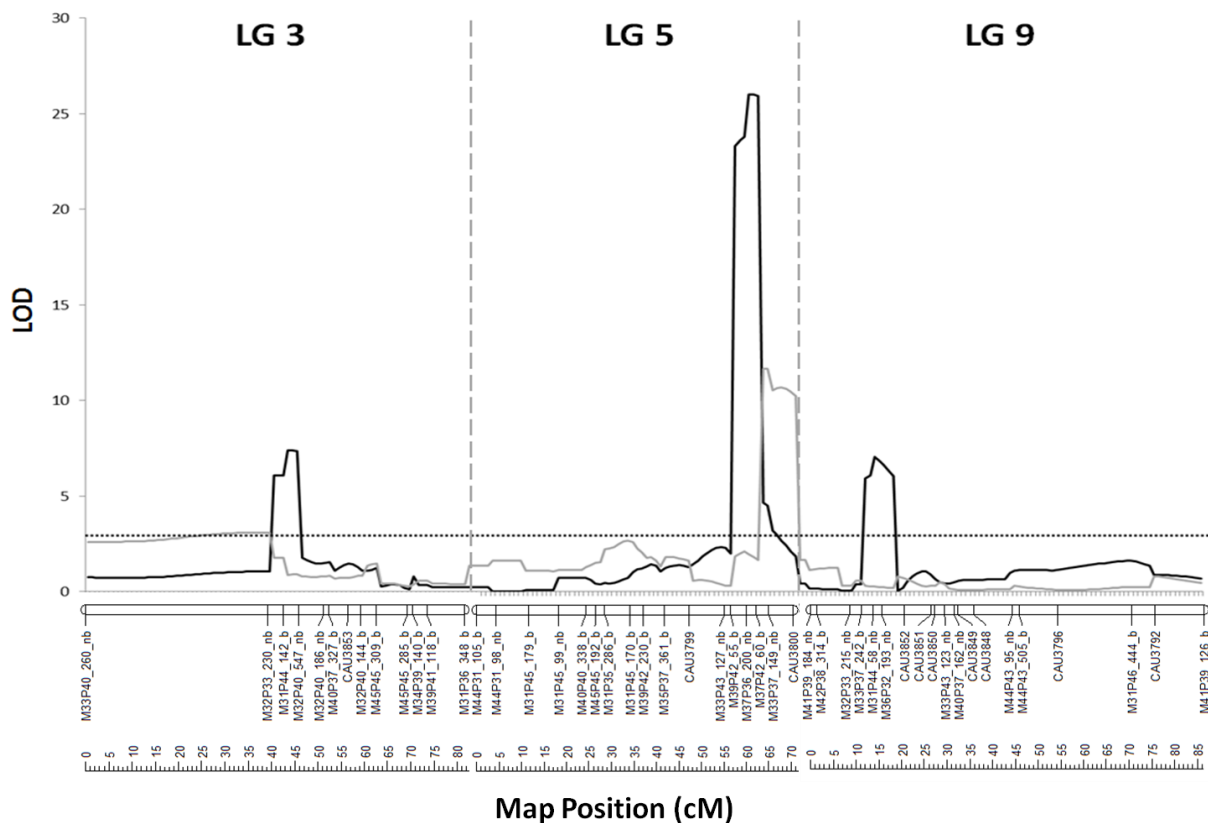


Figure 7: Detailed QTL mapping results for bolting delay (DTB-days to bolt) on linkage groups (LG) 3, 5 and 9. The linkage groups are displayed on the x-axis by the horizontal bar on which marker names are also located. Names of AFLP markers end on nb if the dominant marker allele is derived from the seed parent 82.5523 or on b if derived from the parent 93167P. The LOD curve for DTB under natural overwintering conditions is plotted in solid black, for cold treatment under artificial conditions it is indicated in solid grey. The dashed line indicates the LOD threshold value of 3.0.

Table 3: QTL mapping results for bolting delay as days to bolt (DTB) in F₃ families derived from crossing 82.5523 by 93167P after natural (n=264) and artificial (n=253) cold treatment, respectively. In the experiment under artificial conditions plants were transplanted to the field after artificial cold treatment on May 02, 2012. The onset of bolting was recorded twice a week from mid of May until end of July 2012 for the experiment performed under natural overwintering conditions and from end of May until October 2012 for the experiment performed under artificial cold treatment (see material and methods).

<i>Generation used for phenotyping</i>	<i>QTL name</i>	<i>Linkage group</i>	<i>Position</i>	<i>Flanking markers</i>	<i>Confidence interval</i>	<i>LOD</i>	<i>R²</i>	<i>Additive effects</i>	<i>Dominant effects</i>
F ₃	<i>DTBnat₁</i>	3	43	M31P44_142_b and M32P40_547_nb	42 - 46	7.38	29.8	-2.301	-1.087
F ₃	<i>DTBnat₂</i>	5	61	M37P36_200_nb and M37P42_60_b	59 - 63	26.02	46.3	-4.813	-1.795
F ₃	<i>DTBnat₃</i>	9	16	M36P32_193_nb and CAU3852	14 - 20	7.03	31.1	-2.420	-0.424
							107.2		
F ₃	<i>DTBart₁</i>	3	38	M33P40_260_nband M32P33_230_nb	0 - 40	3.09	14.1	-2.082	1.836
F ₃	<i>DTBart₂</i>	5	63	M37P42_60_b and M33P37_149_nb	62 - 65	11.65	24.6	-2.411	-1.106
							38.7		

R²: coefficient of determination

LOD: logarithm of the odd

3.5 Discussion

Post-winter bolting resistance is an important trait for the development of winter beets with controlled bolting behavior in cold temperate regions. This is the second report of QTL for post-winter bolting behavior. The identification of these QTL is a further step towards developing a winter sugar beet by exploiting natural variation in the *Beta vulgaris* gene pool. Combining these QTL with other genetic factors, like previously published by Pfeiffer et al. (2014), could result in a winter sugar beet variety with complete post-winter bolting resistance.

In our study we aimed to perform a genetic analysis of post-winter bolting resistance. We had observed this trait in the parental leaf beet accession 82.5523 with a post-winter bolting rate of only 0.5. In our mapping population we could observe complete post-winter bolting resistance after artificial vernalization in single F₂ plants. However, we did not observe such a phenotype in the F₃ generation, even in F₃ families derived from F₂ plants, which had not bolted after the first cold treatment (see **Material and methods**). Nevertheless, we observed delay in bolting after winter in the F₂ and F₃ generation. The reason for the lack of bolting resistance might be that the alleles underlying post-winter bolting resistance of accession 82.5523 were only partially transferred during crossing. The occurrence of non bolting plants only in the F₂ generation might then indicate different treatments of the F₂ population compared to the F₃ families. In cereals for example, the pot size has a large impact on the time point of bolting induction, and bolting is accelerated in plants that grow in smaller pots. The F₃ plants analyzed in this study were grown in smaller pots than their ancestor F₂ plants. Accordingly, it is conceivable, that the space limited stress is the reason for the higher number of bolting plants in the F₃. Moreover, the artificial light conditions in the greenhouse after cold treatments (F₂ population) might not have been as bolting inductive compared to natural light condition after transplanting the F₃ families to the field.

Comparing QTL detected with F₃ phenotypes from both experiments shows that under natural overwintering conditions an additional third QTL could be detected. Also, under natural overwintering conditions the detected QTL had larger effects that explain more of the phenotypic variation (54.6 % vs. 20.7 %) compared to artificial cold treatment. This might reflect a smaller experimental error of the experiment with natural overwintering and consequently more precision and power in the respective QTL mapping. On the other hand this result indicates that artificial conditions only reflect natural conditions in part, with light quality, day length and temperature profile as main environmental factors. Despite that, confidence intervals and QTL positions indicate that *DTBnat₁* and *DTBart₁* on linkage group 3 as well as *DTBnat₂* and *DTBart₂* on linkage group 5 reflect the same underlying genetic factors.

3.5.1 Implementation of the detected QTL in winter beet development

While post-winter bolting resistance could not be observed in the F₃ generation, the identification of QTL for bolting delay still is an important result of this study. These QTL could well be related to post-winter bolting resistance. Such a relationship was observed in a previous QTL study with the post-winter bolting resistant sugar beet accession BETA 1773 (Pfeiffer et al. 2014) as one of the population parents. The major QTL detected in that study affects not only post-winter bolting resistance but bolting delay as well. Based on that, it can be speculated that bolting delay contributes to bolting resistance. Moreover, the accumulation of bolting delay QTL could result in a genotype with post-winter bolting delay, especially if combined with the post-winter bolting resistance QTL *BR₁* mapped by Pfeiffer et al. (2014). If this approach proves to be successful, however, a solution for bolting induction of completely

post-winter bolting resistant sugar beets has to be developed as discussed in Pfeiffer et al. (2014).

3.6 Acknowledgements

This study was funded by the German Federal Ministry of Education and Research (BMBF) under the grant No. FKZ 0315465B. We thank Martin Kirchhoff, Meike Friedrichsen, Sabrina Butze, Monika Bruisch and Erwin Danklefsen for support and technical assistance in the lab, greenhouse and field. The Institute for Clinical Molecular Biology (IKMB), University Kiel, Germany, is acknowledged for collaborating in DNA sequencing.

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4 Closing discussion

4.1 Genetic mapping of bolting delay and bolting resistance after winter

For the development of winter sugar beet varieties an effective system for bolting control is of substantial importance. This will allow an extended vegetative growth period for crop production as well as bolting for seed production. To achieve suitable genotypes for this winter cropping system, it is therefore of uttermost importance to acquire a deep understanding of the genetic factors underlying bolting induction as well as post-winter bolting delay and post-winter bolting resistance. In this study I focused on these two post-winter bolting traits.

To my knowledge, no QTL of post-winter bolting delay or post-winter bolting resistance have been reported so far. The results of my study substantially extend the status of current knowledge on the inheritance of these traits by the identification of new QTL.

A first step towards QTL mapping of post-winter bolting traits was the development of two structured mapping populations segregating for post-winter bolting delay and post-winter bolting resistance, respectively. Accordingly, late bolting plants from accessions showing a low post-winter bolting rate were chosen as seed parents for crossing experiments. In this way, two mapping populations were developed and F₂ and F₃ generations were phenotyped for bolting behavior after cold treatment. For each F₂ population, I constructed a genetic map in order to map QTL for bolting delay and bolting resistance after winter. Both maps contain 9 chromosomes and span 737 cM (**Chapter 2**) and 691 cM (**Chapter 3**), which is comparable to already published genetic map sizes of sugar beet (Holtgräwe et al., 2014; Schneider et al., 2007; Würschum et al., 2013).

In total, I could identify four QTL for post-winter bolting delay and one QTL for post-winter bolting resistance and could map these QTL to chromosome 3, 5, and 9. To date, a number of genes (**Table 4**) related to flowering time have been mapped in beet (Abou-Elwafa et al., 2011; Chia et al., 2008; Dally et al., 2014; Gielen et al., 2007; Mutasa-Göttgens et al., 2009; Pin et al., 2010; Pin et al., 2012; Reeves et al., 2007; Schneider et al., 2007).

In a previous unpublished study, five flowering time QTL have already been mapped in sugar beet by a mixed-model approach in multi-parental QTL populations. These QTL were mapped to chromosome 2, 5, 7 and 9 (Müller et al., unpublished). Moreover, the researchers could successfully map in total 14 flowering time genes during this study (Müller et al., unpublished). I compared the linkage groups of the mapping populations developed in the course of this study with those developed by Müller et al. (unpublished) by searching for markers that they have in common.

All genes and QTL mapped by Müller et al. (unpublished data) to the chromosomes 1, 2, 4, 6, 7 and 8 could be directly rejected as candidates for bolting delay and bolting resistance after winter due to the fact, that they are not co-localizing to the QTL identified in this study.

Unfortunately, the linkage maps for chromosomes 3 and 5 of my mapping populations do not show common markers with the linkage maps developed by Müller et al. (unpublished). Accordingly, flowering time genes mapped by Müller et al. (unpublished) to the chromosomes 3 (*BvBFT*, *BvFPA*) and 5 (*BvAGL20*, *BvCDF2*) cannot be rejected as potential candidate genes.

For chromosome 9, I was able to perform a direct comparison of the results from both studies, since the linkage map generated by Müller et al. (unpublished) and those constructed during

Table 4: Mapped flowering time genes in *B. vulgaris*, their chromosome position and their function as far as already known.

<i>B. vulgaris</i> gene	Chromosome	Description	Reference
<i>BvGa20ox1</i>	I	Gibberellic acid pathway gene	Mutasa-Göttgens et al. (2009)
<i>BvGI</i>	I	Photoperiod pathway gene	Müller et al. unpublished
<i>BvLHP1</i>	I	Vernalization pathway gene	Müller et al. unpublished
<i>BvTOL1</i>	II	Unknown function	Pin et al. (2012)
<i>BTC1</i>	II	Bolting gene; repression of <i>BvFT1</i> and activation of <i>BvFT2</i>	Pin et al. (2012)
<i>BvCOL1</i>	II	Photoperiod pathway gene	Chia et al. (2008)
<i>BvBFT</i>	III	Floral integrator gene	Pin et al. (2010), Müller et al. unpublished
<i>BvFPA</i>	III	Autonomous pathway gene	Abou-Elwafa et al. (2011)
<i>BvFT2</i>	IV	Floral integrator gene	Pin et al. (2010)
<i>BvFLK</i>	IV	Autonomous pathway gene	Abou-Elwafa et al. (2011)
<i>BvAGL20</i>	V	Floral integrator gene	Gielen et al. (2007)
<i>BvCDF2</i>	V	Photoperiod pathway gene	Müller et al. unpublished
<i>BvFL1</i>	VI	Vernalization pathway gene	Reeves et al. (2007)
<i>BvLD</i>	VII	Autonomous pathway gene	Schneider et al. (2007), Abou-Elwafa et al. (2011)
<i>BvFVE1</i>	VII	Autonomous pathway gene	Abou-Elwafa et al. (2011)
<i>BvMFT1</i>	VIII	Floral integrator gene	Pin et al. (2010), Müller et al. unpublished
<i>BvBBX19</i>	IX	2 nd bolting gene; repression of <i>BvFT1</i> and activation of <i>BvFT2</i>	Dally et al. (2014)
<i>BvFT1</i>	IX	Floral repressor gene	Pin et al. (2010)
<i>BvGa3ox1</i>	IX	Gibberellic acid pathway gene	Mutasa-Goettgens et al. (2009)
<i>BvLDL1</i>	IX	Autonomous pathway gene	Abou-Elwafa et al. (2011)
<i>BvSWP1</i>	IX	Autonomous pathway gene	Müller (personal communication)

this study have some markers in common (**Figure 8**). This comparison revealed no relation between the QTL DTB_1 and BR_1 and the QTL for full flowering (time when all flowers were open on secondary branches) mapped by Müller et al. (unpublished). By contrast, a relation between the QTL $DTBnat_3$ and one QTL mapped by Müller et al. (unpublished) could not be rejected, since the confidence intervals of the QTL might overlap. All displayed maps of chromosome 9 (**Figure 8**) have a common marker in the floral integrator gene $BvFT1$ as well as in $B2$. For $B2$, only the name of the locus is given, thus the marker is not directly located in the $BvBBX19$ gene albeit closely linked. Moreover, I could map the flowering time candidate gene $BvSWP1$ in the leaf beet population (**Chapter 3**). $BvSWP1$ showed no co-localization with the QTL $DTBnat_3$ and thus, it can be excluded as candidate gene for the QTL detected in my study. Furthermore, I was able to map the gibberellic acid pathway gene $BvGa3ox1$ in both of my mapping populations. In the sugar beet population (**Chapter 2**), this gene is mapped 2.3 cM upstream of DTB_1 and 3.7 cM downstream of BR_1 and can thus be rejected as a candidate gene. However, before addressing possible candidate genes, fine mapping is required to further narrow down the QTL region.

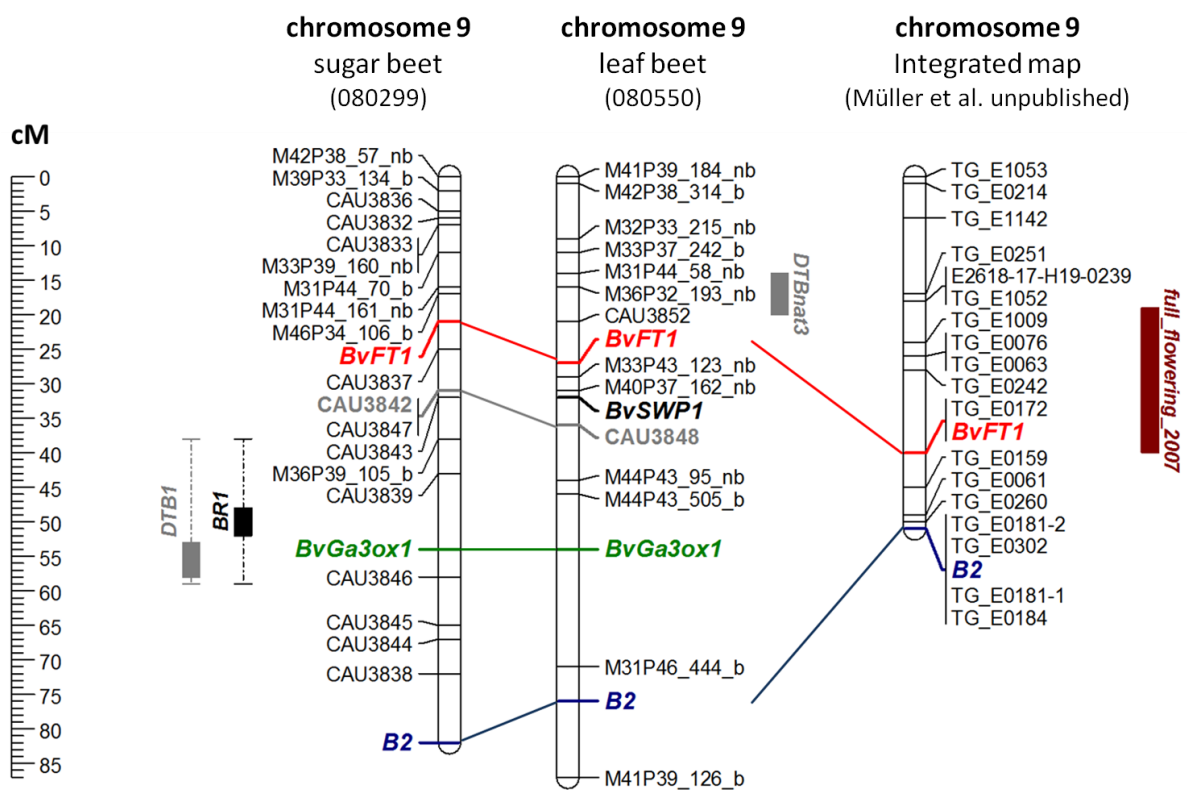


Figure 8: Comparison of genetic maps for sugar beet chromosome 9. Genetics maps generated during this study are designated as Chromosome 9 sugar beet (080299) (**Chapter 2**) and Chromosome 9 leaf beet (080550) (**Chapter 3**). The genetic map constructed by Müller et al. (unpublished) is labeled as ‘chromosome 9 integrated map’. Mapped flowering time candidate genes are indicated in bold and *italics* and traced by distinct colors across maps. The map by Müller et al. (unpublished) is modified by reversing the orientation of the map. All mapped QTL are displayed as bars next to the chromosomes. The scale on the left indicates the genetic distance in cM. The comparison of the maps was generated with the software Map Chart version 2.30.

Accordingly, my results are the first report of a major QTL for post-winter bolting behavior in sugar beet explaining a bolting delay of 82 days and a reduction in post-winter bolting rate by 65 % (**Chapter 2**). The identification of this QTL marked a first step towards the development of a winter sugar beet by exploiting the natural variation within the sugar beet gene pool.

Although post-winter bolting resistance could not be genetically characterized in the leaf beet population (**Chapter 3**), the identification of QTL for bolting delay after winter offers a possibility to accumulate post-winter bolting delay and post-winter bolting resistance alleles that may result in a winter sugar beet with a strong delay in bolting after winter.

In both studies I was not able to explain the complete genotypic variation with the detected QTL. This indicates the presence of minor QTL with an effective size below the detection threshold.

Besides QTL mapping for bolting delay and bolting resistance after winter, a further initial objective of my study was the development of selectable markers flanking the QTL detected in both analyzed mapping populations. The availability of these markers will enable marker-assisted selection of genotypes homozygous or heterozygous for the recessive post-winter bolting resistance alleles. Marker-assisted selection provides valuable opportunities for enhancing the selection response since molecular markers can be applied in a highly precise and cost-effective manner at a very early developmental stage, i.e. the seedling (Miedaner and Korzun, 2012).

Selection markers were successfully identified for the post-winter bolting resistance QTL (*BRI*) in the mapping population derived from sugar beet BETA 1773 (seed code: 080299) (**Chapter 2**). Two molecular markers CAU3839 and CAU3841 (*BvGa3ox1*) flank the *BRI* region in a distance of <5 cM and <1 cM, respectively. Consequently, those markers can be used for marker-assisted selection of the *BRI* locus. CAU3839 is an InDel marker and CAU3841 is a CAPS marker; both marker types can be easily converted into KASP (competitive allele-specific PCR) markers. The KASP genotyping assays enable bi-allelic scoring of SNPs at specific loci and thus provide a system for high throughput marker analysis. An overview of the different aspects of the KASP genotyping platform, its application in crop improvement, and comparison with the chip-based Illumina GoldenGate platform is given by Semagn et al. (2013). The authors demonstrated that KASP offers a cost-effective and scalable flexibility in applications that require small to moderate numbers of markers such as quality control analysis, QTL mapping in bi-parental populations, marker-assisted recurrent selection, marker-assisted backcrossing, and QTL fine mapping (Semagn et al., 2013). Recently, KASP markers were successfully used to develop robust functional marker assays for high-throughput selection of soybean cyst nematode resistance and to differentiate the sources of resistance (Shi et al., 2015).

The second mapping population, derived from leaf beet 82.5523 (seed code: 080550), did not segregate for post-winter bolting resistance, but for post-winter bolting delay. Unfortunately, the development of sequence based markers which can be used as selectable markers for the detected QTL was not as successful as compared to the sugar beet population derived from BETA 1773 (080299). Only one QTL (*DTBnat₃*) is associated with a sequence based InDel marker (CAU3852) that can be easily used for marker-assisted selection. For the remaining QTL (*DTBnat₁*, *DTBart₁*, *DTBnat₂* and *DTBart₂*) no sequence derived selectable marker is available because those QTL are flanked by AFLP markers. For these QTL, further analyses need to be performed in order to develop co-dominant sequence based markers, which are cheaper concerning the costs for development and additionally reveal the heterozygous genotypes.

The *in silico* cloning of candidate genes for post-winter bolting resistance was a further initial objective of my study. *In silico* cloning is defined as the identification of unknown genes by searching DNA databases that provide information on homolog genes and their biological function (Passier and Doevendans, 2004).

Previously, due to the lack of the genome sequence information *in silico* cloning in sugar beet was mainly performed using EST and genomic sequence databases. The availability of the

sugar beet genome sequence substantially facilitates fine-mapping of QTL and identification of candidate genes, since SNP-based markers can now be designed for any region of the genome (Dohm et al., 2012; Dohm et al., 2014). Recently, Li et al. (2015) used an *in silico* approach from EST and genome survey sequences in sugar beet to predict in total 13 mature microRNAs (miRNAs), which impact the plant's salt tolerance. MicroRNAs are endogenous small RNAs (mostly comprising a size of 20-25 bp), which are negatively regulating gene expression at post-transcription levels (Zhang and Wang, 2015). These miRNAs play a crucial role in almost all biological and metabolic processes in the life cycle of plants, and thus may provide the basis for a unique strategy for plant improvement (Zhang and Wang, 2015).

During my study, the initial plan was to search the beet sequence for candidate genes after mapping the QTL underlying the post-winter bolting resistance trait. Unfortunately, this was not possible within the time frame of this study. However, *in silico* cloning of the related candidate genes, as well as the cloning process itself could help to understand their function and their impact on the inheritance of post-winter bolting behavior as well as their influence on the life cycle of sugar beet. These data could be very helpful to increase our understanding of the genetics of bolting behavior after winter and serve as a basis for the introduction of these traits into sugar beet breeding programs.

At the beginning of my study the gene *BvFT1* was hypothesized to constitute an interesting candidate gene for post-winter bolting resistance due to its described function and an observed association between detected sequence variants and their bolting phenotypes (Pfeiffer, 2010). In contrast to only one *FT* gene present in the model plant *Arabidopsis thaliana*, *B. vulgaris* carries a pair of *FT* homologs with antagonistic functions, referred to as *BvFT1* and *BvFT2* (Pin et al. 2010). *BvFT1* acts as a floral repressor and is down-regulated by vernalization. *BvFT2* is a functional ortholog to *FT* in *Arabidopsis* and acts as a major floral integrator. Annual plants in which *BvFT2* was suppressed by RNAi showed only vegetative growth, indicating that *BvFT2* is required for the transition to the generative phase (Pin et al., 2010). Due to the described function of the gene, *BvFT1* was a good candidate gene for post-winter bolting resistance observed in BETA 1773 (080299). Accordingly, I performed a haplotype analysis in order to detect an association between the post-winter bolting resistance phenotype and a *BvFT1* haplotype within sugar beet accession BETA 1773 (080299). While I worked with bi-parental populations in the QTL mapping experiments (**Chapter 2 and 3**) this analysis was performed in a non-structured population.

In the haplotype analysis, post-winter bolting resistance could be associated with *BvFT1*. An expansion of this haplotype analysis was planned in a structured population derived from a heterozygous seed parent. Therefore, selfing progenies should be sown in overwintering field trials, but due harsh weather conditions sowing was not possible. Alternative growing of these plants over winter in the greenhouse was rejected because artificial vernalization might not reflect overwintering conditions in the field. Thus, the field sowing was postponed for one year. In the meantime, I obtained the first QTL results in the bi-parental mapping population derived from BETA 1773 (080299), which indicated that *BvFT1* is linked to the QTL on chromosome 9, but not so closely (> 17 cM) that it plays a major role concerning the inheritance of the trait. Therefore the experiment was not continued, since *BvFT1* could be rejected as a candidate gene for post-winter bolting resistance in BETA 1773 (080299).

In 2014, Dally et al (2014) described the map-based cloning of a candidate gene from the *B2* locus from beet, *BvBBX19*, which is acting epistatically over the *B*-locus and encodes a DOUBLE B-BOX TYPE ZINC FINGER protein. The gene was mapped to chromosome 9 and could thus constitute a candidate gene if it is localized in the region of the *BR₁* (**Chapter 2**) or *DTBnat₃* (**Chapter 3**) QTL mapped during this study. However, due to the available marker data generated for both populations I can reject *BvBBX19* as a candidate

gene for post-winter bolting delay as well as for post-winter bolting resistance, since the marker CAU3784 which is co-segregating with *BvBBX19* in the study of Dally et al. (2014) is not localized in the *BR₁* QTL region (**Chapter 2**).

4.2 Breeding of non bolting winter beets

For breeding winter sugar beets, besides bolting control winter hardiness is of substantial importance. If the varieties are not able to survive the moderate winters in the cold temperate regions of Europe, the plant material can be regarded as not suitable for breeding under this aspect. Reinsdorf et al. (2013) reported that frost tolerance depends on winter conditions as well as on the plant development. Moreover, they showed that the optimum plant size is independent from plant age and that frost tolerance is highest at a maximum taproot diameter of 1–2.5 cm. Furthermore, the concentration of anorganic salts like sodium and potassium as well as organic constituents like amino-N and water amount in the taproot contribute to frost tolerance (Reinsdorf et al., 2013). Recently, Loel and Hoffmann (2015) reported that acclimatization plays an important role concerning the survival rates of beets in overwintering field experiments accompanied by greenhouse experiments. They observed that during the process of acclimatization, betaine, glutamine, proline and raffinose were markedly accumulated in root and leaves and osmolality was thereby enhanced. Moreover, they were able to show that betaine, amino acids and osmolality had a positive correlation to the survival rate of the analyzed beet phenotypes and were thus identified as potential frost protecting substances for sugar beet. On the contrary, the results indicate that raffinose and proline seem to rather act as stress indicators as they were observed to be negatively correlated to beet survival (Loel and Hoffmann, 2015).

In addition to physiological and agronomic studies (Reinsdorf and Koch, 2013) genetic studies, concerning frost tolerance in beet, have been performed. Kirchhoff (2013) performed a QTL study on frost tolerance in a structured mapping population. QTL mapping based on field data revealed two QTL on chromosome 4 and 6 for the trait winter hardiness. These QTL explain 11.7 % of the observed phenotypic variation and none of these QTL was mapped in the vicinity of any mapped candidate gene for cold tolerance (Kirchhoff, 2013).

Phenotypic data from a second test year were used by Uhlmann (2014) for a further QTL mapping. In this way, three significant QTL on chromosome 3, 4 and 9 have been identified explaining a phenotypic trait variation of 35.5 % (Uhlmann, 2014).

The combination of these QTL for winter hardiness in beet with other genetic factors could result in a winter sugar beet which could survive winters in moderate climate regions. However, even if the beet genotypes survive these winter conditions, early bolting of these beets must be avoided. Therefore, I propose two strategies. For strategy one, I suggest to pyramid the QTL for bolting delay (*DTB₁*, *DTBnat₁*, *DTBnat₂*, *DTBnat₃*) in order to generate a beet with a substantial bolting delay. Since these beets are bolting delayed after winter, but not bolting resistant, seed production of those beets is possible and can be used for a winter cropping system. Another strategy could be the integration of the QTL for bolting resistance (*BR₁*) to avoid bolting after winter completely. While the second strategy seems feasible with the QTL detected in this study, the remaining challenge is to enable bolting for seed production. To overcome this challenge, I also performed bolting induction experiments during my study.

My approach was based on the hypothesis that at least one candidate gene interferes with plant hormone metabolism. Gibberellins (GA) are known to play important roles in key physiological processes including stem elongation (bolting) and flowering and are, therefore, potential targets for controlling reproductive growth in sugar beet (Mutasa-Göttgens et al., 2009). In sugar beet, it was shown that GA acts independently of the *B* allele and photoperiod to induce bolting. It has also been demonstrated, that vernalization enables GA action

independently of the *B* allele (Mutasa-Goettgens et al., 2010). In comparison to annual (*BB*, *Bb*) beets, biennial (*bb*) types required vernalization for promotion of continued stem growth by GA. In this case it is assumed that the effect of vernalization on GA-induced stem elongation is independent of *B* (Mutasa-Göttgens et al., 2010). Their results demonstrate no significant major effect of GA on bolting induction under inductive long day conditions. However, this result may be caused by insufficient light intensities in the climate chambers they used. Thus, an investigation of the effects of light intensity and light quality on bolting induction is considered to be absolutely necessary.

Furthermore, the results of GA experiments in sugar beet (Mutasa-Göttgens et al., 2010) demonstrated that GA treatment without prior vernalization does not lead to an increased bolting rate. Due to these facts I assumed that GA application after cold treatment induces bolting. This should result in an increased bolting rate of the biennial (*bb*) genotypes of sugar beet BETA 1773 (080299) and leaf beet 82.5523 (080550), if at least one of the candidate genes for post-winter bolting resistance interferes with the GA metabolism.

Accordingly, I designed different greenhouse experiments, in which vernalized plants from progenies of sugar beet BETA 1773 (080299) were treated with gibberellin, auxin and cytokinin. A group without any hormone treatment served as control group. The bolting rate of plants treated with GA did not statistically deviate from the observed bolting rate of the control group. However, the observed bolting rate of plants treated with cytokinin and auxin was more than twice as high compared to the control group. My experiment corroborated the data of Mutasa-Göttgens et al (2010), i.e. GA treatment might not have a large effect on bolting, while the growth regulating hormones cytokinin and auxin seemed to increase the bolting rate by up to threefold.

One reason for the observed results of the experiment may be that no candidate gene for post-winter bolting resistance interferes with the GA metabolism. However, environmental cues, which may play major roles in the floral transition cannot be excluded. It is well known that biennial beets have a vernalization requirement and that day length and light quality are important factors for the induction of bolting. The experiments for this study were performed in a greenhouse where environmental factors such as temperature, day length and light quality can be controlled. However, experiments in a greenhouse are not comparable to a fully closed system like e.g. a climate chamber. For the continuation of the work on bolting induction by hormone treatment a newly designed experimental protocol in a climate chamber must be developed. An already established test allows a fast characterization of bolting behavior after vernalization. This, however, requires the establishment of optimal vernalization and light conditions (temperature, day length, light quality, light quantity) first.

4.3 Outlook, future experiments

QTL mapping is one strategy to identify quantitative trait loci (genomic regions that are contributing to a specific phenotypic variation as in our case bolting behavior after winter) by calculating the genetic linkage between molecular markers and the genetic factors controlling the QTL segregating in a structured mapping population. Once the chromosomal localization of the QTL has been identified, the region of interest can be saturated with molecular markers to finally obtain a marker which completely co-segregates with the desired trait in a mapping population. By this way, the size of the QTL region will be reduced and thus the chromosomal position of the respective target gene can be determined. This process is called map-based cloning and was recently demonstrated in sugar beet by Dally et al. (2014).

A further approach for the identification of a given gene of interest is a bioinformatic approach as established by Schneeberger et al (2009) for simultaneous mapping and mutation

identification via deep sequencing, the so-called SHOREmap approach. The SHOREmap software supports genome-wide genotyping and candidate-gene sequencing in a single step through analysis of deep sequencing data from a large pool of recombinants and is thus based on the concept of a bulked segregant analysis (BSA). This approach could be also suitable for using F₂ populations segregating for bolting behavior after winter developed during this study (**Chapter 2** and **3**). Sequencing of pooled DNA from post-winter bolting and post-winter bolting resistant plants of the sugar beet mapping population has recently been performed in a different project. I expect these results to enable a fine-mapping of the *BR₁* locus and a substantial reduction of the number of potential candidate genes in this region. However, even if the gene number can be reduced to one single *BR₁* gene, a comprehensive functional analysis is necessary to confirm its function and possible genetic interactions.

Once the underlying gene is determined and sequence data is available, the easiest way to confirm its function is the application of expression analysis. Expression of the candidate gene has to be determined in bolting and bolting resistant plants before and after winter. The remaining F₃ seeds obtained from F₂ plants of the sugar beet population (**Chapter 2**), which showed segregation for bolting after winter (early bolting vs. late bolting), could constitute suitable plant material for this analysis. Expression analysis can be done via quantitative RealTime PCR (RTq-PCR) and may result in different gene expression levels before and after winter in either post-winter bolting or post-winter bolting resistant plants, if the analyzed gene is responsible for the inheritance of the trait.

In addition, after cloning of the target gene a functional analysis is highly important. One way to perform such an analysis is via complementation of mutants lacking the function of interest. Complementation analysis was recently performed in sugar beet by Stracke et al. (2014). The authors described the *R2R3-MYB* gene family by means of *in silico* analysis of the recently published *sugar beet* genome sequence in order to predict protein domain architectures and to validate the extent of divergence and conservation between *A. thaliana* and *B. vulgaris* gene families (Stracke et al., 2014). The *R2R3-MYB* genes encompass one of the largest transcription factor gene families in plants, playing major regulatory roles in plant-specific processes, regarding plant development, accumulation of metabolites and defense responses (Stracke et al., 2014). Validation of the obtained functional classification was achieved by cDNA isolation and subsequent functional analysis by transient transactivation assays and complementation of an *A. thaliana* line mutated for the homologous genes (Stracke et al., 2014). The findings of this study provide a first step towards further understanding of the molecular and biological functions of MYB transcription factors in sugar beet (Stracke et al., 2014).

Typically, the classical genetic approach for exploring biological pathways starts by identifying mutations that cause a phenotype of interest (Prelich, 2012). Mutant phenotypes can also be caused by overexpression or misexpression of a gene product from a wild type (Prelich, 2012).

In 2010, overexpression analysis in sugar beet was successfully performed for functional characterization of the floral integrator gene *BvFT2* (Pin et al., 2010). The authors could demonstrate that expression of *BvFT2* under the constitutive cauliflower mosaic virus (CaMV) 35S promoter in a biennial background leads to early flowering without vernalization and plants started flowering 18 weeks after transformation (Pin et al., 2010).

Additionally, RNAi, a conserved biological response to double-stranded RNA, could be used for regulation of the expression of protein-coding genes. RNAi has been demonstrated to be a useful tool for experimental manipulation of gene expression and for verification of gene functions on a whole genome scale (Hannon, 2002). Pin et al. (2010) used this technique for suppression of *BvFT2* gene expression and successfully demonstrated that RNAi-induced

downregulation of *BvFT2* resulted in transgenic plants that failed to bolt even after cold treatment (Pin et al., 2012).

In recent years, a new technique for targeted genome editing came across and opened a new era for mutation-based methods. Instead of generating genetic variation randomly by radiation treatments or chemical induced mutagenesis, the new genome editing techniques are based on inducing mutations directly at a specific target region of the genome (Ito et al., 2015). These mutations in specified genomic locations are caused by DNA double-strand breaks (DSBs) (Petolino, 2015). This new method cannot only be used for single-celled organisms but also for more complex organisms such as plants and animals (Weeks et al., 2015). DNA repair of such DSBs can lead to gene knockouts or gene replacement by homologous recombination, if exogenously supplied homologous DNA fragments are available (Weeks et al., 2015).

Recently, the CRISPR/Cas9 method has been successfully applied for directed genome editing in many organisms, including model and crop plants (Belhaj et al., 2015). The CRISPR/Cas9 method is based on the type II bacterial clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 (CRISPR-associated) adaptive immune response (Jinek et al., 2012). Cas9, a RNA-guided endonuclease, can be targeted towards specific genomic sequences and cleave the foreign DNA from e.g. phages into short fragments (Belhaj et al., 2015; Bhaya et al., 2011). These DNA fragments can then be incorporated into a CRISPR locus and are subsequently transcribed into RNA (Bhaya et al., 2011). Since only a short RNA sequence must be synthesized to confer recognition of a new target, CRISPR/Cas9 is a relatively cheap, time-saving and easy-implementable technology that has already proven to be extremely multifaceted (Belhaj et al., 2015).

CRISPR/Cas9 could be a game-changing technology that may revolutionize basic research in plant breeding for crop improvement (Belhaj et al., 2015). In plant research, the CRISPR/Cas9 genome editing method was recently successfully applied in several plant species such as e.g. Arabidopsis, potato, soybean, tomato, tobacco, maize, rice, sorghum and wheat (Weeks et al., 2015).

Accordingly, for breeding a winter sugar beet, in the near future it may be conceivable to use the CRISPR/Cas9 method by targeting one of the post-winter bolting delay or post-winter resistance genes underlying the QTL identified in this study.

5 Summary

Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is the only crop grown for sugar production in Europe. Sugar beets are biennials and store the produced sugar in their taproot. A major aim in sugar beet breeding is the increase of sugar yield. This aim could be achieved by extending the growth period in the field, since the plants can use the photosynthetically active radiation longer and more effectively during the year. Thus, breeding of so-called winter beets could play a crucial role in increasing sugar beet yields. In contrast to the current growing system, winter beets would be sown in autumn of the year prior to harvest. The plants then stay on the field over winter and are able to start photosynthesis and sugar storage earlier than regularly grown beets in the following spring. However, in order to implement this growing system in practical agriculture two important requirements have to be fulfilled: i) improvement of winter hardiness, since this trait is indispensable for winter survival of the plants in cool temperate climates, and ii) bolting of sugar beets after winter has to be controlled. Bolting is the elongation of the main shoot as consequence of the transition from vegetative to reproductive growth, which is triggered by vernalization, a prolonged exposure to cold temperatures, followed by long day conditions. Bolting after winter must be avoided, since it leads to consumption of the sugar stored in the taproot during vegetative growth and thus, to serious yield losses. Since overwintering in the field takes place under vernalizing conditions, post-winter bolting resistance is also indispensable for ensuring the continued vegetative growth of the winter beets during the entire cultivation period. However, for breeding and seed production, bolting must be enabled. Thus, the genetics of bolting and flowering and consequently, also the genetic mechanisms driving bolting delay and bolting resistance after winter need to be properly understood.

The aims of my study were the identification and genetic mapping of quantitative genetic factors (QTL) affecting post-winter bolting delay and post-winter bolting resistance in beet. These aims were pursued by QTL mapping in segregating populations derived from crossing parents with noticeable bolting behavior.

In **Chapter 2**, the results of QTL mapping for bolting delay and bolting resistance after winter in a complete sugar beet background are presented. Sugar beet accession BETA 1773 (080299), which showed a low post-winter bolting rate in the field, was crossed with a biennial sugar beet with regular bolting behavior for the development of a F₃ mapping population. The F₃ population was grown in the greenhouse and transplanted to the field after artificial cold treatment. Bolting behavior was consequently recorded from May to October. Bolting behavior was assessed by two different factors, post-winter bolting delay (defined as days to bolt after cold treatment) and post-winter bolting resistance (bolting rate after cold treatment). For days to bolt F₃ family means (n=186) ranged from 25 to 164 days, while for bolting rate after cold treatment F₃ family means ranged from 0 to 1. A linkage map (737 cM) based on 119 AFLP, SSR, InDel and SNP-based CAPS markers was established for QTL mapping. One QTL for each trait (*DTB₁* and *BR₁*), which explained about 65 % of the phenotypic variation was successfully mapped to the same region on chromosome 9 with a partially recessive allele increasing post-winter bolting delay and post-winter bolting resistance.

The research in **Chapter 3** followed a similar strategy, but the traits of interest were transferred from leaf beet accession 82.5523 (080550) exhibiting a low post-winter bolting rate in the field, to the mapping population by crossing with an annual sugar beet. While the F₂ population was segregating for post-winter bolting delay and post-winter bolting resistance, in the F₃ families only post-winter bolting delay could be assessed in two different experiments under natural overwintering conditions (n=265) as well as after artificial cold treatment (n=253). For days to bolt after artificial cold treatment F₃ family means ranged from

27 to 43 days while for days to bolt after natural overwintering conditions F_3 family means ranged from 7 to 29 days. For QTL mapping a linkage map (691 cM) comprising 139 AFLP, SSR, InDel and SNP-based CAPS markers was established. In total, two QTL for bolting delay after artificial cold treatment could be successfully mapped to chromosomes 3 (*DTBart₁*) and 5 (*DTBart₂*) as well as three QTL for bolting delay under natural overwintering conditions (*DTBnat₁*, chromosome 3; *DTBnat₂*, chromosome 5 and *DTBnat₃*, chromosome 9) explaining up to 54,6 % of the observed phenotypic variation. Confidence intervals and QTL positions indicate that *DTBart₁* and *DTBnat₁* on chromosome 3 as well as *DTBart₂* and *DTBnat₂* on chromosome 5 reflect the same underlying genetic factors.

Although post-winter bolting resistance could not be observed in the F_3 generation, the identification of the QTL for bolting delay in this material is an important result of the study. Due to the observed relationship of the QTL mapped in Chapter 2, where the major QTL affects both, post-winter bolting resistance and post-winter bolting delay, the QTL for post-winter bolting delay mapped in Chapter 3 could well be related to post-winter bolting resistance.

The QTL mapped in the sugar beet population (**Chapter 2**) explains only 65 % of the phenotypic variation and is thereby unsuitable for the development of a winter beet with complete post-winter bolting resistance. However, the QTL for post-winter bolting delay mapped in Chapter 2 and Chapter 3 could be pyramided in order to generate a sugar beet with a maximum post-winter bolting delay. Since these beets are delayed but not resistant concerning post-winter bolting, seed production of those beets is possible.

The results of my work extend the knowledge of post-winter bolting delay and post-winter bolting resistance in beet, that are of substantial importance for breeding winter beets with controlled bolting behavior.

6 Zusammenfassung

Die Zuckerrübe (*Beta vulgaris* ssp. *vulgaris*) ist die einzige in Europa zur Zuckerproduktion angebaute Kulturpflanze. Ein wichtiges Ziel in der Zuckerrübenzüchtung ist die Steigerung des Zuckerertrages, welches durch eine Verlängerung der Anbauphase der Rüben im Feld erreicht werden könnte, da die Pflanzen die zur Zuckerbildung benötigte photosynthetisch aktive Strahlung länger und im Jahresverlauf effektiver nutzen könnten. Die Züchtung so genannter Winterrüben könnte somit eine entscheidende Rolle bei der Ertragssteigerung spielen. Im Gegensatz zum derzeitigen Anbausystem würden die Winterrüben schon im Herbst des Erntevorjahres ausgedrillt werden. Die Pflanzen würden im Feld überwintern und könnten im folgenden Frühjahr zeitiger mit der Zuckereinlagerung beginnen als herkömmlich gedrillte Zuckerrüben. Um dieses Anbausystem in der Praxis umzusetzen, müssen allerdings zwei wichtige Faktoren zuvor züchterisch bearbeitet werden: i) die Winterhärte, welche notwendig ist, damit die Pflanzen den Winter in kühlgemäßigten Klimazonen überleben und ii) die Schosskontrolle. Unter Schossen versteht man eine deutliche Streckung der Sprossachse, die den Übergang der Pflanze vom vegetativen zum generativen Wachstum markiert. Das Schossen wird durch die Vernalisation, eine längere Kälteperiode, und darauffolgende Langtagsbedingungen induziert und führt zu gravierenden Ertragsverlusten. Da die Überwinterung im Feld unter vernalisierenden Bedingungen erfolgt, ist eine Schossresistenz, die das vegetative Wachstum der Rüben über die komplette Anbauphase garantiert, für die Züchtung von Winterrüben unabdingbar. Für züchterische Aspekte und die Saatgutproduktion wiederum muss das Schossen der Rüben gewährleistet sein. Daher ist ein grundlegendes Verständnis der genetischen Grundlagen des Schossens, der Schossverzögerung und der Schossresistenz erforderlich.

Ziel meiner Arbeit war daher die genetische Kartierung von quantitativ genetischen Faktoren (QTL), die an der Ausprägung von sowohl verzögertem Schossen als auch vollständiger Schossresistenz beteiligt sind. Dafür wurden bi-parentale Populationen durch Kreuzungen mit in ihrem Schossverhalten variierenden Eltern erstellt.

In Kapitel 2 werden die Ergebnisse der QTL Kartierung in einem vollständigen Zuckerrüben-Hintergrund dargestellt. Für die Erzeugung einer F₃ Kartierungspopulation wurde eine zweijährige, schossverzögerte Pflanze der Zuckerrübenakzession BETA 1773 (080299), die im Feld nach Winter eine verminderte Schossrate zeigte, mit einer Zuckerrübe mit regulärem Schossverhalten nach Kälteeinfluss gekreuzt. Die F₃ Population wurde im Gewächshaus angezogen und nach einer künstlichen Kältebehandlung ins Feld gepflanzt. Das Schossverhalten der F₃ Familien wurde von Mai bis Oktober durchgängig phänotypisiert, wobei die Merkmale Schossverzögerung (Tage bis zum Schossen nach erfolgter Kältebehandlung) sowie Schossresistenz (Schossrate nach erfolgter Kältebehandlung) erfasst wurden. Für die Schossverzögerung variierten die Mittelwerte der 186 untersuchten F₃ Familien von 25 bis 164 Tagen, während die Schossrate von 0 bis 1 variierte. Für die QTL-Kartierung wurde eine genetische Karte (737 cM) basierend auf 119 AFLP-, SSR-, InDel- und SNP basierten CAPS-Markern erstellt. Für jedes der beiden untersuchten Merkmale konnte ein QTL (*DTB₁* und *BR₁*), der 65 % der beobachteten Variation genetisch erklärt, in der gleichen Region von Chromosom 9 kartiert werden.

Kapitel 3 beschreibt den gleichen Ansatz, jedoch wurden hier die Merkmale Schossverzögerung und Schossresistenz aus einem Mangoldelter 82.2253 (080550) eingekreuzt. Während die F₂ Population für beide Merkmale aufspaltete, konnte in der F₃ Generation nur eine Aufspaltung für das Merkmal Schossverzögerung beobachtet werden. Die Schossverzögerung der F₃ Familien wurde in zwei verschiedenen Experimenten, zum einen unter natürlichen Überwinterungsbedingungen (n=265) sowie nach künstlicher Kältebehandlung (n=253), als Tage bis zum Schossen erfasst. Die Mittelwerte der F₃ Familien

variieren nach künstlicher Kältebehandlung zwischen 27 und 43 Tagen sowie zwischen 7 und 29 Tagen unter natürlichen Überwinterungsbedingungen. Für die QTL Kartierung wurde eine genetische Karte (691 cM) basierend auf 139 AFLP-, SSR-, InDel- und SNP basierten CAPS-Markern erstellt. Insgesamt konnten zwei QTL für Schossverzögerung nach künstlicher Kältebehandlung auf den Chromosomen 3 (*DTBart₁*) und 5 (*DTBart₂*) sowie drei QTL für Schossverzögerung unter natürlichen Überwinterungsbedingungen auf den Chromosomen 3 (*DTBnat₁*), 5 (*DTBnat₂*) und 9 (*DTBnat₃*) kartiert werden. Diese erklären bis zu 54,6 % der phänotypischen Variation. Die Konfidenzintervalle sowie die Positionen der QTL *DTBart₁* und *DTBart₂* auf Chromosom 3 und der QTL *DTBart₂* und *DTBnat₂* auf Chromosom 5 deuten darauf hin, dass es sich hierbei um die gleichen genetischen Faktoren handelt.

Obwohl in der F₃ Generation keine vollständige Schossresistenz beobachtet werden konnte, ist die Identifikation von QTL für das Merkmal Schossverzögerung ein wichtiges Ergebnis dieser Arbeit, da die in Kapitel 2 erzielten Ergebnisse die Schlussfolgerung zulassen, dass der kartierte QTL sowohl das Merkmal Schossverzögerung als auch das Merkmal Schossresistenz bedingt.

Da der in der Zuckerrübenpopulation kartierte QTL (Kapitel 2) nur insgesamt 65 % der beobachteten phänotypischen Variation genetisch erklärt und daher allein nicht ausreichend ist, um eine vollständige Schossresistenz zu erzielen, könnte ein Ansatz weiterer Untersuchungen sein, die in beiden Population kartierten QTL für das Merkmal Schossverzögerung zu pyramidisieren, um eine möglichst lange Schossverzögerung zu garantieren. Da Zuckerrüben mit einer extremen Schossverzögerung jedoch über keine vollständige Schossresistenz verfügen würden, könnten sie daher auch für die Züchtung und Saatgutvermehrung genutzt werden.

Durch die in dieser Arbeit generierten Ergebnisse konnten grundlegende Kenntnisse zur Schossverzögerung und vollständigen Schossresistenz nach Winter gewonnen werden. Diese können für die Züchtung von Winterrüben von wesentlicher Bedeutung sein.

7 Appendix

7.1 Supplementary material for chapter 2

7.1.1 Supplementary tables for chapter 2

Supplementary Table 1: Distribution of AFLP and sequence based markers in the linkage map used for QTL analysis.

Linkage group	AFLP	Sequence based marker	Length (cM)	Largest gap (cM)
1	8	2	85.7	21.1
2	11	2	76.0	21.9
3	14	1	80.0	13.9
4	6	7	86.8	14.2
5	7	6	100.1	19.3
6	11	4	79.3	13.3
7	4	5	69.9	14.3
8	8	1	78.3	30.9
9	7	15	81.5	10.7
Total	79	43	737	-

Supplementary Table 2: Overview of crossing parents used for the development of a segregating F₂ mapping population, seed codes are given in brackets (). For detailed bolting behavior of F₂ plants see **Supplementary Table 3**

Seed parent	Pollinator	F ₁	F ₂
biennial sugar beet BETA 1773 (080299)	biennial sugar beet 93161P (930176)	(090757/03)	(102946)

Supplementary Table 3: Phenotypic data of F₂ single plants of population 102946 and their F₃ offspring after cold treatment. pwbr: post-winter bolting resistant phenotype; incb: incomplete bolting phenotype. DTB: days to bolt after cold treatment; BR: bolting rate. For F₃ families' least square means were calculated by SAS PROC MIXED (SAS Institute Inc., 2009, Cary, USA, Version 9.2). Marker data of the sequence based flanking marker CAU3839, CAU3841 and CAU3846, that flank the QTL *BR₁* and *DTB₁* on linkage group 9, is given. 'b' and 'a' indicate marker genotypes homozygous for the marker allele (M1) derived from the biennial sugar beet parent 93161P (930176), or homozygous for the marker allele (M2) derived from the biennial sugar beet parent BETA 1773 (080299), respectively. 'h' indicates heterozygous marker genotypes. 'na': not available.

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean	F ₃ BR family mean	CAU 3839	CAU 3841	CAU 3846
102946/001	bolting	51	na	na	na	na	na	na
102946/002	bolting	45	112070	87.82	0.65	na	na	na
102946/003	bolting	37	112697	na	na	na	na	na
102946/004	bolting	41	na	na	na	na	na	na
102946/005	bolting	41	112071	80.87	0.73	h	h	h
102946/006	bolting	34	112072	na	na	na	na	na
102946/007	bolting	34	112073	32.64	1.00	b	b	b
102946/008	bolting	51	na	na	na	na	na	na
102946/009	bolting	31	112074	na	na	na	na	na
102946/010	bolting	45	112075	na	na	na	na	na
102946/011	bolting	31	112076	29.62	1.00	b	b	b
102946/012	bolting	31	112077	na	na	na	na	na
102946/013	bolting	45	112078	64.90	0.84	h	b	b
102946/014	bolting	45	na	na	na	na	na	na
102946/015	bolting	41	112079	77.86	0.73	na	h	na
102946/016	bolting	41	112080	na	na	na	na	na
102946/017	bolting	62	na	na	na	na	na	na
102946/018	bolting	41	112698	59.34	0.85	h	h	h
102946/019	bolting	51	112081	na	na	na	na	na
102946/020	bolting	45	112082	66.08	0.74	h	h	h
102946/021	bolting	37	112699	75.19	0.77	h	h	h
102946/022	bolting	41	112083	51.48	0.96	b	b	b
102946/023	bolting	41	112084	46.68	0.97	b	b	b
102946/024	bolting	37	112085	78.26	0.69	na	h	h
102946/025	bolting	41	112086	51.94	0.90	na	b	na
102946/026	bolting	41	112087	42.96	0.98	b	b	na
102946/027	bolting	41	112088	65.95	0.80	b	h	na
102946/028	bolting	41	112089	40.64	1.00	b	b	b
102946/029	bolting	62	na	na	na	na	na	na
102946/030	bolting	41	112090	54.21	0.85	b	h	h
102946/031	bolting	34	112091	35.76	0.98	b	b	b
102946/032	bolting	41	112092	na	na	na	na	na
102946/033	bolting	31	112093	36.13	0.99	b	b	b
102946/034	pwbr	>165	na	na	na	a	a	a
102946/035	bolting	45	112094	113.80	0.40	h	h	na
102946/036	bolting	45	112095	80.25	0.69	na	h	na

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean	F ₃ BR family mean	CAU 3839	CAU 3841	CAU 3846
102946/037	bolting	41	112096	36.67	1.00	b	b	b
102946/038	bolting	41	112097	36.72	1.00	b	b	b
102946/039	bolting	41	112098	na	na	na	na	na
102946/040	bolting	45	112099	na	na	na	na	na
102946/041	bolting	51	112100	na	na	na	na	na
102946/042	incb	65	na	na	na	a	a	a
102946/043	bolting	37	112101	44.12	0.92	b	b	b
102946/044	bolting	41	112700	na	na	na	na	na
102946/045	bolting	41	112701	33.60	1.00	na	b	na
102946/046	bolting	34	112102	30.22	1.00	b	b	b
102946/047	bolting	51	112103	na	na	na	na	na
102946/048	bolting	41	na	na	na	na	na	na
102946/049	bolting	45	112104	na	na	na	na	na
102946/050	bolting	37	112105	76.06	0.68	na	h	na
102946/051	bolting	51	112106	133.90	0.30	a	a	a
102946/052	incb	34	na	na	na	a	a	a
102946/053	bolting	65	na	na	na	na	na	na
102946/054	bolting	62	112107	159.83	0.06	a	a	a
102946/055	bolting	34	112108	na	na	na	na	na
102946/056	bolting	34	112109	35.19	1.00	b	b	b
102946/057	bolting	51	112110	107.82	0.46	h	h	h
102946/058	bolting	51	112111	85.70	0.70	h	h	h
102946/059	bolting	51	112112	94.40	0.60	h	h	h
102946/060	bolting	31	112113	47.94	0.89	b	b	b
102946/061	bolting	51	112114	144.25	0.19	h	a	a
102946/062	bolting	31	112115	50.67	0.84	h	b	b
102946/063	bolting	55	112116	162.49	0.05	a	a	a
102946/064	bolting	41	112117	63.84	0.82	na	h	na
102946/065	bolting	65	112118	163.50	0.00	a	a	a
102946/066	bolting	41	112119	67.66	0.79	na	h	h
102946/067	bolting	51	112120	na	na	na	na	na
102946/068	bolting	37	112121	86.34	0.72	h	h	h
102946/069	bolting	37	112122	na	na	na	na	na
102946/070	bolting	45	112123	118.94	0.40	na	h	h
102946/071	bolting	31	112702	35.61	0.98	na	na	na
102946/072	bolting	41	112124	80.86	0.75	h	h	na
102946/073	bolting	37	112125	86.29	0.68	h	h	h
102946/074	bolting	37	112126	69.48	0.75	h	h	h
102946/075	bolting	51	112127	na	na	na	na	na
102946/076	pwbr	>165	na	na	na	a	a	a
102946/077	bolting	31	112128	na	na	na	na	na
102946/078	bolting	34	112129	56.59	0.82	h	h	h
102946/079	pwbr	>165	na	na	na	a	a	a
102946/080	bolting	134	na	na	na	a	a	na

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean	F ₃ BR family mean	CAU 3839	CAU 3841	CAU 3846
102946/081	bolting	37	112130	107.12	0.47	b	a	na
102946/082	bolting	41	112131	57.22	0.83	h	h	h
102946/083	bolting	62	112132	na	na	na	na	na
102946/084	bolting	37	112133	na	na	na	na	na
102946/085	bolting	41	112134	49.27	0.92	h	b	b
102946/086	bolting	51	112135	na	na	na	na	na
102946/087	bolting	51	112703	133.46	0.30	na	a	a
102946/088	bolting	55	112136	na	na	na	na	na
102946/089	bolting	55	112137	na	na	na	na	na
102946/090	bolting	31	112138	45.16	0.89	b	b	b
102946/091	bolting	41	112139	34.02	1.00	b	b	b
102946/092	bolting	37	112140	na	na	na	na	na
102946/093	bolting	76	na	na	na	na	na	na
102946/094	bolting	41	112141	45.59	0.92	h	b	b
102946/095	bolting	45	112142	71.76	0.73	h	b	b
102946/096	bolting	37	112143	na	na	na	na	na
102946/097	bolting	31	112144	35.70	1.00	b	b	b
102946/098	bolting	51	112145	na	na	na	na	na
102946/099	incb	65	na	na	na	h	na	h
102946/100	bolting	45	112704	na	na	na	na	na
102946/101	bolting	41	112146	73.35	0.79	h	h	h
102946/102	pwbr	>165	na	na	na	a	a	a
102946/103	bolting	31	112147	na	na	na	na	na
102946/104	bolting	31	112148	na	na	na	na	na
102946/105	bolting	31	112149	35.94	0.98	h	b	b
102946/106	bolting	45	112150	na	na	na	na	na
102946/107	bolting	37	112151	na	na	na	na	na
102946/108	bolting	34	112152	na	na	na	na	na
102946/109	pwbr	>165	124278	na	na	a	a	a
102946/110	bolting	37	112153	na	na	na	na	na
102946/111	bolting	41	112154	na	na	na	na	na
102946/112	bolting	62	112155	na	na	na	na	na
102946/113	incb	45	na	na	na	na	na	na
102946/114	bolting	37	112156	35.91	1.00	b	b	na
102946/115	bolting	45	112157	113.58	0.46	b	h	na
102946/116	bolting	37	112158	na	na	na	na	na
102946/117	bolting	31	112159	na	na	na	na	na
102946/118	bolting	41	112160	43.86	0.98	b	b	b
102946/119	bolting	51	na	na	na	na	na	na
102946/120	bolting	37	112161	60.98	0.80	h	na	na
102946/121	bolting	62	112162	na	na	na	na	na
102946/122	bolting	41	112163	52.57	0.92	b	b	na
102946/123	bolting	41	112164	na	na	na	na	na

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean	F ₃ BR family mean	CAU 3839	CAU 3841	CAU 3846
102946/124	incb	62	na	na	na	na	na	na
102946/125	bolting	41	112705	na	na	na	na	na
102946/126	bolting	51	112165	92.26	0.63	h	h	h
102946/127	bolting	31	112166	31.38	1.00	h	h	na
102946/128	bolting	51	112167	na	na	na	na	na
102946/129	bolting	34	112706	na	na	na	na	na
102946/130	bolting	45	112168	na	na	na	na	na
102946/131	bolting	31	112169	na	na	na	na	na
102946/133	incb	65	124279	na	na	na	na	na
102946/134	pwbr	>165	na	na	na	a	a	a
102946/135	bolting	45	112170	54.17	0.89	h	h	na
102946/136	bolting	51	112171	na	na	na	na	na
102946/137	bolting	76	na	na	na	na	na	na
102946/138	bolting	45	112172	101.69	0.58	h	h	na
102946/139	bolting	41	112173	na	na	na	na	na
102946/140	bolting	45	112174	52.87	0.86	h	h	h
102946/141	bolting	37	112175	58.94	0.82	h	h	h
102946/142	bolting	41	112176	na	na	na	na	na
102946/143	bolting	45	112177	73.34	0.81	h	h	h
102946/144	bolting	45	112178	52.52	0.90	h	a	a
102946/145	bolting	37	112179	na	na	na	na	na
102946/146	bolting	41	112180	na	na	na	na	na
102946/147	bolting	41	112181	96.11	0.58	h	h	na
102946/148	bolting	34	112707	na	na	na	na	na
102946/149	pwbr	>165	na	na	na	a	a	a
102946/150	bolting	34	112708	na	na	na	na	na
102946/151	incb	65	na	na	na	na	na	na
102946/152	bolting	31	112182	28.00	1.00	b	b	na
102946/153	bolting	31	112183	51.01	0.89	b	b	na
102946/154	bolting	55	na	na	na	na	na	na
102946/155	bolting	34	112184	na	na	na	na	na
102946/156	bolting	41	112185	na	na	na	na	na
102946/157	bolting	34	112186	na	na	na	na	na
102946/158	pwbr	>165	na	na	na	a	a	a
102946/159	bolting	45	112187	84.24	0.69	h	h	na
102946/160	bolting	41	112188	76.11	0.71	h	h	h
102946/161	bolting	45	112189	na	na	na	na	na
102946/162	bolting	62	112190	na	na	na	na	na
102946/163	bolting	62	na	na	na	na	na	na
102946/164	bolting	76	na	na	na	na	na	na
102946/165	bolting	45	112191	119.08	0.44	h	h	h
102946/166	pwbr	>165	na	na	na	a	a	a
102946/167	bolting	37	112192	67.58	0.75	na	h	h
102946/168	bolting	62	112193	na	na	na	na	na

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean	F ₃ BR family mean	CAU 3839	CAU 3841	CAU 3846
102946/169	bolting	45	112709	92.15	0.73	h	h	na
102946/170	incb	55	na	na	na	na	na	na
102946/171	bolting	34	na	na	na	na	na	na
102946/172	bolting	41	112710	na	na	na	na	na
102946/173	bolting	37	112711	na	na	na	na	na
102946/174	bolting	41	112194	95.26	0.59	h	a	h
102946/175	bolting	34	112195	na	na	na	na	na
102946/176	bolting	41	112196	na	na	na	na	na
102946/177	bolting	41	na	na	na	na	na	na
102946/178	bolting	37	112197	70.13	0.82	h	b	h
102946/179	bolting	34	112198	68.34	0.73	na	h	na
102946/180	bolting	37	112199	40.62	1.00	h	h	h
102946/181	bolting	37	112200	na	na	na	na	na
102946/182	bolting	45	na	na	na	na	na	na
102946/183	bolting	55	112201	na	na	na	na	na
102946/184	bolting	37	112202	90.36	0.71	h	h	h
102946/185	bolting	34	112203	na	na	na	na	na
102946/186	bolting	45	112204	109.67	0.55	h	h	na
102946/187	bolting	34	112205	na	na	na	na	na
102946/188	bolting	45	112206	147.29	0.19	a	a	na
102946/189	bolting	41	112207	55.97	0.88	h	h	h
102946/190	bolting	45	112208	70.21	0.81	h	h	h
102946/191	bolting	37	112209	74.18	0.76	h	h	h
102946/192	bolting	34	na	na	na	na	na	na
102946/193	bolting	55	112210	163.50	0.00	na	a	a
102946/194	bolting	41	112211	89.69	0.62	h	h	h
102946/195	bolting	51	na	na	na	na	na	na
102946/196	bolting	34	112212	38.44	0.98	b	b	na
102946/197	bolting	37	112712	na	na	na	na	na
102946/198	bolting	41	112213	101.71	0.50	h	h	h
102946/199	bolting	41	112713	na	na	na	na	na
102946/200	bolting	31	112214	na	na	na	na	na
102946/201	bolting	41	112714	na	na	na	na	na
102946/202	bolting	41	112215	na	na	na	na	na
102946/203	bolting	37	112216	41.13	0.99	h	h	h
102946/204	bolting	51	112217	129.55	0.28	a	a	na
102946/205	bolting	41	112715	na	na	na	na	na
102946/206	bolting	45	112218	60.56	0.84	h	na	h
102946/207	bolting	55	na	na	na	na	na	na
102946/208	bolting	45	112219	58.79	0.89	h	h	h
102946/209	bolting	62	na	na	na	na	na	na
102946/210	bolting	62	na	na	na	na	na	na
102946/211	bolting	45	112220	99.40	0.53	h	na	h

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean	F ₃ BR family mean	CAU 3839	CAU 3841	CAU 3846
102946/212	bolting	45	112221	na	na	na	na	na
102946/213	bolting	34	112222	25.72	1.00	b	b	na
102946/214	bolting	34	112716	42.00	0.92	b	b	b
102946/215	bolting	41	112717	na	na	na	na	na
102946/216	pwbr	>165	na	na	na	a	a	a
102946/217	bolting	45	112223	na	na	na	na	na
102946/218	bolting	37	112224	na	na	na	na	na
102946/219	bolting	34	112225	na	na	na	na	na
102946/220	bolting	41	112226	na	na	na	na	na
102946/221	pwbr	>165	na	na	na	a	a	na
102946/222	bolting	41	112227	63.64	0.79	h	h	h
102946/223	bolting	45	112228	76.36	0.70	h	h	h
102946/224	incb	62	na	na	na	na	na	na
102946/225	bolting	31	112229	na	na	na	na	na
102946/226	bolting	45	112230	49.88	0.91	na	h	na
102946/227	bolting	41	112231	58.93	0.88	h	h	h
102946/228	bolting	51	na	na	na	na	na	na
102946/229	bolting	51	112232	126.68	0.32	a	a	a
102946/230	bolting	41	112233	59.17	0.80	h	h	na
102946/231	bolting	41	na	na	na	na	na	na
102946/232	bolting	45	112718	na	na	na	na	na
102946/233	bolting	41	112234	na	na	na	na	na
102946/234	bolting	41	112235	na	na	na	na	na
102946/235	bolting	41	112236	na	na	na	na	na
102946/236	pwbr	>165	na	na	na	h	a	a
102946/237	bolting	41	112237	na	na	na	na	na
102946/238	bolting	55	112238	111.74	0.57	a	a	a
102946/239	bolting	31	112239	na	na	na	na	na
102946/240	bolting	55	112240	na	na	na	na	na
102946/241	pwbr	>165	na	na	na	na	na	na
102946/242	bolting	31	112241	29.99	1.00	b	h	b
102946/243	bolting	37	112242	na	na	na	na	na
102946/244	bolting	41	112243	na	na	na	na	na
102946/245	bolting	41	112244	64.31	0.78	h	h	h
102946/246	bolting	41	112245	na	na	na	na	na
102946/247	bolting	41	112246	na	na	na	na	na
102946/248	bolting	31	112247	52.92	0.88	b	b	na
102946/249	bolting	62	112248	144.42	0.25	a	a	a
102946/250	bolting	31	112249	46.08	0.92	b	b	b
102946/251	bolting	37	112250	91.23	0.64	h	h	na
102946/252	bolting	48	na	na	na	na	na	na
102946/253	bolting	41	112251	na	na	na	na	na
102946/254	bolting	48	112252	85.95	0.64	h	h	h
102946/255	bolting	41	112253	64.79	0.78	h	h	h

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean	F ₃ BR family mean	CAU 3839	CAU 3841	CAU 3846
102946/256	bolting	51	na	na	na	na	na	na
102946/257	bolting	51	112254	52.63	0.88	na	h	na
102946/258	bolting	41	112255	55.60	0.92	h	h	h
102946/259	bolting	41	112256	41.41	1.00	h	h	h
102946/260	incb	41	na	na	na	na	a	a
102946/261	bolting	37	112257	74.83	0.73	na	na	na
102946/262	bolting	41	112258	64.53	0.80	h	h	na
102946/263	bolting	37	112259	na	na	na	na	na
102946/264	bolting	41	112719	na	na	na	na	na
102946/265	bolting	37	112720	43.50	1.00	b	b	b
102946/266	bolting	37	112721	58.34	0.83	h	b	b
102946/267	bolting	41	112260	50.56	0.92	h	h	h
102946/268	bolting	34	112261	70.81	0.77	h	h	na
102946/269	bolting	51	na	na	na	na	na	na
102946/270	bolting	34	112262	38.17	1.00	h	b	b
102946/271	bolting	45	112263	84.81	0.66	h	h	h
102946/272	bolting	41	112264	na	na	na	na	na
102946/273	bolting	41	112265	na	na	na	na	na
102946/274	bolting	34	112266	57.24	0.83	h	h	na
102946/275	bolting	37	112267	46.83	0.90	na	h	h
102946/276	bolting	37	112268	31.77	1.00	na	b	na
102946/277	bolting	41	112269	na	na	na	na	na
102946/278	bolting	41	112270	80.17	0.66	na	h	h
102946/279	incb	65	na	na	na	na	na	na
102946/280	bolting	51	na	na	na	na	na	na
102946/281	bolting	37	112271	75.46	0.69	h	h	h
102946/282	bolting	41	112272	na	na	na	na	na
102946/283	bolting	65	112273	163.50	0.00	na	a	a
102946/284	bolting	41	112722	na	na	na	na	na
102946/285	incb	83	na	na	na	na	na	na
102946/286	bolting	45	112274	75.69	0.78	na	b	b
102946/287	bolting	41	112275	83.68	0.72	h	h	h
102946/288	bolting	45	112276	141.99	0.24	na	a	na
102946/289	bolting	37	112277	na	na	na	na	na
102946/290	bolting	37	112723	na	na	na	na	na
102946/291	bolting	41	112278	na	na	na	na	na
102946/292	bolting	41	112279	81.04	0.64	h	a	a
102946/293	pwbr	>165	124280	na	na	a	a	a
102946/294	bolting	41	112724	na	na	na	na	na
102946/295	bolting	76	na	na	na	na	na	na
102946/296	bolting	45	112280	na	na	na	na	na
102946/297	pwbr	>165	124281	na	na	h	a	na
102946/298	incb	65	na	na	na	na	na	na

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean	F ₃ BR family mean	CAU 3839	CAU 3841	CAU 3846
102946/299	bolting	55	na	na	na	na	na	na
102946/300	bolting	45	112281	na	na	na	na	na
102946/301	bolting	45	112282	86.01	0.70	na	h	h
102946/302	bolting	51	na	na	na	na	na	na
102946/303	bolting	41	112283	na	na	na	na	na
102946/304	bolting	41	112725	96.46	0.54	h	h	h
102946/305	bolting	45	112726	39.06	0.94	h	b	b
102946/306	bolting	37	112284	25.50	1.00	b	b	b
102946/307	bolting	37	112727	80.12	0.68	h	h	h
102946/308	bolting	37	112728	61.70	0.81	h	h	h
102946/309	bolting	55	112285	114.30	0.49	a	a	na
102946/310	bolting	45	112729	na	na	na	na	na
102946/311	bolting	37	112286	26.24	1.00	b	b	b
102946/312	bolting	51	na	na	na	na	na	na
102946/313	pwbr	>165	na	na	na	h	h	h
102946/314	pwbr	>165	na	na	na	a	a	a
102946/315	bolting	34	112287	86.58	0.63	h	h	b
102946/316	bolting	37	112288	na	na	na	na	na
102946/317	pwbr	>165	124282	na	na	h	a	a
102946/318	bolting	41	112289	na	na	na	na	na
102946/319	bolting	34	112290	na	na	na	na	na
102946/320	bolting	45	112730	na	na	na	na	na
102946/321	incb	104	na	na	na	na	na	na
102946/322	bolting	41	112731	na	na	na	na	na
102946/323	bolting	31	112291	35.59	0.97	na	b	b
102946/324	bolting	37	112292	42.48	0.94	na	h	h
102946/325	bolting	51	na	na	na	na	na	na
102946/326	bolting	41	112293	64.52	0.84	h	h	h
102946/327	bolting	41	112294	78.59	0.70	na	h	na
102946/328	bolting	41	112295	44.50	0.92	na	b	b
102946/329	bolting	41	112296	68.83	0.81	na	h	h
102946/330	bolting	41	na	na	na	na	na	na
102946/331	bolting	41	112297	80.69	0.65	h	h	h
102946/332	bolting	34	112298	na	na	na	na	na
102946/333	bolting	51	na	na	na	na	na	na
102946/334	bolting	41	112299	40.27	0.97	h	b	b
102946/335	bolting	41	112732	na	na	na	na	na
102946/336	bolting	37	112300	53.37	0.85	h	h	na
102946/337	bolting	51	112301	71.21	0.79	a	a	na
102946/338	pwbr	>165	na	na	na	a	na	a
102946/339	bolting	62	112302	na	na	na	na	na
102946/340	bolting	48	112303	109.48	0.51	h	h	na
102946/341	bolting	51	na	na	na	na	na	na
102946/342	bolting	41	112304	na	na	na	na	na

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean	F ₃ BR family mean	CAU 3839	CAU 3841	CAU 3846
102946/343	bolting	31	112733	na	na	na	na	na
102946/344	incb	83	na	na	na	na	a	na
102946/345	incb	65	na	na	na	na	na	na
102946/346	bolting	41	112305	na	na	na	na	na
102946/347	bolting	34	na	na	na	na	na	na
102946/348	bolting	37	112306	103.98	0.50	na	h	na
102946/349	bolting	37	112307	98.64	0.57	h	h	h
102946/350	bolting	45	112308	77.40	0.77	b	h	h
102946/351	bolting	45	112309	63.55	0.77	b	h	na
102946/352	bolting	31	112310	na	na	na	na	na
102946/353	bolting	41	112734	na	na	na	na	na
102946/354	bolting	37	112735	41.02	1.00	b	b	na
102946/355	bolting	48	112311	101.62	0.51	h	h	h
102946/356	incb	83	na	na	na	na	na	na
102946/357	pwbr	>165	na	na	na	a	a	na
102946/358	pwbr	>165	na	na	na	a	a	a
102946/359	bolting	34	112312	45.35	1.00	b	b	na
102946/360	bolting	45	112313	na	na	na	na	na
102946/361	bolting	41	112314	81.49	0.74	b	na	b
102946/362	bolting	55	112315	150.46	0.16	a	a	na
102946/363	bolting	37	112736	74.92	0.75	a	h	h
102946/364	incb	51	na	na	na	na	na	na
102946/365	pwbr	>165	na	na	na	a	a	a
102946/366	incb	51	na	na	na	na	na	na
102946/367	bolting	51	112737	141.81	0.18	a	a	a
102946/368	bolting	31	112316	48.02	0.96	b	b	na
102946/369	bolting	45	112317	82.46	0.72	b	a	na
102946/370	bolting	37	112318	na	na	na	na	na
102946/371	bolting	37	na	na	na	na	na	na
102946/372	bolting	34	112319	27.53	1.00	b	b	na
102946/373	incb	111	na	na	na	na	na	na
102946/374	bolting	37	112320	39.58	0.98	na	b	b
102946/375	bolting	41	112738	49.23	0.95	b	na	h
102946/376	bolting	41	112321	78.86	0.71	h	h	na
102946/377	bolting	41	112322	na	na	na	na	na
102946/378	bolting	31	112323	36.40	1.00	na	b	b
102946/379	bolting	34	112324	103.17	0.50	h	a	na
102946/380	bolting	37	112325	na	na	na	na	na
102946/381	bolting	41	112739	na	na	na	na	na
102946/382	bolting	37	112326	36.53	1.00	b	na	na
102946/383	bolting	31	112327	31.02	1.00	na	h	na
102946/384	bolting	41	na	na	na	na	na	na
102946/385	bolting	55	na	na	na	na	na	na

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean	F ₃ BR family mean	CAU 3839	CAU 3841	CAU 3846
102946/386	bolting	45	112328	67.47	0.81	h	h	na
102946/387	bolting	37	112329	53.65	0.92	h	h	na
102946/388	bolting	31	112330	na	na	na	na	na
102946/389	bolting	62	112331	na	na	na	na	na
102946/390	bolting	48	112332	na	na	na	na	na
102946/391	bolting	34	112333	28.78	1.00	h	b	na
102946/392	bolting	45	112334	na	na	na	na	na
102946/393	pwbr	>165	na	na	na	a	a	a
102946/394	bolting	41	112335	na	na	na	na	na
102946/395	bolting	41	112336	48.38	0.92	h	h	na
102946/396	bolting	41	112337	na	na	na	na	na
102946/397	bolting	62	na	na	na	na	na	na
102946/398	pwbr	>165	na	na	na	a	a	a
102946/399	bolting	41	112338	75.42	0.75	b	h	na
102946/400	bolting	41	112339	43.79	0.96	b	b	b
102946/401	bolting	51	112340	na	na	na	na	na
102946/402	bolting	41	112341	na	na	na	na	na
102946/403	bolting	51	112342	na	na	na	na	na
102946/404	bolting	34	112740	24.67	1.00	h	b	b
102946/405	bolting	41	112343	64.76	0.79	na	h	h
102946/406	pwbr	>165	na	na	na	a	na	a
102946/407	bolting	41	112344	na	na	na	na	na
102946/408	bolting	41	112345	na	na	na	na	na
102946/409	pwbr	>165	na	na	na	na	na	a
102946/410	bolting	34	112741	41.07	0.98	na	na	na

Supplementary Table 4: Sequence derived markers used for construction of the genetic map of F₂ population 102946. Beside marker names, map position, primer combinations, PCR fragment sizes, annealing temperature and in case of CAPS markers the restriction enzymes are listed. ‘TD’: touch-down PCR protocol was used. For primer sequence information see **Supplementary Table 5**.

Marker name	Map position	Primer combination	PCR fragment size seed parent	PCR fragment size pollinator	Mapping technique	Annealing temperature (°C)	Reference
CAU 3793	6	N133 N134	445 bp + 360 bp	805 bp	CAPS, <i>BpiI</i>	61	<i>BvSVP</i> , N. Dally
CAU 3794	1	N313 N314	163 bp	177 bp	InDel	56	scaffold00041 (RefBeet 0.9)
CAU 3795	1	N197 N198	163 bp	151 bp	InDel	55	scaffold00039 (RefBeet 0.9)
CAU 3797	2	A245 A245	695 bp	496 bp + 199 bp	CAPS, <i>AluI</i>	58	<i>BvCOL1</i> , A.Müller
CAU 3798	2	N241 N242	128 bp	152 bp	InDel	56	scaffold00074 (RefBeet 0.9)
CAU 3801	3	N319 N320	132 bp	157 bp	InDel	57	scaffold00113 (RefBeet 0.9)
CAU 3802	4	N203 N204	71 bp	254 bp	InDel	54	scaffold00054 (RefBeet 0.9)
CAU 3805	4	N051 N052	126 bp + 93 bp	219 bp	CAPS, <i>HpaII</i>	59	<i>BvFT2</i> , S. Frerichmann
CAU 3806	4	N237 N238	220 bp	235 bp	InDel	57	scaffold00191 (RefBeet 0.9)
CAU 3807	4	N269 N270	141 bp	132 bp	InDel	56	scaffold00917 (RefBeet 0.9)
CAU 3808	4	N249 N250	113 bp	122 bp	InDel	58	scaffold00357 (RefBeet 0.9)
CAU 3809	5	N317 N318	134 bp	154 bp	InDel	58	scaffold00252 (RefBeet 0.9)
CAU 3810	5	N205 N206	254 bp	265 bp	InDel	60	scaffold00326 (RefBeet 0.9)
CAU 3811	5	N283 N284	130 bp	138 bp	InDel	57	scaffold00006 (RefBeet 0.9)
CAU 3812	5	N233 N234	162 bp	141 bp	InDel	58	scaffold00017 (RefBeet 0.9)
CAU 3813	5	N263 N264	175 bp	196 bp	InDel	57	scaffold00060 (RefBeet 0.9)
CAU 3814	6	N207 N208	170 bp	190 bp	InDel	53	scaffold00022 (RefBeet 0.9)
CAU 3815	6	N291 N292	161 bp	172 bp	InDel	55	scaffold00050 (RefBeet 0.9)
CAU 3816	7	N277 N278	180 bp	196 bp	InDel	53	scaffold00202 (RefBeet 0.9)
CAU 3817	7	N275 N276	218 bp	232 bp	InDel	57	scaffold00008 (RefBeet 0.9)
CAU 3818	7	N253 N254	346 bp	366 bp	InDel	58	scaffold00023 (RefBeet 0.9)

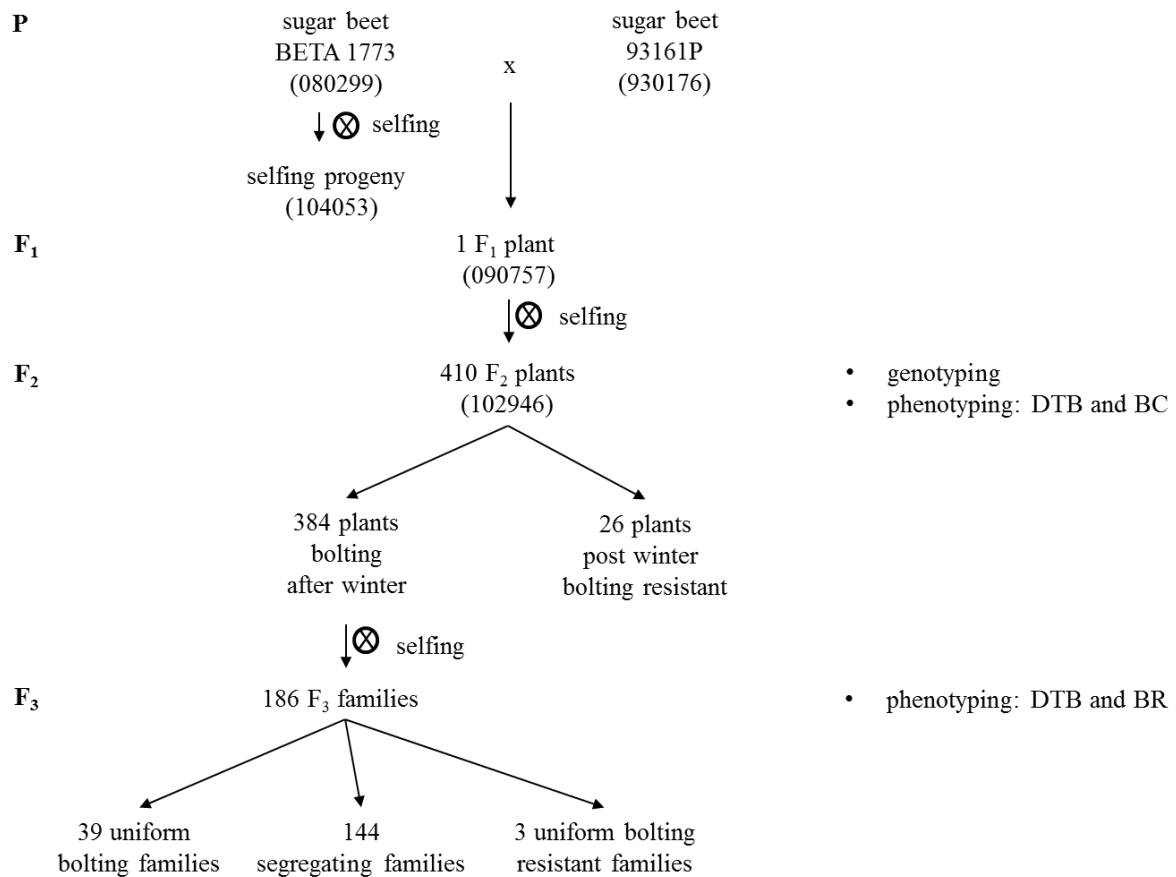
Marker name	Map position	Primer combination	PCR fragment size seed parent	PCR fragment size pollinator	Mapping technique	Annealing temperature (°C)	Reference
CAU 3820	5	N257 N258	130 bp	147 bp	InDel	58	scaffold00002 (RefBeet 0.9)
CAU 3821	8	N251 N252	198 bp	212 bp	InDel	57	scaffold00101 (RefBeet 0.9)
CAU 3823	7	N115 N116	~240 bp	~170 bp	InDel	59	BQ589879
CAU 3824	7	K195 K195	642 bp	460 bp + 182 bp	CAPS	57	GS923113, M. Kirchhoff
CAU 3825	6	K899 K900	893 bp	597 bp + 296 bp	CAPS, <i>VspI</i>	57	GS923346, M. Kirchhoff
CAU 3826	4	K235 K236	321 bp + 299 bp	620 bp	CAPS, <i>Alw26I</i>	59	GS923128, M. Kirchhoff
CAU 3828	4	N081 N082	253 bp + 142 bp	395 bp	CAPS, <i>BseDI</i>	60	BQ586741
CAU 3832	9	N297 N298	154 bp	167 bp	InDel	58	scaffold00281 (RefBeet 0.9)
CAU 3833	9	N299 N300	169 bp	182 bp	InDel	52	scaffold00037 (RefBeet 0.9)
CAU 3835	9	N149 N150	670 bp + 205 bp	875 bp	CAPS, <i>BsuRI</i>	58	<i>BvFT1</i> , scaffold00007 (RefBeet 0.9)
CAU 3836	9	N281 N282	203 bp	224 bp	InDel	55	scaffold00100 (RefBeet 0.9)
CAU 3837	9	N229 N230	195 bp	179 bp	InDel	57	scaffold00007 (RefBeet 0.9)
CAU 3838	9	N279 N280	159 bp	141 bp	InDel	56	scaffold00167 (RefBeet 0.9)
CAU 3839	9	N247 N248	110 bp	128 bp	InDel	55	scaffold00013 (RefBeet 0.9)
CAU 3840	9	G646 G647	777 bp	829 bp	InDel	58	TG_E0140, G. Capistrano
CAU 3841	9	A920 A921	393 bp + 246 bp	639 bp	CAPS, <i>Eco31I</i>	58	<i>BvGa3ox1</i> , scaffold00076 (RefBeet 0.9)
CAU 3842	9	B017 B018	~350 bp	~330 bp	SSR	TD	FDSB1001, B. Büttner
CAU 3843	9	N305 N306	115 bp	131 bp	InDel	58	scaffold00197 (RefBeet 0.9)
CAU 3844	9	N331 N332	182 bp	209 bp	InDel	58	scaffold00359 (RefBeet 0.9)
CAU 3845	9	N333 N334	172 bp	195 bp	InDel	57	scaffold00359 (RefBeet 0.9)
CAU 3846	9	N329 N330	109 bp	109 bp + 101 bp	InDel	57	scaffold00076 (RefBeet 0.9)
CAU 3847	9	N335 N336	213 bp	189 bp	InDel	57	scaffold00359 (RefBeet 0.9)

Supplementary Table 5: Primer used for PCR in order to generate sequence derived marker information. Primer sequences are displayed in 5' → 3' orientation.

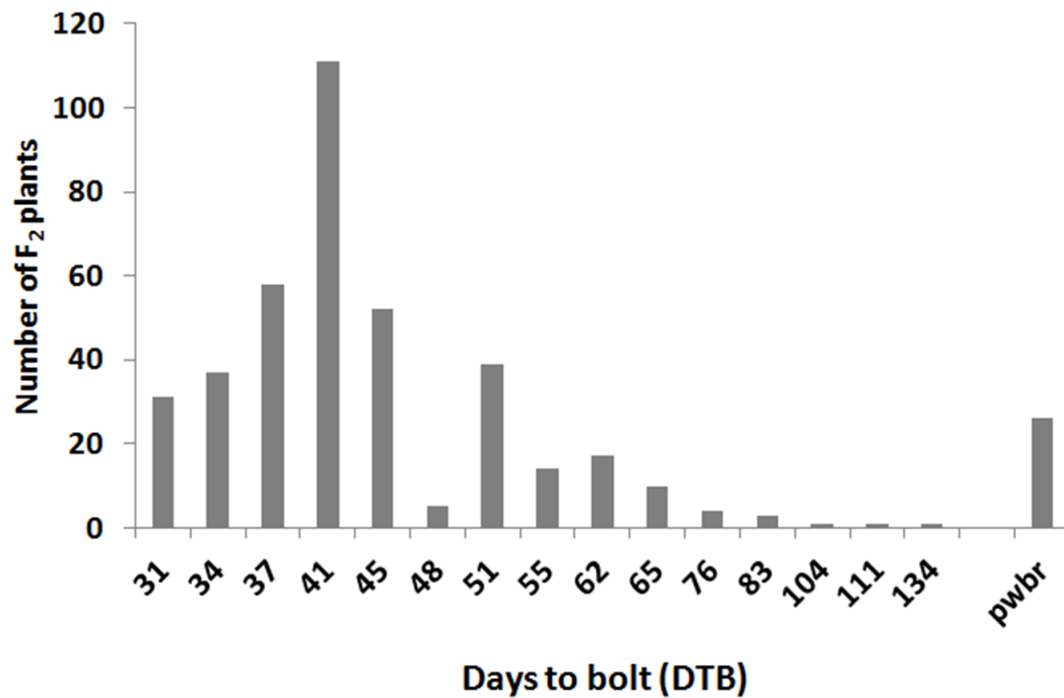
Primer name	Sequence (5' → 3')
A245	GTACAATCTAAAGCCACTC
A245	CTCCTTCTCTCATTGGAACC
A920	ACCCGAGAGCACAACTTAT
A921	TTTATTTTCTTGTGTAGTGGACCAT
B017	ACTTCAACCACTATCACAAAGTGAG
B018	ATCTTATGCTGCCATGACCA
G646	CCTCTCTTTCTCTCTCTGCAAC
G647	ACACTGCAACACGATCAGG
K195	GGTGAACTTACCAACTTGAAC
K195	CGGTTATGAAACAAGTTGAGG
K235	CATTGAGAGCAGAAACCACTC
K236	CCAGGGACA ACTACTCTTTAG
K899	TGGCTTTTGATGAGAGGTTC
K900	ACCCTGGAGCTTTGTTATC
N051	GATGCAACATGAGATGCGATG
N052	GATCTCTTGGTGCTCTAGGC
N081	GACGACTGTTCAAGGACTGAAG
N082	GAATACATTCCACACTACTACCAC
N115	GGTTCGCCGTAAAAGCTCG
N116	GCTTTGGA ACTTCGGCGTG
N133	GA ACTTCGCCACTCATCCTTAC
N134	CTTGGATGCATGGCTTAAAGGC
N149	GATCCAGTAGTTTTGGGTCTC
N150	GAACGATATTTGGTCAGATAGG
N197	GATGCAAGAGTGTGGTAAC
N198	CATCTGACTCATCAATACCC
N203	GCATCGAGACGAATCTAAC
N204	GTGATCTCATGCAACATATG
N205	GTAGTCTGCTGTGTCAAGTG
N206	GAGACATTCCATCTCACGCAG
N207	CGTTTGCAAATGATTGAGTC
N208	GTTACCTTTGCCTAATTGAG
N229	GAGCAAAGTGTTTCATGTTCCC
N230	CTTAAGTACCTGTGTTAGATCC
N233	CTATGGTCAAACAGGCTGAAG
N234	GTTATCTTCATGAAGCGTGCC
N237	CCAAAAGAAAACCCTGTCCTG
N238	CCATTTGCTTCTTCTGCC
N241	CTTCGTCTGAGTTAATCTGC
N242	CAATGTCACCAACACACTAAC
N247	CATTATGGAACGGAGGAAG
N248	CCCCTGTAGGTCTTTAAG

Primer name	Sequence (5' → 3')
N249	GTTGGGTCATTTGGAATTAGTG
N250	GGTTCATGGTACAACCTGTTTCG
N251	CTTCCATATGCTGATTGCATG
N252	CACCATTTGAGTCATCAGCG
N253	CAAATGTCATGCACCAATGAAC
N254	CTCAGAACTGATTCCAGGATC
N257	CCCTGTGAAATCAGTCATTATC
N258	GGGTAGTACGGTAGCCTAG
N263	GGAATTGCTGTATACCCTGG
N264	CACATGGTACACTCGAGTC
N269	CCAGAAGCGCCTCAATTAC
N270	CTTTAATGGCGCTTCTTCC
N275	GGAGTCAGCCTAAAATATCC
N276	GGTGTCACTCCCATGTTTC
N277	GATCATCTCGTTTCAAGTC
N278	GTTGGCAGAGTATMTTTCG
N279	CCTTGCAGAACTATAATCCAC
N280	GTTCTTTTGATGACCCAACG
N281	GGTCATCACAGGATCAATAG
N282	CTCGTTCCATTGACTATTCG
N283	CTCAGATGCAAGAGGCAAAC
N284	GTCCTTTGTTGCTTTCTTAGG
N291	GGAATCCAAAGCTCCTATAG
N292	GCTATCCCTTGGCAATAATG
N297	GCAACAGTAGCTACAACAGC
N298	CTACAGGTGTGCAATAAGACG
N299	GAATTAGGGAGTTAGCATC
N300	CTAACCCTAGTTTCGTC
N305	GACATAAAATCGGAAGGGTGG
N306	GTGACACAGTGAAAAGGATCC
N313	CGATGGCTAGTACCCTAG
N314	CCTTGAGTCTWAATTGCATCG
N317	CCTTTCATGCTAGTCTAGCG
N318	CATAAGTAGCATGGAACCCTC
N319	GGGACCAAACCTGTTTTTC
N320	CCAACCTGGTCACTCTATGTC
N329	GCTGACTTAGATCACTTTCTTC
N330	CTTTCAAACCTCTATGCAGTCAG
N331	CATGTCGATTACTCCCAAAGC
N332	CAGTTTGAGCATCCGACTTTC
N333	CTTGGAGRTTKTGTAACCGC
N334	GCATTTAACTTGGAAGGATGG
N335	GCACGTTCTCGTTTTAGTTTGC
N336	CGTTTCCAGAAGTCATCGTTG

7.1.2 Supplementary figures for chapter 2



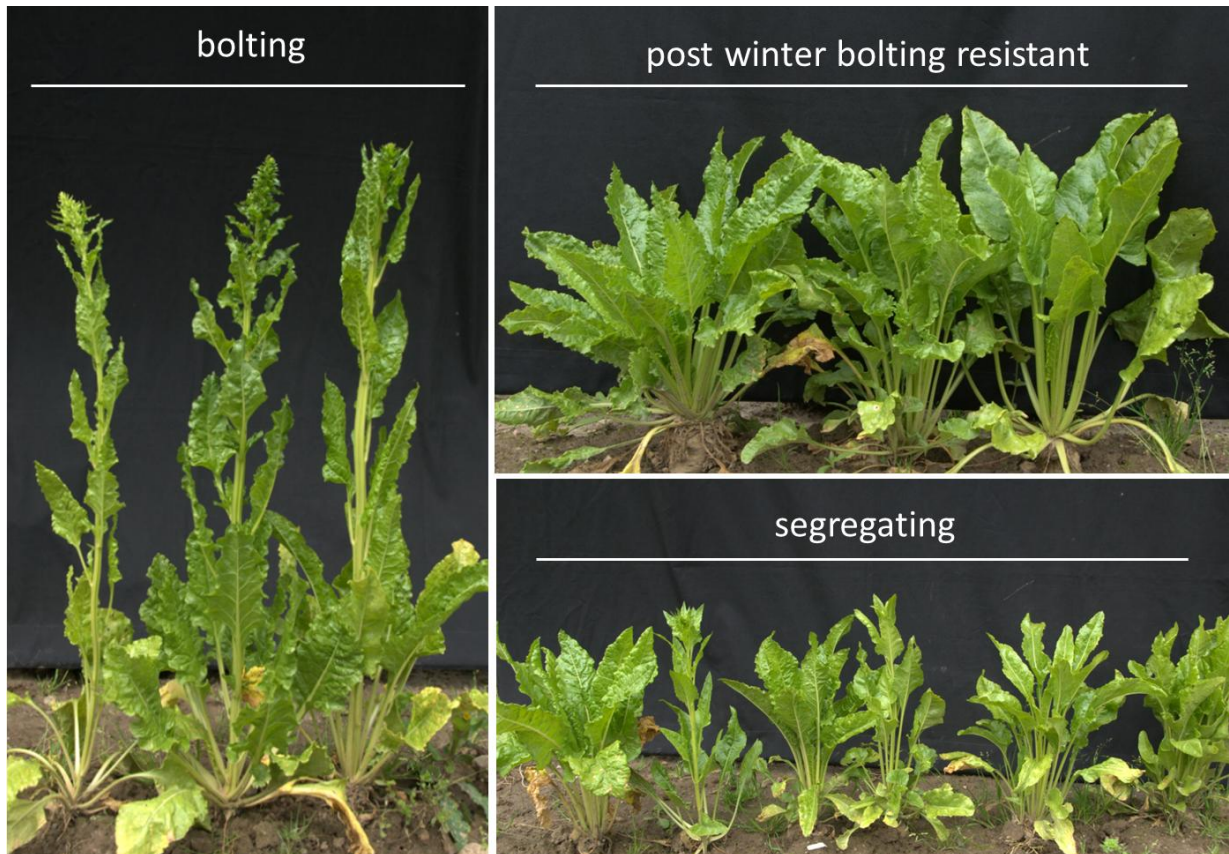
Supplementary Figure 1: Development of F₂ and F₃ populations segregating for bolting behavior. F₂ plants were derived from a cross of sugar beet accessions BETA 1773 and 93161P. F₃ families were derived by selfing of F₂ plants. F₂ plants were phenotyped for DTB, which are defined as days to bolting after the end of artificial cold treatment and for BC (bolting code; 0: post winter bolting resistant; 1: bolting after winter). F₃ families were phenotyped for DTB and post winter bolting rate (BR). 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). Bolting rate was recorded at October 15, 2012. Internal seed codes are shown in brackets. During F₃ phenotyping parental accessions (104053 and 930176) were carried along the experiments as controls.



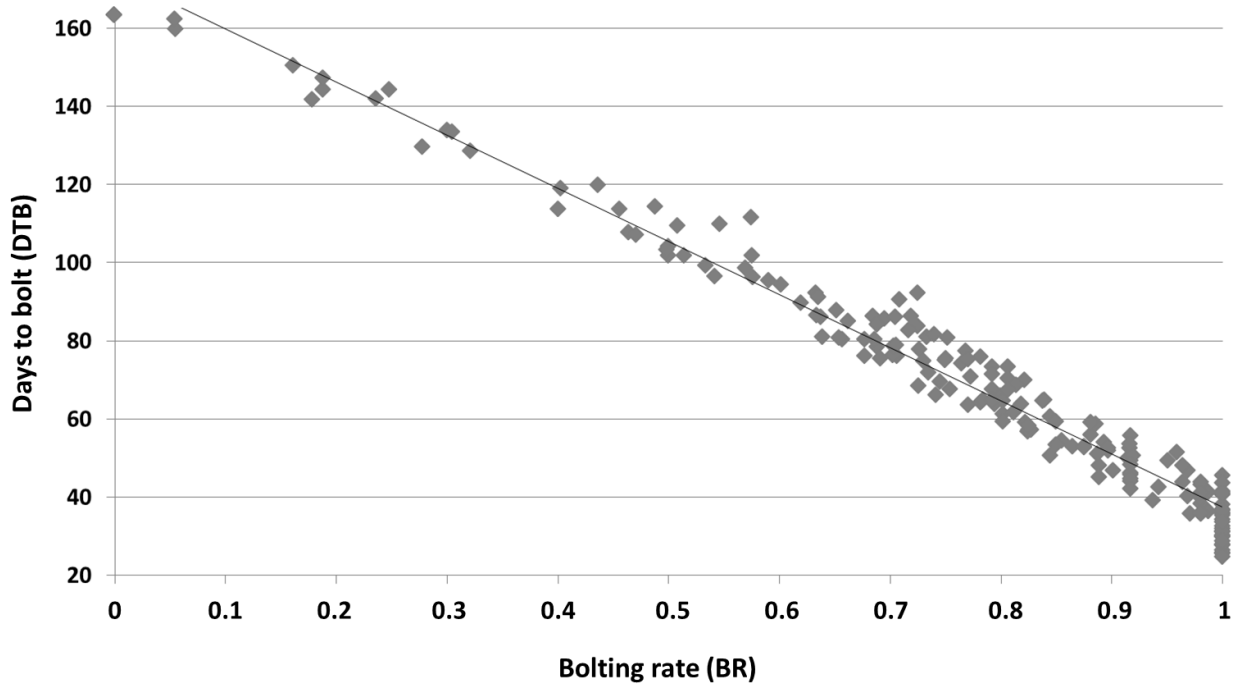
Supplementary Figure 2: Phenotypic variation for bolting time after cold treatment in the F₂ population. Days to bolt (DTB) are defined as at first visible elongation of main stem (BBCH 51). Population size n=410; sowing date: November 1, 2010; planting to the field: April 19, 2011. Plants that are post-winter bolting resistant are designated by pwbr.



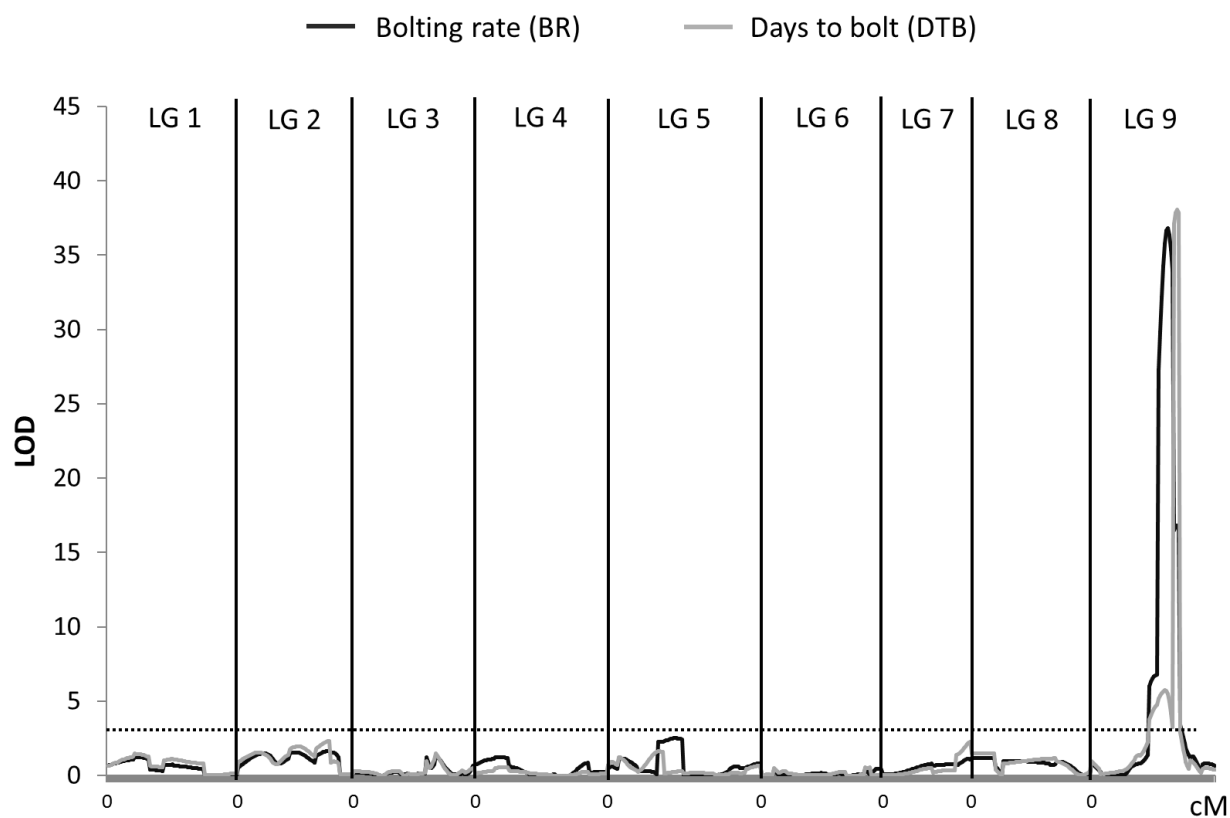
Supplementary Figure 3: Observed bolting phenotypes in F₂ and F₃ generations. On the left a post-winter bolting resistant plant is displayed, in the middle an incompletely bolting plant and on the right a bolting and flowering plant.



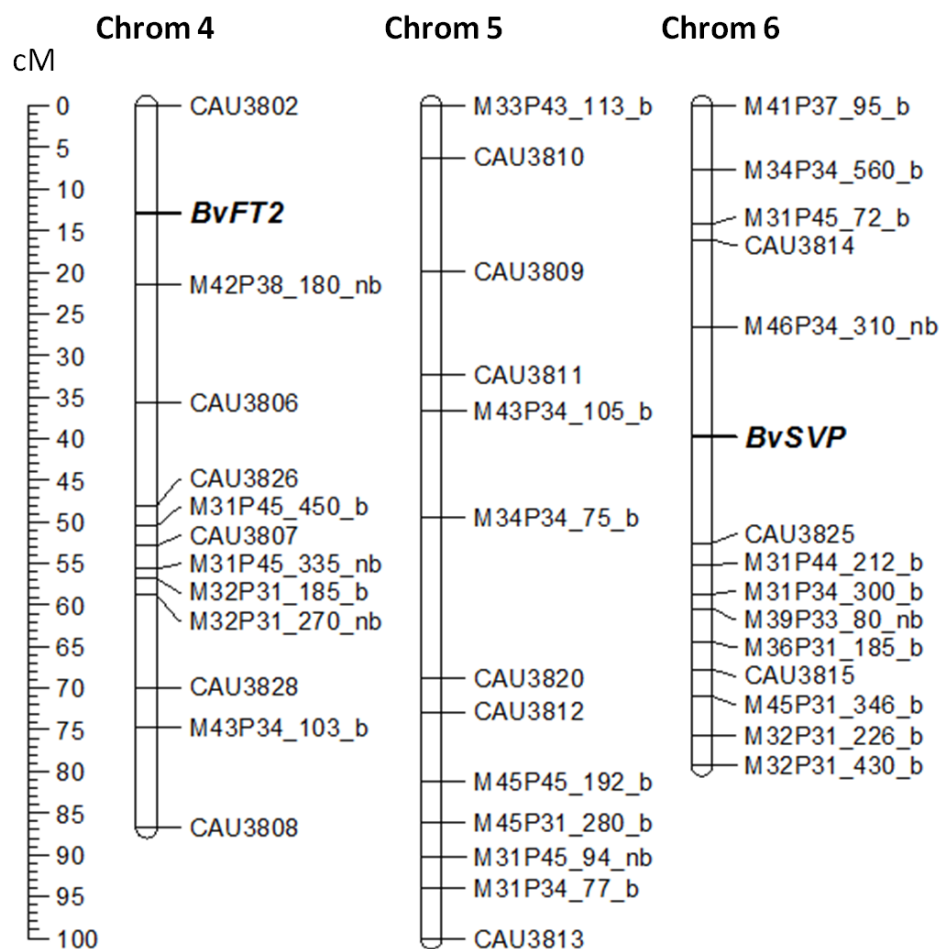
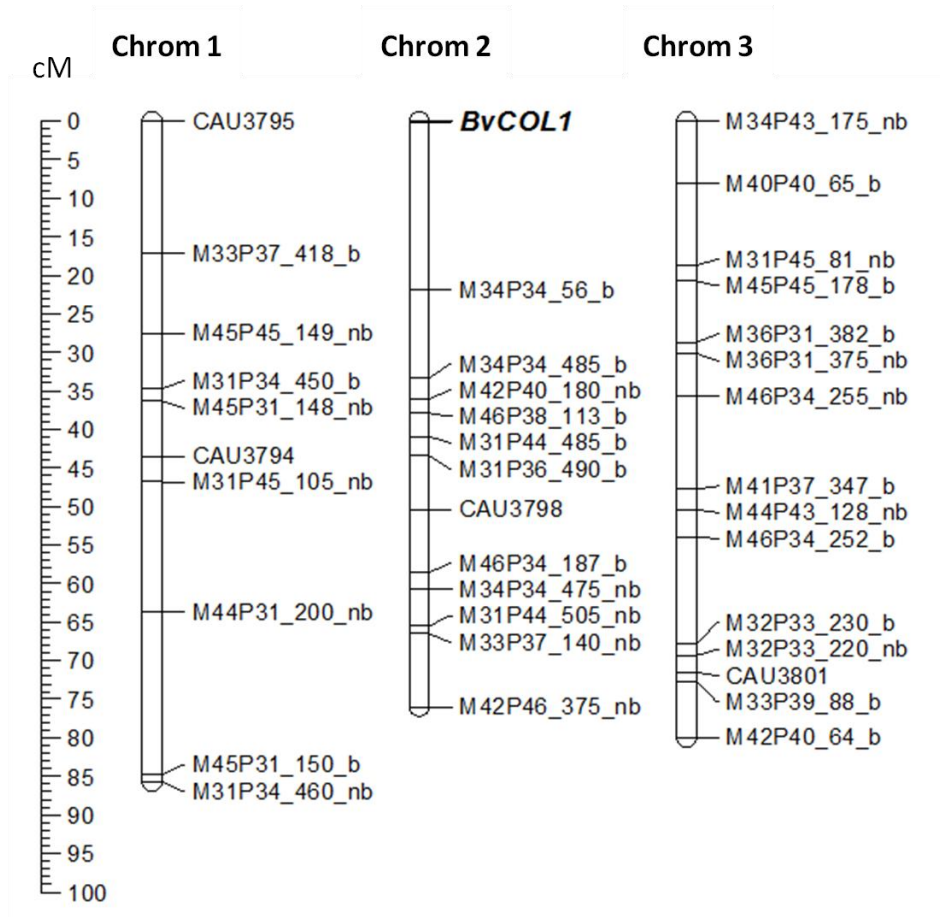
Supplementary Figure 4: Bolting phenotypes of F_3 families. The plants were planted to the field after artificial cold treatment and phenotyped twice a week from end of May 29 until mid of October 15, 2012. On the left side bolting plants of the same F_3 family are displayed, on the top right side a family with complete post-winter bolting resistance and below a segregating family.

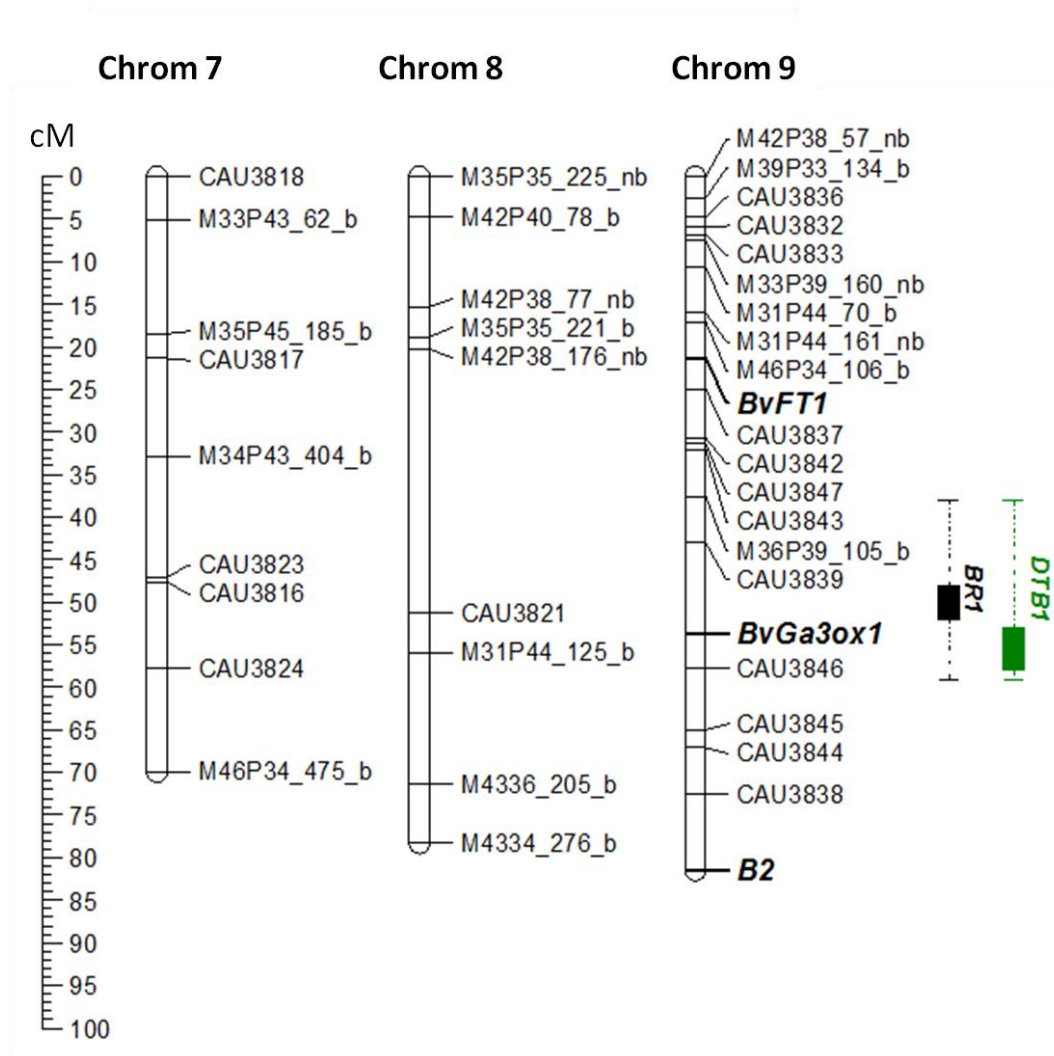


Supplementary Figure 5: Biplot of the correlation ($r=-0.986$) between days to bolt (DTB) and bolting rate (BR) after winter of 186 F_3 families.



Supplementary Figure 6: QTL mapping results among all nine linkage groups for the traits bolting delay (DTB – indicated in grey) and post-winter bolting rate (BR - indicated in black). The linkage groups are displayed on the x-axis, the LOD (logarithm of the odds) is displayed on the y-axis. The dashed line indicates the LOD threshold 3.0; LG: linkage group.





Supplementary Figure 7: Genetic map of F_2 population 102946 derived from a cross of sugar beet accessions BETA 1773 (080299) and 93161P (930176). The linkage map in cM is indicated by the vertical bars on which also marker names are located. Names of AFLP markers end on 'nb' if the dominant marker allele is derived from the parent BETA 1773 (080299) or on 'b' if derived from the parent 93161P (930176). Chrom: chromosome. Mapped QTL for post-winter bolting resistance (*BR₁*) and post-winter bolting delay (*DTB₁*) are indicated by the black and green bar, respectively. Mapped flowering time genes or bolting loci are indicated in bold and *italics*. *BvCOL1* (CAU3797), *BvFT2* (CAU3805), *BvSVP* (CAU3793), *BvFT1* (CAU3835), *BvGa3ox1* (CAU3841), *B2* (CAU3840).

7.2 Supplementary material for chapter 3

7.2.1 Supplementary tables for chapter 3

Supplementary Table 6: Distribution of AFLP and sequence based markers in the linkage map used for QTL analysis.

Linkage group	AFLP	Sequence based marker	Length (cM)	Largest gap (cM)
1	13	1	82.8	13.0
2	10	1	77.2	21.2
3	12	1	81.5	39.0
4	15	4	83.0	12.7
5	15	2	70.4	7.9
6	19	2	71.0	9.4
7	13	1	70.6	8.8
8	10	1	67.7	23.1
9	12	7	86.5	16.3
Total	119	20	690.7	-

Supplementary Table 7: Overview of crossing parents used for the development of a segregating F₂ mapping population, seed codes are given in brackets (). For detailed bolting behavior of F₂ plants see **Supplementary Table 8**.

Seed parent	Pollinator	F ₁	F ₂
biennial leaf beet 82.5523 (080550)	annual sugar beet 93167P (001684)	(090972/08)	(100436)

Supplementary Table 8: Phenotypic data of F₂ single plants of population 100436 and their F₃ offspring. pwbr: post-winter bolting resistant phenotype; DTB for F₂ plants: days to bolt after cold treatment; DTB in the F₃ were recorded as days to bolt after cold treatment (art. cond. = artificial cold treatment) or days to bolt after May 1, 2012 (nat. cond. = natural overwintering conditions). For F₃ families least square means were calculated by SAS PROC MIXED (SAS Institute Inc., 2009, Cary, USA, Version 9.2). Marker data of the sequence based marker CAU3852, that flank the QTL *DTBnat₃* on chromosome 9, is given. 'b' and 'a' indicate marker genotypes homozygous for the marker allele (M1) derived from the annual sugar beet parent 93167P (001684), or homozygous for the marker allele (M2) derived from the biennial leaf beet parent 82.5523 (080550), respectively. 'h' indicates heterozygous marker genotypes. 'na': not available.

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean (art. cond)	F ₃ DTB family mean (nat. cond)	CAU 3852
100436/0001	bolting	23	110704	29.63	42.62	h
100436/0007	bolting	na	114598	30.79	na	h
100436/0011	bolting	23	110705	29.84	40.22	h
100436/0013	bolting	14	110706	27.40	41.19	na
100436/0016	bolting	23	110707	27.67	42.83	h
100436/0017	bolting	33	110708	28.21	44.49	a
100436/0018	bolting	28	110709	33.52	46.71	a
100436/0020	bolting	26	110710	27.75	39.42	h
100436/0024	bolting	14	110711	27.51	34.93	h
100436/0027	bolting	12	110712	27.00	33.87	h
100436/0028	bolting	19	110713	27.01	33.79	h
100436/0037	bolting	23	110714	27.00	37.82	h
100436/0042	bolting	21	110715	na	na	a
100436/0045	bolting	19	110716	27.71	40.23	a
100436/0046	bolting	19	110717	30.50	37.34	b
100436/0048	bolting	42	110718	35.17	46.57	a
100436/0049	bolting	61	na	na	na	a
100436/0054	bolting	16	110719	27.62	40.22	a
100436/0059	bolting	12	110720	32.49	40.38	b
100436/0063	bolting	21	110721	27.01	32.89	h
100436/0064	bolting	19	110722	27.14	36.26	h
100436/0069	bolting	16	110723	28.93	45.55	h
100436/0070	bolting	12	110724	27.00	31.96	h
100436/0075	bolting	26	110725	na	47.80	h
100436/0076	bolting	19	110726	27.33	38.63	h
100436/0081	pwbr	85	114592	35.61	na	a
100436/0082	pwbr	85	114593	na	na	a
100436/0083	bolting	14	110727	26.99	37.17	h
100436/0085	bolting	26	110728	29.10	42.22	h
100436/0088	bolting	14	110729	27.00	37.78	h
100436/0091	bolting	26	110730	27.25	36.00	b
100436/0092	bolting	19	110731	27.75	42.23	h
100436/0094	bolting	16	110732	27.80	33.42	b
100436/0095	bolting	19	110733	29.08	40.32	h
100436/0097	bolting	19	110734	27.01	40.10	h
100436/0101	bolting	na	114599	27.50	na	a
100436/0114	bolting	14	110735	27.38	37.54	b
100436/0116	bolting	19	110736	27.89	39.17	h
100436/0118	bolting	12	110737	27.00	37.47	b

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean (art. cond)	F ₃ DTB family mean (nat. cond)	CAU 3852
100436/0123	bolting	19	110738	27.67	36.37	h
100436/0126	bolting	21	110739	27.50	39.32	a
100436/0130	bolting	28	110740	na	37.20	h
100436/0134	bolting	28	110741	27.01	33.04	h
100436/0137	pwbr	85	114594	na	na	a
100436/0141	bolting	19	110742	30.46	42.11	h
100436/0147	bolting	21	110743	30.00	36.96	h
100436/0154	bolting	26	110744	28.34	45.00	a
100436/0156	bolting	14	110745	27.33	38.18	h
100436/0160	bolting	26	110746	27.50	39.37	h
100436/0162	bolting	21	110747	29.66	44.41	b
100436/0177	bolting	14	110748	28.73	38.61	a
100436/0188	bolting	12	110749	27.00	35.56	h
100436/0189	bolting	14	110750	27.13	35.33	h
100436/0190	bolting	26	110751	36.41	43.06	a
100436/0191	bolting	14	110752	27.33	33.67	na
100436/0198	bolting	19	110753	28.99	na	h
100436/0202	bolting	16	110754	27.20	33.50	b
100436/0204	bolting	23	110755	37.25	45.51	a
100436/0206	bolting	12	110756	27.14	31.83	b
100436/0207	bolting	19	110757	27.17	39.06	h
100436/0212	bolting	12	110758	27.00	33.56	b
100436/0214	bolting	19	110759	31.06	40.88	a
100436/0221	bolting	16	110760	27.33	36.47	h
100436/0225	bolting	19	110761	26.99	35.15	h
100436/0226	bolting	12	110762	27.40	36.57	h
100436/0234	bolting	19	110763	29.93	46.01	a
100436/0235	bolting	19	110764	30.75	38.74	h
100436/0241	bolting	16	110765	28.61	39.90	a
100436/0247	bolting	21	110766	na	42.83	h
100436/0250	bolting	26	110767	27.00	35.42	b
100436/0254	bolting	16	110768	28.80	40.26	a
100436/0258	bolting	14	110975	27.93	39.07	h
100436/0259	bolting	26	110769	na	30.80	h
100436/0261	bolting	16	110770	27.00	36.50	a
100436/0264	bolting	16	110771	na	35.17	h
100436/0267	bolting	33	110772	na	46.05	a
100436/0268	bolting	16	110773	27.43	30.08	b
100436/0273	pwbr	85	114595	35.75	na	a
100436/0276	bolting	19	110774	na	35.06	h
100436/0277	bolting	19	110775	29.18	45.72	h
100436/0279	bolting	21	110776	29.24	45.00	a
100436/0286	bolting	14	110777	28.28	39.93	h
100436/0290	bolting	23	110778	28.12	38.45	h
100436/0291	bolting	16	110779	28.91	39.26	h
100436/0294	bolting	14	110780	27.79	41.44	a

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean (art. cond)	F ₃ DTB family mean (nat. cond)	CAU 3852
100436/0300	bolting	16	110781	36.49	38.58	b
100436/0301	bolting	14	110782	27.17	36.00	h
100436/0305	bolting	16	110783	27.41	36.04	b
100436/0312	bolting	19	110784	29.00	42.37	h
100436/0313	bolting	19	110785	28.71	40.48	h
100436/0315	bolting	19	110786	28.80	39.82	h
100436/0316	bolting	19	110787	31.00	47.71	h
100436/0319	bolting	19	110788	30.29	44.43	a
100436/0322	bolting	14	110789	27.65	41.27	h
100436/0326	bolting	12	110790	27.00	32.96	b
100436/0330	bolting	14	110791	na	43.00	b
100436/0334	bolting	16	110792	27.40	39.32	h
100436/0339	bolting	19	110793	27.83	38.84	h
100436/0341	bolting	19	110794	27.29	37.47	b
100436/0345	bolting	19	110795	27.34	34.14	h
100436/0347	bolting	14	110796	27.13	35.55	b
100436/0348	bolting	19	110797	28.25	43.20	h
100436/0358	bolting	23	110798	32.46	42.06	h
100436/0360	bolting	12	110799	29.19	39.58	h
100436/0362	bolting	16	110800	27.57	36.69	h
100436/0365	bolting	12	110801	27.33	43.26	h
100436/0368	bolting	26	110802	29.86	38.21	a
100436/0371	bolting	30	110803	30.56	37.94	h
100436/0374	bolting	26	110804	29.00	39.65	h
100436/0378	bolting	26	110805	27.25	42.13	h
100436/0383	bolting	19	110806	27.00	40.96	a
100436/0385	bolting	21	110807	27.25	35.75	b
100436/0386	bolting	16	110808	27.43	35.90	h
100436/0390	bolting	16	110809	29.00	40.78	a
100436/0395	bolting	19	110810	27.83	41.42	h
100436/0396	bolting	19	110811	27.25	43.78	a
100436/0399	bolting	14	110812	27.68	37.50	h
100436/0402	bolting	19	110813	27.83	39.93	a
100436/0403	bolting	12	110814	27.13	36.11	h
100436/0413	bolting	26	110815	29.37	42.95	a
100436/0414	bolting	14	110816	29.17	40.85	h
100436/0417	bolting	19	110817	31.40	44.22	h
100436/0418	bolting	16	110818	27.40	35.97	h
100436/0428	bolting	14	110819	31.33	41.26	h
100436/0431	bolting	23	110820	27.66	37.43	h
100436/0434	bolting	21	110821	27.00	35.43	h
100436/0442	bolting	16	110822	27.00	32.37	b
100436/0444	bolting	26	110823	38.17	51.01	a
100436/0453	bolting	19	110825	29.47	36.47	h
100436/0455	bolting	33	110826	27.00	35.46	a
100436/0456	bolting	19	110827	27.34	41.49	h
100436/0459	bolting	19	110828	27.14	32.92	h

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean (art. cond)	F ₃ DTB family mean (nat. cond)	CAU 3852
100436/0460	bolting	14	110829	27.13	34.61	h
100436/0461	bolting	19	110830	27.17	37.95	h
100436/0463	bolting	16	110831	27.81	39.85	h
100436/0469	bolting	14	110832	27.45	36.67	b
100436/0470	bolting	26	110833	29.00	36.93	h
100436/0472	bolting	26	110834	32.76	47.40	h
100436/0478	bolting	19	110835	27.50	39.17	b
100436/0480	bolting	12	110836	28.60	38.00	h
100436/0481	bolting	16	110837	27.71	42.05	h
100436/0486	bolting	33	110838	na	39.00	b
100436/0492	bolting	37	110839	na	na	a
100436/0493	bolting	16	110840	27.38	35.43	b
100436/0495	bolting	26	110841	na	39.46	na
100436/0497	bolting	12	110842	27.75	35.93	h
100436/0499	bolting	16	110843	27.00	37.90	h
100436/0500	bolting	12	110844	27.77	43.62	a
100436/0502	bolting	14	110845	27.00	34.69	h
100436/0503	bolting	16	110846	27.25	34.19	h
100436/0504	bolting	12	110847	27.14	34.19	h
100436/0505	bolting	19	110848	27.25	35.73	a
100436/0507	bolting	19	110849	29.30	43.25	a
100436/0514	bolting	26	110850	30.50	46.52	h
100436/0515	pwbr	85	114596	na	na	a
100436/0516	bolting	26	110851	29.81	42.93	a
100436/0523	bolting	51	na	na	na	a
100436/0524	bolting	12	110852	28.75	37.41	b
100436/0528	bolting	26	110853	27.50	36.23	b
100436/0534	bolting	33	110854	na	na	b
100436/0541	bolting	12	110855	27.00	31.64	b
100436/0542	bolting	12	110856	28.97	35.03	h
100436/0543	bolting	12	110857	27.00	32.33	h
100436/0544	pwbr	85	116348	na	na	a
100436/0545	bolting	33	110858	na	52.00	a
100436/0551	bolting	16	110859	33.21	41.77	h
100436/0558	bolting	26	110860	31.93	39.77	h
100436/0561	bolting	19	110861	27.80	34.15	h
100436/0563	bolting	19	110862	29.92	37.55	h
100436/0567	bolting	26	110863	33.01	45.93	h
100436/0571	bolting	16	110864	27.00	31.74	b
100436/0572	bolting	21	110865	26.99	42.88	b
100436/0573	bolting	19	110866	na	36.66	b
100436/0575	bolting	23	110867	29.90	43.77	b
100436/0578	bolting	14	110868	27.00	41.18	h
100436/0581	bolting	23	110869	29.79	37.52	h
100436/0584	pwbr	85	114597	37.68	na	a
100436/0587	bolting	21	110870	27.00	35.14	b

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean (art. cond)	F ₃ DTB family mean (nat. cond)	CAU 3852
100436/0591	bolting	19	110871	27.20	31.77	h
100436/0593	bolting	19	110872	28.38	36.41	b
100436/0594	bolting	21	110873	29.80	38.99	a
100436/0600	bolting	16	110874	28.00	42.93	h
100436/0601	bolting	26	110875	27.75	33.71	a
100436/0606	bolting	28	110876	28.38	42.56	a
100436/0607	bolting	19	110877	31.00	36.74	b
100436/0608	bolting	16	110878	26.99	41.11	a
100436/0609	bolting	19	110879	27.95	41.00	a
100436/0617	bolting	16	110880	28.07	37.21	h
100436/0624	bolting	37	110881	na	na	h
100436/0625	bolting	26	110882	39.49	45.57	a
100436/0628	bolting	19	110883	33.26	41.24	h
100436/0631	bolting	16	110884	29.33	37.75	b
100436/0640	bolting	19	110885	28.15	33.39	a
100436/0642	bolting	19	110886	28.33	36.33	b
100436/0648	bolting	19	110887	38.60	49.83	h
100436/0649	bolting	23	110888	28.92	45.56	h
100436/0650	bolting	14	110889	27.00	38.56	b
100436/0653	bolting	16	110890	29.42	41.51	h
100436/0656	pwbr	85	116349	na	na	a
100436/0660	pwbr	85	116350	na	na	a
100436/0664	bolting	19	110891	38.14	41.57	b
100436/0665	bolting	23	110892	30.17	42.92	a
100436/0666	bolting	19	110893	na	43.66	h
100436/0671	bolting	26	110894	na	41.57	h
100436/0678	bolting	33	110895	30.17	46.33	a
100436/0680	bolting	16	110896	33.75	43.71	b
100436/0684	bolting	19	110897	26.99	36.86	h
100436/0686	bolting	19	110898	27.48	40.89	h
100436/0687	bolting	16	110899	28.13	35.00	a
100436/0690	bolting	14	110900	26.99	31.37	b
100436/0693	bolting	26	110901	27.50	35.63	h
100436/0697	bolting	42	110902	na	na	a
100436/0699	bolting	19	110903	29.21	41.37	a
100436/0701	bolting	12	110904	27.00	41.79	b
100436/0702	bolting	35	110905	28.76	42.24	a
100436/0705	bolting	16	110906	27.68	39.45	a
100436/0706	bolting	19	110907	28.00	34.91	b
100436/0709	bolting	19	110908	29.43	37.10	b
100436/0716	bolting	19	110909	28.87	35.62	h
100436/0717	bolting	35	110910	na	na	a
100436/0718	bolting	12	110911	27.25	36.70	h
100436/0721	bolting	14	110912	29.43	3.93	h
100436/0723	bolting	16	110913	27.17	36.47	h
100436/0724	bolting	16	110914	29.49	33.32	b
100436/0730	bolting	12	110915	28.32	38.67	b

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean (art. cond)	F ₃ DTB family mean (nat. cond)	CAU 3852
100436/0733	bolting	19	110916	30.10	39.80	a
100436/0735	bolting	26	110917	30.23	42.21	b
100436/0738	bolting	14	110918	27.64	35.24	h
100436/0739	bolting	28	110919	27.50	35.75	h
100436/0741	bolting	26	110920	27.66	37.17	a
100436/0742	bolting	21	110921	39.00	48.71	a
100436/0743	bolting	23	110922	29.19	46.60	a
100436/0744	bolting	14	110923	26.99	38.38	a
100436/0751	bolting	19	110924	na	38.67	h
100436/0761	bolting	19	110925	27.33	41.42	b
100436/0763	bolting	21	110926	27.52	42.19	h
100436/0767	bolting	21	110927	30.50	40.42	h
100436/0768	bolting	26	110928	33.25	39.88	a
100436/0774	bolting	26	110929	27.79	39.50	b
100436/0776	bolting	16	110930	27.29	37.69	b
100436/0780	bolting	12	110931	27.00	34.88	h
100436/0783	bolting	19	110932	27.00	38.73	a
100436/0785	bolting	14	110933	28.00	40.37	b
100436/0787	bolting	26	110934	28.07	37.97	h
100436/0789	bolting	12	110935	27.01	36.15	h
100436/0791	bolting	23	110936	26.99	37.30	a
100436/0794	bolting	19	110937	28.55	42.41	h
100436/0796	bolting	23	110938	28.79	40.00	h
100436/0803	bolting	19	110939	28.64	39.08	h
100436/0804	bolting	na	110940	27.43	40.96	h
100436/0810	bolting	16	110941	28.18	37.89	b
100436/0813	pwbr	85	116351	na	na	a
100436/0815	bolting	14	110942	28.17	41.40	h
100436/0817	bolting	21	110943	29.42	40.88	h
100436/0820	bolting	16	110944	27.29	40.72	h
100436/0821	bolting	16	110945	28.58	33.60	b
100436/0825	bolting	14	110946	28.75	38.97	b
100436/0828	bolting	19	110947	28.25	42.82	a
100436/0830	bolting	40	110948	33.13	49.61	h
100436/0831	bolting	21	110949	30.87	43.76	h
100436/0835	bolting	19	110950	27.43	40.71	h
100436/0836	bolting	16	110951	27.38	39.31	h
100436/0837	bolting	26	110952	27.30	36.21	a
100436/0838	bolting	47	110953	30.04	44.54	a
100436/0839	bolting	19	110954	30.00	42.21	h
100436/0840	bolting	23	110955	27.00	42.21	a
100436/0842	bolting	61	na	na	na	a
100436/0845	bolting	23	110956	26.99	32.05	h
100436/0846	bolting	19	110957	na	38.69	b
100436/0848	bolting	16	110958	27.00	37.21	b
100436/0849	bolting	16	110959	27.91	39.24	h

F ₂ single plant	F ₂ phenotype	F ₂ DTB	F ₃ seedcode	F ₃ DTB family mean (art. cond)	F ₃ DTB family mean (nat. cond)	CAU 3852
100436/0850	bolting	14	110960	28.30	38.81	h
100436/0851	bolting	19	110961	28.88	40.30	h
100436/0853	bolting	19	110962	27.67	35.09	b
100436/0855	bolting	16	110963	27.00	40.05	h
100436/0856	bolting	14	110964	28.33	40.78	a
100436/0857	bolting	33	110965	27.25	38.00	h
100436/0900	bolting	na	114600	27.00	na	na
100436/0977	bolting	19	110966	27.83	40.32	h
100436/0979	bolting	12	110967	na	36.13	a
100436/0989	bolting	19	110968	29.86	48.14	h
100436/1030	bolting	14	110969	27.00	31.94	h
100436/1050	bolting	26	110970	28.91	43.28	a
100436/1054	bolting	14	110971	27.14	37.38	a
100436/1074	bolting	33	110972	na	35.50	a
100436/1100	bolting	23	110973	27.20	37.77	b
100436/1139	bolting	26	110974	30.24	48.33	h

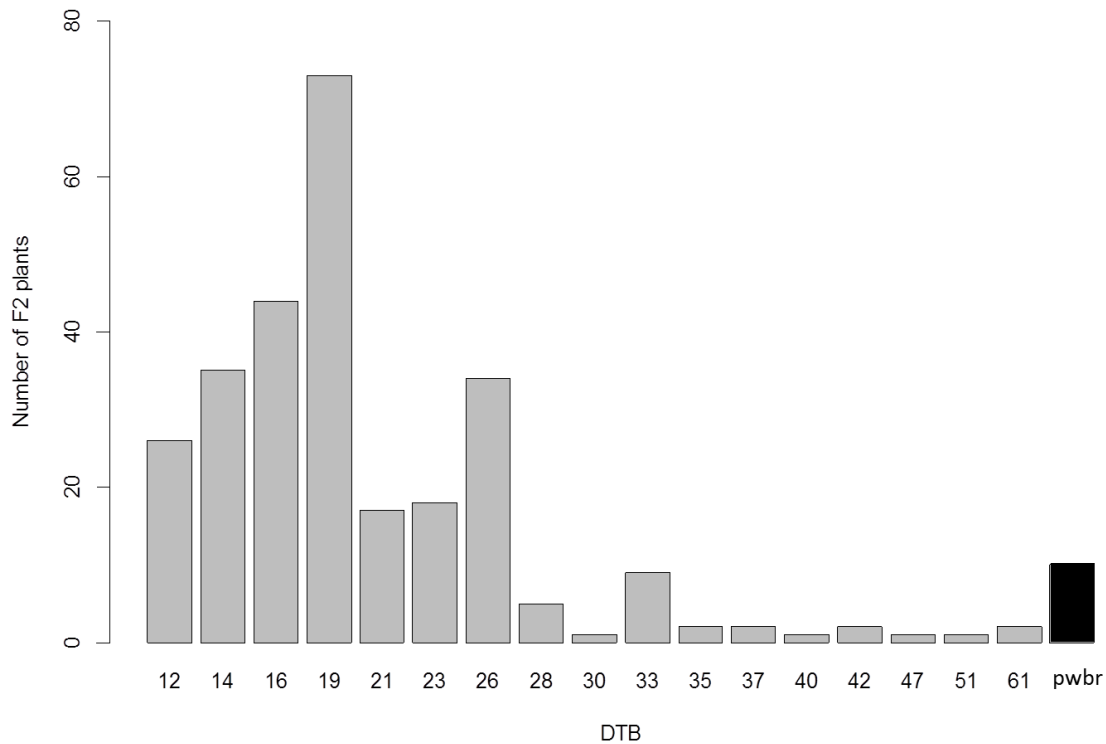
Supplementary Table 9: Sequence derived markers used for construction of the genetic map of F₂ population 100436. Beside marker names, map position, primer combinations, PCR fragment sizes, annealing temperature and in case of CAPS markers the restriction enzymes are listed. ‘TD’: Touch-down-PCR protocol was used. ‘na’: not available. For primer sequence information see **Supplementary Table 10**.

Marker name	Map position	Primer combination	PCR fragment size seed parent	PCR fragment size pollinator	Mapping technique	Annealing temperature (°C)	Reference
CAU 3792	9	G646/ G647	777 bp	829 bp	Indel	58	TG_E0140, G. Capistrano
CAU 3796	9	A920/ A921	502 bp + 137 bp	639 bp	CAPS, <i>AccI</i>	58	<i>BvGa3ox1</i> , scaffold00076 (RefBeet 0.9)
CAU 3799	5	N061/ N062	552 bp	341 bp + 211 bp	CAPS, <i>Bsh1236I</i>	58	TG_E503
CAU 3800	5	A978/ A979	~150 bp	~170 bp	SSR	TD	SB156
CAU 3803	4	N051/ N052	~203 bp	~219 bp	Indel	58	<i>BvFT2</i> , Pin et al. 2012
CAU 3804	4	K847/ K848	503 bp	301 bp + 202 bp	CAPS, <i>Sau3AI</i>	57	M. Kirchhoff
CAU 3819	4	A991/ A992	250 bp	245 bp	Indel	TD	FDSB1023
CAU 3827	7	N115/ N116	~240 bp	~170 bp	InDel	60	BQ589879
CAU 3829	1	N055/ N056	~400 bp	~440 bp	InDel	59	TG_E0532
CAU 3831	6	ZRT78 ZRT81	917 bp	645 bp + 272 bp	CAPS, <i>HhaI</i>	58	<i>BvFL1</i> , Reeves et al. 2007
CAU 3834	6	N107/ N108	961 bp	501 bp + 460 bp	CAPS, <i>SspI</i>	58	BQ586664
CAU 3848	9	B017/ B018	~350 bp	~330 bp	SSR	TD	FDSB1001, B. Büttner
CAU 3849	9	N095/ N096	421 bp + 90 bp	511 bp	CAPS, <i>Eco81I</i>	60	<i>BvSWPI</i> , A. Müller
CAU 3850	9	N031/ N032	492 bp + 262 bp	469 bp + 262 bp + 23 bp	CAPS, <i>AluI</i>	58	<i>BvFT1</i> , scaffold00007 (RefBeet 0.9)
CAU 3851	9	N147/ N148	839 bp	454 bp + 385 bp	CAPS, <i>BstUI</i>	58	<i>BvFT1</i> , scaffold00007 (RefBeet 0.9)
CAU 3852	9	A995/ A996	~250 bp	~240 bp	SSR	58	FDSB1033, B. Büttner
CAU 3853	3	na	na	na	SNP- Assay	TD	KASP SNP-Assay, G. Capistrano
CAU 3854	8	na	na	na	SNP- Assay	TD	KASP SNP-Assay, scaffold00009 (RefBeet 0.9)
CAU 3855	2	N315/ N316	202 bp	174 bp	InDel	55	scaffold00251 (RefBeet 0.9)

Supplementary Table 10: Primer used for PCR in order to generate sequence derived marker information. Primer sequences are displayed in 5' → 3' orientation.

primer name	Sequence (5' → 3')
A920	ACCCGAGAGCACAACAACCTTAT
A921	TTTATTTTCTTGTGTAGTGGACCAT
A978	CACCCAGCCTATCTCTCGAC
A979	GTGGTGGGCAGTTTTAGGAA
A991	TCTCTCTCCCCCTAAAAGTTCA
A992	GTAGCTAGTTCAGCAATCTTCGC
A995	GCTGAGATGATGTTTGTAGGGC
A996	TTCAAATCGCCATCTCCCAG
B017	ACTTCAACCACTATCACAAAGTGAG
B018	ATCTTATGCTGCCATGACCA
G646	CCTCTCTTTCTCTCTCTGCAAC
G647	ACACTGCAACACGATCAGG
K847	AGAATCGGTGTTGGGGATG
K848	GGTAAGAAAGCGGGTTGGA
N031	GGAACCGTATTGCACACGCATAAG
N032	CTGCTCGATTTGGAGGTCTCTG
N051	GATGCAACATGAGATGCGATG
N052	GATCTCTTGGTGCTCTAGGC
N055	GGATTCTCACACAAGTCAGTAG
N056	CTGATGCTGAAGTGTGGAGTG
N061	CACACACCAACACCATCACTC
N062	CCAAGTTTACCCCTTTATCTCTC
N095	GGAATCCACTTGGGGTATTGG
N096	CCACCTGCATAAACCAACACC
N107	GCACCTCAACAAACACATCC
N108	CGAGTTCAAATCAAGAATGGC
N115	GGTTCGCCGTAAAAGCTCG
N116	GCTTTGGAACCTTCGGCGTG
N147	GAAGATGGGGTGGGTGTTTG
N148	GTGTAGGGGTTGAATATAGGTC
N315	CCATAAAAGTTGACGCTTGC
N316	GTCAGTTTTGAGTCAGTGAG
ZRT78	AACTCTATCCAATCCTCACATGTTGTGCA
ZRT81	CCATTAACCACCGAACCAATTCAACAAAGC

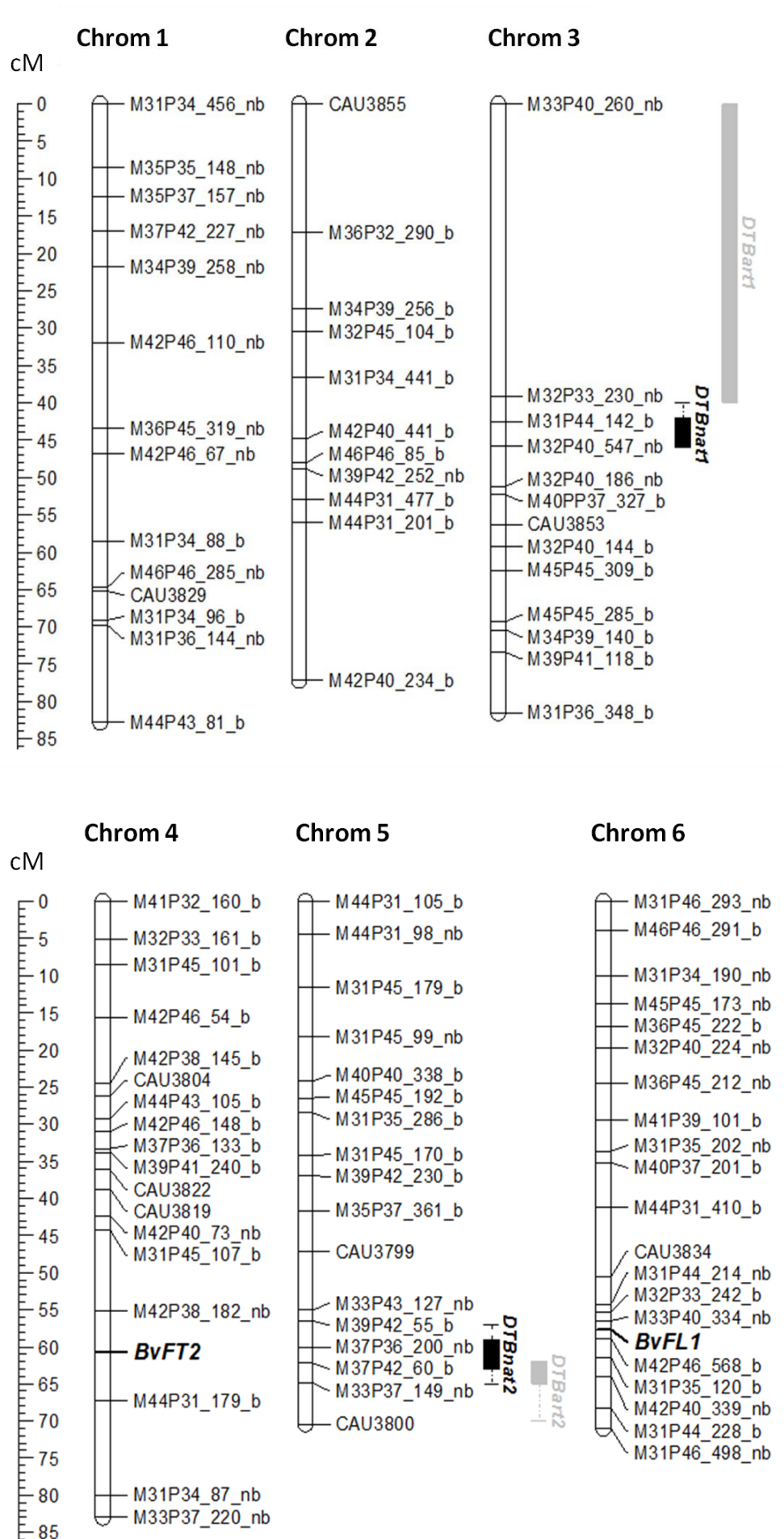
7.2.2 Supplementary figures for chapter 3

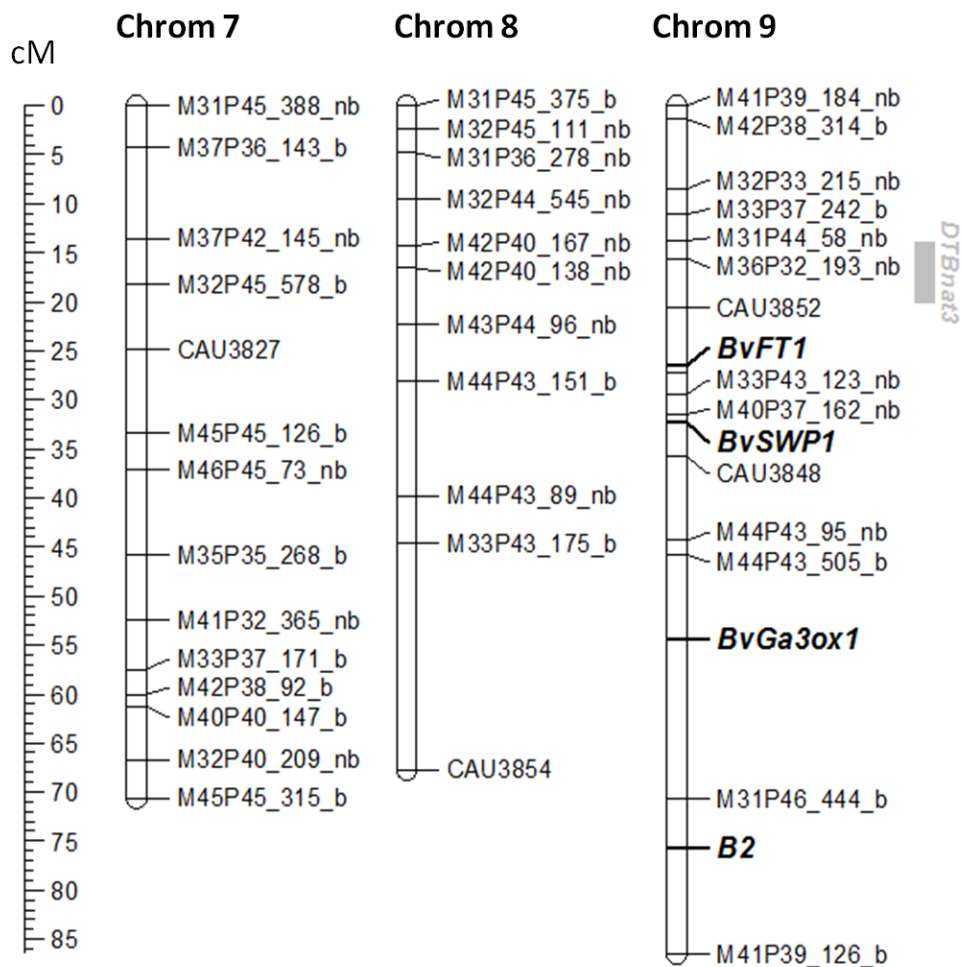


Supplementary Figure 8: Phenotypic variation for bolting time after cold treatment in the F₂ population. Days to bolt (DTB) are defined as at first visible elongation of main stem (BBCH 51) after plants left the cold chamber. Population size n=291; sowing date: May 21, 2010; cold treated in a cold chamber at 5°C under 22 h light; after 1 week of acclimatization at 8 °C and 22 h light transferred from the cold chamber back to the greenhouse and kept under long day conditions at 20 °C under 22 h light. Plants that are post-winter bolting resistant are designated by pwbr.



Supplementary Figure 9: Observed bolting phenotypes in F₂ and F₃ generations. On the left a post-winter bolting resistant plant is displayed and on the right a bolting plant.





Supplementary Figure 10: Genetic map of F_2 population 100436 derived from a cross of leaf beet accession 82.5523 (080550) and sugar beet accession 93167P (001684). The linkage map in cM is indicated by the vertical bars on which also marker names are located. Names of AFLP markers end on 'nb' if the dominant marker allele is derived from the parent 82.5523 (080550) or on 'b' if derived from the parent 93167P (001684). Chrom: chromosome. Mapped QTL for post-winter bolting delay under natural overwintering conditions (*DTBnat₁*, *DTBnat₂* and *DTBnat₃*) and post-winter bolting delay after artificial cold treatment (*DTBart₁* and *DTBart₂*) are indicated by the black and grey bars, respectively. Mapped flowering time genes or bolting loci are indicated in bold and italics. *BvFT2* (CAU3803), *BvFL1* (CAU3831), *BvFT1* (CAU3851), *BvSWP1* (CAU3849), *BvGa3ox1* (CAU3796), *B2* (CAU3792).

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9 Curriculum vitae and publications

9.1 Curriculum Vitae

Personal data

Name	Nina Pfeiffer
Date of birth	18.09.1983
Place of birth	Uslar, Germany
Nationality	German

Professional experience

Since 2013/11	Assistant Wheat Breeder at KWS LOCHOW GMBH, Wetze, Germany
2010 – 2013	Ph.D. student at Plant Breeding Institute, Christian-Albrechts University of Kiel, Germany
2003 – 2005	Apprenticeship as state certified agricultural-technical assistant at KWS SAAT AG, Einbeck, Germany

Education

2010/03	M.Sc. in Agronomy with specification on Plant Sciences Christian Albrechts University of Kiel, Germany Master thesis: "Bestimmung allelischer Varianten von Blühgen-Kandidaten in <i>Beta vulgaris</i> Herkünften mit Schossverzögerung"
2008 – 2010	M.Sc. courses in agriculture at Christian-Albrechts University of Kiel; Germany
2008/11	B.Sc. in Agronomy with specification on Plant Sciences Christian Albrechts University of Kiel, Germany Bachelor thesis: "Vergleich verschiedener Methoden zur schnellen DNA-Extraktion aus Pflanzen für die markergestützte Selektion in der Pflanzenzüchtung"
2005 – 2008	B.Sc. courses in agriculture at Christian-Albrechts University of Kiel; Germany
1996 – 2003	"Gymnasium Uslar" grammar school, Uslar, Germany Degree: Abitur
1994 – 1996	"Orientierungsstufe Uslar" orientation stage, Uslar, Germany
1990 – 1994	"Grundschule Uslar" elementary school, Uslar, Germany

9.2 Publications

9.2.1 Articles

Pfeiffer, N., A.E. Müller, C. Jung 2016. Genetic analysis of delayed bolting after winter in leaf beet (*Beta vulgaris* L.). Under review, Plant Breeding

Pfeiffer N., C. Tränkner, I. Lemnian, I. Grosse, A.E. Müller, C. Jung, F.J. Kopisch-Obuch 2014. Genetic analysis of bolting after winter in sugar beet. Theoretical and applied Genetics 127: 2479-2489

9.2.2 Oral presentations

Tränkner, C., N. Emrani, **N. Pfeiffer**, M. Kirchhoff, F. Kopisch-Obuch, H. van Dijk, M. Schilhabel 2016. Two major QTL for seasonal bolting time detected in *Beta vulgaris* populations that segregate for mono- and polycarpy. SPP 1530 workshop, Circadian rhythms, flowering time genes and crop plant adaptation, Berlin, January 20-22, 2016

Lemnian, I., C. Tränkner, **N. Pfeiffer**, N. Emrani, C. Weinholdt, F.J. Kopisch-Obuch, M. Schilhabel, C. Jung, I. Grosse, 2014. Analysis of whole-genome sequencing data for elucidating floral transition and discovery of non-bolting genes in *Beta vulgaris*. Genetic Variation of Flowering Time Genes and Applications for Crop Improvement. Bielefeld, March 24-26, 2014

Emrani N., I. Lemnian, C. Weinholdt, **N. Pfeiffer**, F. Kopisch-Obuch, C. Jung, M. Schilhabel, I. Grosse, C. Tränkner. 2014. Identification of bolting failure candidate genes in sugar beet (*Beta vulgaris*) by Next Generation Sequencing. Genetic Variation of Flowering Time Genes and Applications for Crop Improvement, Bielefeld, March 24-26, 2014.

Kopisch-Obuch, F., M. Kirchhoff, F. Uhlmann, **N. Pfeiffer**, J.O. Ogutu, E. Orsini, A. Schechert, C. Jung, 2014. QTL for Winter Hardiness in Sugar Beet (*Beta vulgaris* ssp. *vulgaris* L.). Plant and Animal Genome XXII, San Diego CA, January, 10-15, 2014.

Lemnian, I., C. Tränkner, **N. Pfeiffer**, C. Weinholdt, F. Kopisch-Obuch, M. Schilhabel, I. Grosse. 2013. Analysis of deep-sequencing data for elucidating floral transition in *Beta* species. Workshop Floral Transition in Arabidopsis, Tübingen, September 16-18, 2013.

***Pfeiffer N.,** A.E. Müller, C. Jung and F.J. Kopisch-Obuch 2013. Genetic and phenotypic characterization of bolting failure in *Beta vulgaris*. PP 1530 2nd Annual Consortium Meeting, Köln, January 23-24, 2013.

Tränkner, C., I. Lemnian, **N. Pfeiffer**, F. Kopisch-Obuch, M. Schilhabel, H. van Dijk, I. Grosse. 2013. Unraveling a mechanism for floral transition control in annual, biennial, and perennial *Beta* species. PP 1530 2nd Annual Consortium Meeting, Köln, January 23-24, 2013.

***Pfeiffer N.,** A.E. Müller, C. Jung and F.J. Kopisch-Obuch 2013. Genetic and phenotypic characterization of bolting failure in *Beta vulgaris*. Plant and Animal Genome XXI, San Diego CA, January, 12-16, 2013.

Tränkner, C., **N. Pfeiffer**, M. Schilhabel, F. Kopisch-Obuch, M. Kirchhoff, I. Lemnian, I. Große. 2012. Strategy for mapping bolting repressor genes of sugar beet by next generation sequencing. 1st Young Investigator Meeting PP 1530, Halle/Saale, June 18-20, 2012.

Jung, C., S. Frerichmann, N. Dally, **N. Pfeiffer**, S. Vogt and A.E. Müller, 2011. Key regulators for flowering time control in a biennial crop species. 11. Botanikertagung, Berlin, September 18-23, 2011.

*presented by Nina Pfeiffer

9.2.3 Posters

Tränkner, C., I. Lemnian, N. Emrani, **N. Pfeiffer**, S. Prasad Tiwari, F. Kopisch-Obuch, M. Schilhabel, C. Jung, I. Grosse, 2016. Sequence-based mapping of the post-winter bolting resistance locus *BR₁* of sugar beet (*Beta vulgaris* L.) SPP 1530 workshop, Circadian rhythms, flowering time genes and crop plant adaptation, Berlin, January 20-22, 2016

Tränkner, C., I. Lemnian, **N. Pfeiffer**, N. Emrani, S. Vogt, M. Schilhabel, C. Weinholdt, A.E. Müller, C. Jung, F.J. Kopisch-Obuch and I. Große. 2014. Identification of post winter bolting resistance genes of sugar beet (*Beta vulgaris*) by bulked segregant Next Generation Sequencing. GPZ 2014: Genetic Variation in Plant Breeding, Kiel, September 23-25, 2014.

Lemnian I., C. Tränkner, **N. Pfeiffer**, N. Emrani, C. Weinholdt, F. Kopisch-Obuch, C. Jung, M. Schilhabel and I. Grosse. 2014. Analysis of whole-genome sequencing data for elucidating floral transition and detecting non-bolting genes in *Beta vulgaris*. Genetic Variation of Flowering Time Genes and Applications for Crop Improvement, Bielefeld, March 24-26, 2014.

Kopisch-Obuch, F., M. Kirchhoff, **N. Pfeiffer**, F. Uhlmann, J.O. Ogutu, E. Orsini, A. Schechert and C. Jung, 2014. Genetic mapping of winter hardiness and bolting resistance in sugar beet. Plant and Animal Genome XXII, San Diego CA, January, 10-15, 2014.

***Pfeiffer, N.**, A.E. Müller, C. Jung and F.J. Kopisch-Obuch 2013. Genetic and phenotypic characterization of bolting failure in *Beta vulgaris*. Plant 2030 Status Seminar, Potsdam March 6-8, 2013

Tränkner, C. I. Lemnian, **N. Pfeiffer**, C. Weinholdt, M. Kirchhoff, F. Kopisch-Obuch, M. Schilhabel, I. Große. 2012. Towards mapping of bolting failure genes of sugar beet (*Beta vulgaris* ssp. *vulgaris*) by next generation sequencing. GPZ Genome Research Working Group 10. Conference - Plant Stress and Genomics, Halle, September 18-20, 2012.

Tränkner, C., **N. Pfeiffer**, M. Kirchhoff, F. Kopisch-Obuch, M. Schilhabel, H. van Dijk, I. Lemnian, I. Grosse. 2012. Unraveling a mechanism for floral transition control in annual, biennial, and perennial *Beta* species. 1st Young Investigator Meeting PP 1530, Halle/Saale, June 18-20, 2012.

***Pfeiffer N.**, A.E. Müller, C. Jung and F.J. Kopisch-Obuch 2012. Genetic and phenotypic characterization of bolting failure in *Beta vulgaris*. GPZ Annual Conference – Breeding Crops for Sustainable Agricultural Production, Giessen, February 28 – March 1, 2012.

*presented by Nina Pfeiffer

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