GEFÖRDERT VOM

Bundesministerium
für Bildung
und Forschung

Veröffentlichung der Ergebnisse von Forschungsvorhaben im BMBF-Programm

GABI-FUTURE-Verbundvorhaben:' Funktionelle Genomforschung an Blühgenen zur gezielten genetischen Modifikation des Blühzeitpunkts in Zuckerrübe - GABI - GENOFLOR' (Teilprojekt A)

Förderkennzeichen: 0315058A

Zuwendungsempfänger: Christian-Albrechts-Universität zu Kiel, 24098 Kiel

Ausführende Stelle: Christian-Albrechts-Universität zu Kiel - Agrar- und Ernährungswissenschaftliche Fakultät - Institut für Pflanzenbau und Pflanzenzüchtung, 24098 Kiel

Projektleitung: Herr Dr. Müller

Laufzeit: 01.02.2008 bis 31.12.2011

"Das diesem Bericht zugrundeliegende BMBF-Forschungsvorhaben wurde mit Mitteln des Bundesministeriums für Bildung und Forschung unter dem Förderkennzeichen 0315058A gefördert. Die Verantwortung für den Inhalt dieser Veröffentlichung liegt beim Autor".

I. Kurze Darstellung zu

1. Aufgabenstellung

Floral transition is a major developmental switch that is tightly controlled by a network of proteins that perceive and integrate developmental and environmental signals to promote or inhibit the transition to flowering. In sugar beet and other crop species, induction and timing of flowering greatly affect yield and the respective crops' potential for cultivation under extensive conditions. In particular, cultivation of sugar beet over winter is expected to result in significant gains in root yield but is not possible in Germany and other central European countries due to induction of flowering by cold temperatures. However, suitable genotypes that on the one hand are resistant to cold induction, but on the other hand can be induced to flower for seed production and multiplication under defined conditions are difficult or impossible to identify by traditional selection and breeding approaches. Therefore, the main objectives of the proposed project are: a) genome-wide identification and expression profiling of floral transition genes in sugar beet, b) RNAi, overexpression and TILLing of select floral transition genes, and c) phenotyping for altered flowering time and bolting resistance to identify viable strategies for the development of winter cultivars. The first-time use of the SuperSAGE technology to study vernalization response in plants was expected to identify novel target genes for genetic modification of floral transition. The participation of two breeding companies allows immediate transfer of know-how, constructs and demonstration plant material for the future development of prototypes for integration into commercial breeding programs.

2. Voraussetzungen, unter denen das Vorhaben durchgeführt wurde

The project was initiated in the light of the competitive nature of the sugar market (sugar beet *vs.* sugar cane), recent and pending changes of its regulation in Europe, and a strong interest by breeding companies to improve the yield potential of sugar beet and its value for alternative uses such as bioenergy production by the development of winter beets. All major sugar beet breeding companies in Europe expressed interest in the project proposal and engaged in discussions with CAU Kiel. Strube and SESVanderHave joined the project. The project fell within the funding module 'GABI-Bridge' and aimed at the transfer, and expansion, of the extensive knowledge on flowering time control in the model plant *Arabidopsis thaliana* to an agronomically important crop species.

3. Planung und Ablauf des Vorhabens

Project A started August 1, 2008 with the hiring of a technician at CAU Kiel. A Ph.D. student was hired on October 1, 2008. Plant material for SuperSAGE had been grown and harvested at CAU Kiel in preparation of the project and was sent to GenXPro ahead of the project start. Sugar beet transformants were produced for all eleven transgene cassettes generated in the project. All transformants were fully molecularly characterized as proposed, which included the expression analysis of all target genes at CAU Kiel. Phenotypically, the transgenic beets derived from seven of the eleven transformation series were fully characterized as proposed, while the phenotypic characterization of the remaining four transformation series is still ongoing. However, it was explicitely stated in the project proposal that the lengthy transformation procedure and the nature of the trait of interest does not allow the phenotypic characterization of all transformants within the project period. The industrial partners therefore had explicitely stated their commitment to continue the phenotypic evaluation of transformants beyond the end of the project period. Rotating annual meetings were held at all project partners.

4. wissenschaftlichem und technischem Stand, an den angeknüpft wurde, insbesondere
• Angabe bekannter Konstruktionen, Verfahren und Schutzrechte, die für die Durchführung des Vorhabens benutzt wurden,
• Angabe der verwendeten Fachliteratur sowie der benutzten Informations- und Dokumentationsdienste

Many of the key genes that control flowering time have been identified and functionally characterized in *A. thaliana* (reviewed in He and Amasino, 2005; Bäurle and Dean, 2006), and plant genome and EST sequencing projects have begun to unveil the presence and evolutionary conservation of these genes across taxa (Albert *et al*., 2005; Hecht *et al*., 2005). For several genes equivalent or related functions in species as diverse as *A. thaliana* and *Oryza sativa* have been demonstrated, but inter-species comparisons also revealed flowering time genes that may have undergone functional divergence during the evolution of flowering behaviour (Putterill *et al*., 2004; Lee *et al*., 2005).

In *B. vulgaris*, the genes and pathways that regulate flowering time were largely unknown at the start of the project. The tendency for early bolting (without vernalization requirement) is under the control of a single dominant gene which was cloned in the PI's group shortly before the start of the project and identified as the pseudo-response regulator gene *BvBTC1*. In consideration of several observations detailed in II.1.1 and our results from the SuperSAGE analysis (s. II.1.1), this gene was included in the functional analysis of floral transition genes in the current project. Furthermore, in biennial beets, induction and timing of flowering depends on vernalization and requires appropriate photoperiodic and developmental conditions, but at the start of the project there was only one published report on a candidate regulatory gene in beet, which was the *A. thaliana Flowering Locus C* (*FLC*) homolog *BvFL1* (Reeves *et al*., 2007). A patent search on the search portals of the European Patent Office and the World Intellectual Patent Organization revealed that *FLC* and a downstream target gene, *AGL20*, were also the subject of research at Syngenta (WO 2007/122086). The complex nature of flowering time control, however, is likely to require research on multiple genes and modification strategies. In addition, and importantly, the approach that was followed in the current project for the first time employed the use of the SuperSAGE technology (Matsumura *et al*., 2003; 2005; 2006) to identify previously unrecognized targets for genetic modification of floral transition in beet. There were several genomic resources available for sugar beet that allowed identification of candidate genes on the basis of homology to flowering time genes from model species, and facilitated SuperSAGE data analysis and exploitation. These resources included public and proprietary EST collections and BAC libraries that were available in-house. Also, whole-genome physical mapping and sequencing of the sugar beet genome was underway or planned as part of GABI projects and was expected to allow a comprehensive annotation of the SuperSAGE data in due time.

5. Zusammenarbeit mit anderen Stellen

The project comprised key experimental work packages that were divided among the partners, subcontractors and collaborators according to the respective parties' areas of expertise as follows: Identification and molecular characterization of flowering time genes and transgenes generated in the project (CAU Kiel); genome-wide expression profiling by SuperSAGE (GenXPro as subcontractor of CAU Kiel); identification of allelic variants in candidate genes by TILLing (collaboration with the GABI-TILL consortium); sugar beet transformation with RNAi and overexpression constructs and pre-selection of transgenic events (SESVanderHave); regeneration of plants, vernalization and phenotyping for altered bolting behavior (Strube Research).

II. Eingehende Darstellung

1. der Verwendung der Zuwendung und des erzielten Ergebnisses im Einzelnen, mit Gegenüberstellung der vorgegebenen Ziele

II.1.1 Genome-wide identification and expression profiling of floral transition genes

CAU Kiel's first objective was to identify candidate genes for targeted genetic modification of flowering time in sugar beet on the basis of homology to known flowering time genes (Figure 1) and responsiveness of gene expression to vernalization. To this end, we performed tblastn-based sequence similarity searches and bidirectional best hit analyses in GenBank (http://blast.ncbi.nlm.nih.gov), the public sugar beet EST database BvGI (versions 1.0 to 3.0, http://compbio.dfci.harvard.edu/tgi), and the GABI Primary Database (http://www.gabipd.org) with flowering time control genes from *A. thaliana* and other model species. This analysis identified several putative orthologs of floral regulators in *A. thaliana* from all major regulatory pathways. In addition, work in other projects in the PI's group identified two potential key regulators of floral transition, i.e. (i) the long-sought 'bolting gene', which we termed *BvBTC1* (*BOLTING TIME CONTROL 1*; Pin *et al*., 2012), and (ii) a sugar beet homolog of the floral integrator gene *FT*. Although *BvBTC1* was first thought to be the central regulator of annuality and bolting without a requirement for vernalization, several data indicated that it may also have a regulatory function in biennials, including our observations that it has a full-length coding sequence, biennial haplotypes do not differ from annual haplotypes at evolutionarily conserved positions, and diurnal *BvBTC1* expression is similar (albeit not identical) in biennials as in annuals. *FT* is a central regulator of flowering time in *A. thaliana* and integrates signals from various floral regulatory pathways, and *ft* mutants are late-flowering and non- or only weakly responsive to vernalization (Koornneef *et al*., 1991, 1998; Kardailsky *et al*., 1999). A putative ortholog of *FT*, for which ESTs were absent from public EST collections, was identified by RT-PCR and RACE in a previous project (Eurotrans-Bio-Blossom). To further characterize this gene and facilitate a functional analysis by TILLing, we identified the full-length genomic sequence of ~12kb including ~3kb of promoter sequence, exon-intron structure, and

~2kb of the downstream intergenic region by BAC library screening and primer walking. This gene was later found to correspond to one of two *FT*-like genes recently described in sugar beet (Pin *et al*., 2010) and will be referred to as *BvFT1* in accordance with the nomenclature by Pin and colleagues. Both *BvBTC1* and *BvFT1* were deemed prime candidates for targeted modification of flowering time in sugar beet and further analyzed in the current project. Furthermore, sugar beet homologs of the vernalization pathway genes *FLC* and *LHP1* were also selected for further analysis. *FLC* is a central repressor of floral transition in Arabidopsis and is down-regulated by vernalization, and overexpression of *FLC* leads to a delay in flowering after vernalization. Shortly before the begin of the current project, an *FLC* homolog (*BvFL1*) was isolated from beet, shown to be functionally related to *FLC* in Arabidopsis and regulated by vernalization in beet (Reeves *et al*., 2007), but a functional analysis in beet was lacking. *LHP1* is involved in the maintenance of the inactive chromatin state of *FLC* following vernalization but also represses *FT*, and *lhp1* mutants in Arabidopsis have either early-flowering or late-flowering phenotypes depending on the genetic background of the mutants and the environmental conditions analyzed. A full-length *LHP1* homolog in sugar beet was isolated by the PI's group in the Eurotrans-Bio-Blossom project and its expression shown to be regulated by vernalization. Thus, together, the genes listed above constitute a first set of candidate genes and include the only two vernalization pathway gene homologs identified at the time in beet, *BvFL1* and *BvLHP1*, a homolog of the key floral integrator gene *FT*, and the central regulator of bolting in beet, *BvBTC1*.

In a complementary approach, floral transition candidate genes were selected on the basis of genome-wide expression profiling of vernalization response by SuperSAGE (Matsumara *et al*., 2008). Young immature leaves harvested at various time points during a vernalization time-course experiment (Figure 2) were used to produce nine SuperSAGE libraries containing 1.7 to 2.2 x10^6 sequenced tags/library (giving a total of 17.4 x10^6 tags; Table 1). Immature (non-expanded) leaves were chosen because they were postulated by grafting (Stout, 1945; Curtis and Hornsey, 1964) and defoliation experiments (Crosthwaite and Jenkins, 1993) to be the site of vernalizing temperature perception and early events during the transition to the flowering competent phase in sugar beet. Expression data from Arabidopsis and temperate cereals suggested that key regulators of vernalization response are differentially regulated in response to vernalization. For example, the floral inducer gene *VRN1* in cereals is gradually up-regulated during prolonged periods of cold temperatures (Danyluk *et al*., 2003; Trevaskis *et al*., 2003; Yan *et al*., 2003). We therefore used gradual expression changes during vernalization as indicator for a function in vernalization-dependent control of flowering. To facilitate annotation of tags and using cost-effective second generation sequencing technology (Illumina), we sequenced cDNA pooled from all nine samples and generated a database of 20106 cDNA contigs which combines publicly available sugar beet EST data and the newly generated cDNA sequences (Table 2).

Table 1. Tag counts in nine SuperSAGE libraries produced in the vernalization time-course experiment.

	Number of SuperSAGE tags per library									
Library	T_0	T_1	T_2	T_3	T_4	T_{1V}	T_{2V}	T_{3V}	T_{4V}	Total
Tags (x10^6)	2.01	2.23	2.12	1.74	2.17	1.74	1.85	1.77	1.78	17.41
Unique tags (x10^3)	54.9	54.3	50.2	56.6	57.5	54.9	57.5	56.5	55.5	67.5

Table 2. Reference cDNA sequences for tag annotation.

	Number of Illumina reads	Number of contigs	Average size of contigs [bp]	Maximal size of contigs [bp]
GABI-GENOFLOR cDNA library	75.167.775	81.127	416	3.548
Combined with public EST/TCs (MIRA assembly)	n.a.	20.106	798	3.850

Figure 3 shows the criteria for identification of tags which are significantly up-regulated in several library comparisons. Among 67515 unique tags identified by SuperSAGE, and using the p-statistics for transcript profiles devised by Audic and Claverie (1997; 99% confidence level), 490 tags are continuously upregulated during vernalization, while also being upregulated when compared to non-vernalized controls (Figure 4a; Table 3). 176 tags were identified when corresponding criteria for down-regulation of gene expression were used (Figure 4b; Table 3). We used RNAi for functional analyses and suppression of bolting in transgenic plants and had proposed to target candidate genes for floral promoter genes. We further reasoned that genes which are up-regulated by vernalization, a major stimulus of flowering in plants, are likely to include floral promoter genes, whereas genes which are down-regulated by vernalization may have a repressive effect on flowering. This assumption is consistent with expression data in the literature for the floral promoter gene *VRN1* in cereals (s. above) and the floral repressor genes *FLC* in Arabidopsis and *BvFT1* in beet, as shown recently by Pin *et al.* (2010). Thus, candidate floral promoter genes were selected among genes which are positively regulated by vernalization. Of the 490 tags which are gradually up-regulated, 169 could be assigned to a cDNA/EST contig in cDNA database generated in the current project. These contigs were annotated using BLAST and BLAST2GO (http://www.blast2go.org; Figure 5). Among these, three genes were annotated as having transcription factor activity (Figure 5). By further sequence analysis, the corresponding cDNA contigs were identified as the *FLC* homoglog *BvFL1* (Reeves et al, 2007) and homologs of other transcription factors which will be referred to as *BvTFCs*. Up-regulation of *BvFL1* is in contrast to the previously published report by Reeves *et al.* (2007) and the regulation of *FLC* in Arabidopsis, where the gene is down-regulated by vernalization, suggesting that *FLC* in *A. thaliana* and *BvFL1* may not be functionally equivalent. Further GO and sequence analysis of all cDNA contigs identified one gene which was annotated with the GO terms “nucleic acid binding” (“molecular function", level 3; Figure 5) and “regulation of transcription” (“biological process”, level 7). Intriguingly, a second vernalization-responsive gene in the same GO subcategory is *BvBTC1*, further supporting the notion that this gene may play a functional role in biennials (s. above) and may also be involved in the vernalization response. The remaining genes were assigned to a broad spectrum of molecular functional categories and included a considerable fraction of putative stress response genes including e.g. oxido-reductases and homologs of various metabolic pathway genes and enzymes without known functions in flowering time control. Finally, by using less stringent criteria for up-regulation of gene expression during vernalization, a homolog of a vernalization response gene in *Arabidopsis thaliana* was identified as an additional candidate gene. This gene is only very weakly expressed in all samples of the vernalization time course analyzed by SuperSAGE except for time point t3V (three months after begin of vernalization treatment), where it is significantly up-regulated according to a statistical analysis using the p-statistics by Audic and Claverie (1997).

In conclusion, sequence and expression analyses and literature reviews led to selection of eight candidate genes (*BvBTC1*, *BvFT1*, *BvFL1*, *BvLHP1*, *BvTFC1-4*) which were used for construction of 11 transformation vectors for functional analyses in transgenic beets (s. below). Several further genes whose expression is regulated by vernalization may also contribute to flowering time regulation but were not further considered here because of the more speculative nature of this possibility and the limited number of transgene cassettes planned in the project. According to our milestone plan for the project, it was intended to identify 15-20 *B. vulgaris* floral transition gene candidates and to select the best candidate genes on the basis of the available information for construction of 10 transgene cassettes. Thus, these project results are in good agreement with the original objectives. In further accordance with the milestone plan, the expression profiles of vernalization-responsive candidate genes identified by SuperSAGE were verified by RT-qPCR analysis.

Table 3. Numbers of tags which are differentially expressed at $p \leq 0.01$ (as determined using DiscoverySpace; Robertson *et al.*, 2007) during the vernalization time-course experiment.

Pairwise comparison of libraries	No. of differentially expressed tags	No. of tags up-regulated	No. of tags down-regulated
T4V vs T4	7.165	2.877	4.288
T3V vs T3	12.103	7.717	4.386
T2V vs T2	13.575	8.698	4.877
T1V vsT1	9.536	4.343	5.193
T3V vs T2V	6.988	4.110	2.878
T2V vs T1V	12.442	8.420	4.022

II.1.2 Generation of transformation vectors

The function of floral transition candidate genes was analyzed by transformation of RNAi and/or overexpression constructs into sugar beet. While the majority of candidate genes were considered to be putative floral promoters and were thus targeted by RNAi, the available data for *BvFL1* were ambiguous. *BvFL1* was identified by Reeves *et al.* (2007) as a putative ortholog and functional relative of the central regulator of vernalization response and floral repressor gene *FLC* in Arabidopsis, and overexpression of *AtFLC* in sugar beet was reported to result in a delay in bolting following vernalization (International Patent Application Number WO 2007/122086). Moreover, under continuous low light conditions, *BvFL1* was found to be down-regulated during vernalization, which is consistent with *FLC* regulation in Arabidopsis. These data suggested that overexpression of *BvFL1* in sugar beet may also delay, or suppress bolting. However, our SuperSAGE data had revealed gradual up-regulation of *BvFL1* during vernalization under short-day conditions. In accordance with the data by Reeves *et al.*, our SuperSAGE data showed that *BvFL1* expression after vernalization is reset to pre-vernalization levels. In consideration of the conflicting expression data and complex control of *BvFL1*, and to further analyze the functional role of *BvFL1* in vernalization-responsive bolting control, we included both a *BvFL1* overexpression construct (using splice variant 3 which was shown by Reeves *et al.* to complement the phenotype of an Arabidopsis *flc* mutant) and an RNAi construct (targeting the 3'UTR of *BvFL1* to maximize target gene specificity) in the set of constructs for transformation into sugar beet. A total of three RNAi cassettes were constructed for *BvBTC1*. While one of the constructs was designed to target the 5' UTR of *BvBTC1* to reduce the risk of off-target effects and thus facilitate the functional analysis of this gene, two additional cassettes targeted the two conserved domains in *BvBTC1*, the response regulator receiver (REC) domain and the CONSTANS, CONSTANS-LIKE and TOC1 (CCT) domain. These domains were targeted in an attempt to co-silence additional members of the same family and maximize the intended suppressive effect on bolting, the rationale being that homologous pseudo-response regulator genes in Arabidopsis have partially overlapping and redundant functions and that CCT domains are also found in other floral promoter genes. All other genes were targeted by one RNAi construct each. In total, 24 plasmids were constructed, of which 11 vectors carried the final overexpression or RNAi transgene cassettes for transformation into sugar beet, which is consistent with the milestone plan.

II.1.3 TILLing of candidate genes

To start exploring the feasibility of the use of EMS mutants as a non-transgenic alternative to sugar beet transformants for future winter beet breeding programs, the candidate genes were also screened for sequence variation by TILLing in collaboration with the sugar beet group of the GABI-TILL consortium. Because we primarily selected putative floral promoter genes and mutagenesis generally results in loss-of-function (or reduction-of-function) mutations, EMS mutations in our candidate genes may be expected to delay or suppress bolting. A TILLing screen identified a total of 51 sequence variants in six different target fragment analyzed. However, because of a high degree of heterogeneity in the accession used for mutagenesis, which became apparent during the course of the study, sequencing of the putative mutants identified by TILLing revealed that the majority of sequence variants appeared to result from naturally occurring allelic variation.

II.1.4 Target gene expression analysis in sugar beet transformants

The eleven constructs were introduced into sugar beet by polyethylene glycol-mediated DNA transfer. Independent transformants were pre-selected for the presence of transgene inserts and low transgene copy numbers by PCR, TaqMan assays and Southern analysis. Transformants with low copy numbers (one to three) were multiplied by cloning and analyzed for bolting time or bolting resistance following vernalization and reduction in target transcript accumulation by RT-qPCR.

A total of 97 transgenic events derived from transformation with all eleven constructs (transformation series GFA to GFK; Table 6) were analyzed, with each construct being represented by 7-13 independent events. For each transgenic event, leaf samples of three clones each (with one exception, event number GFB087, for which only one clone could be regenerated) were harvested from non-vernalized plants in the greenhouse at Strube at Zeitgeber time (ZT) 6h - 8h and used for RNA extraction and cDNA synthesis. In total, approximately 300 plants were analyzed, including the transgenic plants and 18 non-transgenic biennial control plants. Three technical replicates were performed for each RT-qPCR reaction. To determine RT-qPCR efficiencies and serve as positive controls, the endogenous target transcript regions analyzed by RT-qPCR were cloned into the cloning vector pGEM-T. All plant samples were assayed for expression of

the respective RNAi or overexpression target gene and the housekeeping gene *BvGAPDH*, which was used as a reference gene for normalization of the RT-qPCR data.

The results of the RT-qPCR analysis are shown in Table 4. All three constructs targeting *BvBTC1* (pS163, pS165, pS166) resulted in strong down-regulation of the target gene in a subset of transgenic events. Expression analysis of *BvPRR7*, a *BvBTC1* homolog which we identified among the cDNA contigs generated by Illumina sequencing in the present study, revealed that down-regulation of *BvBTC1* by RNAi transgenes targeting the conserved CCT domain region did not result in concomitant down-regulation of the putative "off-target" gene *BvPRR7*. For the target gene *BvLHP1* (construct pS164), the expression data indicated only very weak down-regulation of the target gene, suggesting that the corresponding RNAi construct was not effective or that strong down-regulation of the target gene may be detrimental to the plant. With the exception of *BvLHP1*, the RT-qPCR expression analysis demontrated the successful down-regulation of all RNAi target genes under study in 20% to 92% of the transgenic events analyzed. Furthermore, seven out of eight events derived from transformation with the *BvFL1* over-expression construct indeed showed strong up-regulation of *BvFL1* expression. In general, the observed changes in expression levels were largely consistent among the three clones representing a given transgenic event.

According to the project proposal it was intended to analyze target gene expression in 5-10 events per construct. Here, we analyzed 7-13 events per construct and were able to show the expected down- or up-regulation of the respective target gene in approximately half of all 97 transgenic events analyzed. Thus, in conclusion, this major and final original milestone was fully met.

II.1.5 Identification of downstream target genes by gene expression analysis

We expanded the molecular characterization of select transformants in which the respective target genes were down- or up-regulated by also assaying expression changes of genes presumed to be potential downstream target genes within regulatory cascades controlling floral transition. In addition, because all our RNAi target genes are regulated by vernalization, we harvested leaf tissue samples at two additional time points, i.e. at the end of vernalization and four weeks post-vernalization. Expression analysis of putative downstream genes at different time points may facilitate the detection of regulatory interactions which otherwise may go undetected. For example, the downstream effects of RNAi targeting a gene which is only lowly expressed prior to vernalization, but which is highly expressed after vernalization, may be more easily detectable in samples harvested after vernalization. This analysis was performed for the four target genes of the seven transformation series GFA to GFG. All three sets of transformants produced with *BvBTC1* RNAi constructs included transformants which failed to bolt for more than five months after vernalization under the climate chamber conditions tested. In addition, besides clones which did not bolt, five events also comprised clones where stem elongation reached or passed the threshold value. However, bolting was delayed for these clones compared to the non-transgenic controls. Furthermore, for most of the respective transgenic events only a small subset of clones reached the threshold stem size, and these clones had a stunted appearance. The phenotypic data are highly consistent with the RT-qPCR expression analysis in that all events in which *BvBTC1* was down-regulated showed bolting suppression, whereas events in which the target gene was not down-regulated comprised only clones that bolted. Gene expression analysis of downstream candidate genes in RNAi plants before and after vernalization (Figures 6 and 7) revealed critical regulatory interactions between *BvBTC1*, *BvFT1* and *BvFT2* and enabled us to propose a model for bolting control in biennial beets (Figure 8). By contrast, our data also suggest that a beet homolog of the central vernalization pathway gene *FLC* in Arabidopsis appears to play only a relatively minor role in bolting control, and that it is not a major regulator of components of the regulatory *BvBTC1/BvFT1/BvFT2* module. Together, these data demonstrate evolutionary diversification of bolting control in the model dicot species *A. thaliana* and the only distantly related dicot crop species *B. vulgaris*, and constitute a significant advancement of our knowledge of bolting control in beet. The results of the RNAi analysis of *BvBTC1* are reported in Pin *et al.* (2012). A manuscript describing the results for *BvFL1*, *BvFT1* and *BvLHP1* is in preparation. As originally proposed, the phenotypic evaluation of the transformants derived from the final four transformation series is currently being continued by the industrial partners.

Table 4. Primary sugar beet transformants analyzed by RT-qPCR.

Vector	Construct type	Trans-formation series	Transgenic event code number	PAT gene copy number[1]	Effector transgene copy number[1]	Target gene expression level
pS163	RNAi	GFA	001-01F	1	2	not down
pS163	RNAi	GFA	001-02B	2	2	down
pS163	RNAi	GFA	001-11H	2	3	down
pS163	RNAi	GFA	002-10H	3	2	not down
pS163	RNAi	GFA	GFA011	2	3	not down
pS163	RNAi	GFA	GFA036	2	2	not down
pS163	RNAi	GFA	GFA057	2?	1?	down
pS163	RNAi	GFA	GFA068	2	2	not down
pS163	RNAi	GFA	GFA080	2?	1	down
pS164	RNAi	GFB	004-10G	3	1-2	not down
pS164	RNAi	GFB	008-10F	1-2	1	not down
pS164	RNAi	GFB	009-06D	3	3	not down
pS164	RNAi	GFB	009-11H	3	3	not down
pS164	RNAi	GFB	009-12G	1	3	down?
pS164	RNAi	GFB	GFB028	1?	1?	not down
pS164	RNAi	GFB	GFB070	1	2	not down
pS164	RNAi	GFB	GFB087	4	4	down
pS164	RNAi	GFB	GFB124	2	2?	down?
pS165	RNAi	GFC	005-01G	2	1	not down
pS165	RNAi	GFC	005-10A	2	2	not down
pS165	RNAi	GFC	005-11C	2	2	not down
pS165	RNAi	GFC	005-11E	3	3	down
pS165	RNAi	GFC	006-02C	2-3	3	down
pS165	RNAi	GFC	006-04D	1	2-3	not down
pS165	RNAi	GFC	006-07H	3	3	down
pS165	RNAi	GFC	006-11D	0-1	2	not down
pS165	RNAi	GFC	GFC068	1?	1?	not down
pS165	RNAi	GFC	GFC093	1?	1?	not down
pS165	RNAi	GFC	GFC134	3?	1?	not down
pS166	RNAi	GFD	010-09C	1	1	down
pS166	RNAi	GFD	010-09H	1-2	1	down
pS166	RNAi	GFD	010-12B	1	1	down
pS166	RNAi	GFD	011-01F	1	1	down
pS166	RNAi	GFD	011-03E	1	2	down
pS166	RNAi	GFD	011-04E	3	1	not down
pS166	RNAi	GFD	015-08D	2	3	down
pS167	RNAi	GFE	014-01C	2	2	not down
pS167	RNAi	GFE	014-02G	1	1	not down
pS167	RNAi	GFE	014-07F	1-2	1	down
pS167	RNAi	GFE	014-08B	3	2	not down
pS167	RNAi	GFE	018-06E	1	2	down
pS167	RNAi	GFE	018-09A	2	1	down
pS167	RNAi	GFE	018-12H	2	1	not down
pS167	RNAi	GFE	019-01E	3-4	3	down
pS167	RNAi	GFE	020-01C	2	1	down
pS167	RNAi	GFE	020-01E	2	2	down
pS167	RNAi	GFE	020-05G	2-3	2	down
pS168	overexpression	GFF	016-05C	1	1	up
pS168	overexpression	GFF	016-10A	2	3	up
pS168	overexpression	GFF	016-11C	2	1	up
pS168	overexpression	GFF	017-06C	2-3	2-3	up
pS168	overexpression	GFF	017-07B	1	1	up
pS168	overexpression	GFF	017-07C	1	2	up

Vector	Construct type	Trans-formation series	Transgenic event code number	PAT gene copy number[1]	Effector transgene copy number[1]	Target gene expression level
pS168	overexpression	GFF	021-01G	1	1	not up
pS168	overexpression	GFF	021-09F	1	2-3	up
pS169	RNAi	GFG	019-07G	2	2	not down
pS169	RNAi	GFG	019-10E	1	1	not down
pS169	RNAi	GFG	021-11G	2	1	down
pS169	RNAi	GFG	021-12A	2	2	not down
pS169	RNAi	GFG	021-12H	2	2	not down
pS169	RNAi	GFG	022-06B	1	1?	not down
pS169	RNAi	GFG	022-10F	2	3	not down
pS169	RNAi	GFG	022-11A	2	2	not down
pS169	RNAi	GFG	024-11E	1	1	not down
pS169	RNAi	GFG	024-12E	1	1	down
pFT063	RNAi	GFH	027-01D	1	3	down
pFT063	RNAi	GFH	027-01H	1	1?	down
pFT063	RNAi	GFH	027-04C	1	1-2	down
pFT063	RNAi	GFH	028-03C	2	1	not down
pFT063	RNAi	GFH	029-03E	1	2-3	weakly down
pFT063	RNAi	GFH	029-02D	1	1	weakly down
pFT063	RNAi	GFH	029-04D	1?	1?	weakly down
pFT063	RNAi	GFH	029-02C	1	3	down
pFT063	RNAi	GFH	029-12H	1	2	weakly down
pFT063	RNAi	GFH	031-02D	1	1?	weakly down
pFT063	RNAi	GFH	031-04G	1-2	1-2	weakly down
pFT063	RNAi	GFH	032-12E	1	2	weakly down
pFT063	RNAi	GFH	034-05G	1	2	weakly down
pFT065	RNAi	GFI	027-09A	3?	2?	not down
pFT065	RNAi	GFI	027-09H	n.d.	n.d.	not down
pFT065	RNAi	GFI	027-10B	1-2	2	not down
pFT065	RNAi	GFI	027-11D	1-2	3	down
pFT065	RNAi	GFI	027-11F	3	2-3	down
pFT065	RNAi	GFI	030-10F	n.d.	n.d.	not down
pFT065	RNAi	GFI	030-12A	n.d.	n.d.	not down
pFT065	RNAi	GFI	030-12H	n.d.	n.d.	not down
pFT065	RNAi	GFI	033-05E	n.d.	n.d.	not down
pFT065	RNAi	GFI	033-10A	3	2	not down
pFT068	RNAi	GFJ	035-03D	2-3	1	down
pFT068	RNAi	GFJ	035-04E	3	1	down
pFT068	RNAi	GFJ	035-05G	1-2	1-2	down
pFT068	RNAi	GFJ	035-10A	3	2	down
pFT068	RNAi	GFJ	035-05H	1	1	down
pFT068	RNAi	GFJ	036-04E	n.d.	n.d.	down
pFT068	RNAi	GFJ	037-07C	1	1	down
pFT068	RNAi	GFJ	037-04D	n.d.	n.d.	not down
pFT068	RNAi	GFJ	036-01G	4	2-3	not down
pFT068	RNAi	GFJ	040-01F	n.d.	n.d.	not down
pFT070	RNAi	GFK	036-08E	3	3	down
pFT070	RNAi	GFK	038-11H	n.d.	n.d.	down
pFT070	RNAi	GFK	039-03C	n.d.	n.d.	down
pFT070	RNAi	GFK	037-09G	1-2	1	down
pFT070	RNAi	GFK	037-12F	2	?	not down
pFT070	RNAi	GFK	037-12G	n.d.	n.d.	down?
pFT070	RNAi	GFK	041-05E	n.d.	n.d.	down?

[1] Question marks indicate non-unambiguous data.

References

Abou-Elwafa, S.F., Büttner, B., Chia, T.Y.P., Schulze-Buxloh, G., Hohmann, U., Mutasa-Göttgens, E., Jung, C., Müller, A.E. (2011) Conservation and divergence of autonomous pathway genes in the flowering regulatory network of *Beta vulgaris*. *J. Exp. Bot.* 62:3359–3374.

Albert, V.A., Soltis, D.E., Carlson, J.E., Farmerie, W.G., Wall, P.K., Ilut, D.C., Solow, T.M., Mueller, L.A., Landherr, L.L., Hu, Y., Buzgo, M., Kim, S., Yoo, M.J., Frohlich, M.W., Perl-Treves, R., Schlarbaum, S.E., Bliss, B.J., Zhang, X., Tanksley, S.D., Oppenheimer, D.G., Soltis, P.S., Ma, H., de Pamphilis, C.W., Leebens-Mack, J.H. (2005) Floral gene resources from basal angiosperms for comparative genomics research. *BMC Plant Biol.* 5:5.

Audic, S. and Claverie, J.M. (1997) The significance of digital gene expression profiles. *Genome Res.* 7:986-995.

Bäurle, I. and Dean, C. (2006) The timing of developmental transitions in plants. *Cell* 125:655-664.

Chia, T.Y.P., Müller, A., Jung, C., Mutasa-Göttgens, E.S. (2008) Sugar beet contains a large *CONSTANS-LIKE* gene family including a *CO* homologue that is independent of the early-bolting (*B*) gene locus. *J. Exp. Bot.* 59:2735-2748.

Crosthwaite, S.K. and Jenkins, G.I. (1993) The role of leaves in the perception of vernalizing temperatures in sugar-beet. *J. Exp. Bot.* 44:801-806.

Curtis, G.J. and Hornsey, K.G. (1964) Graft-transmissible induction of elongation and flowering in scions of sugar beet bred for resistance to bolting. *Nature* 202:1238.

Danyluk, J., Kane, N.A., Breton, G., Limin, A.E., Fowler, D.B., Sarhan, F. (2003) TaVRT-1, a putative transcription factor associated with vegetative to reproductive transition in cereals. *Plant Physiol.* 132:1849-1860.

He, Y.H. and Amasino, R.M. (2005) Role of chromatin modification in flowering-time control. *Trends Plant Sci.* 10:30-35.

Hecht, V., Foucher, F., Ferrandiz, C., Macknight, R., Navarro, C., Morin, J., Vardy, M.E., Ellis, N., Beltran, J.P., Rameau, C., Weller, J.L. (2005) Conservation of Arabidopsis flowering genes in model legumes. *Plant Physiology* 137:1420-1434.

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

Kardailsky, I., Shukla, V.K., Ahn, J.H., Dagenais, N., Christensen, S.K., Nguyen, J.T., Chory, J., Harrison, M.J., Weigel, D. (1999) Activation tagging of the floral inducer *FT*. *Science* 286:1962-1965.

Koornneef, M., Hanhart, C.J., van der Veen, J.H. (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 229:57-66.

Koornneef, M., Alonso-Blanco, C., Blankestijn-de Vries, H., Hanhart, C.J., Peeters, A.J.M. (1998) Genetic interactions among late-flowering mutants of Arabidopsis. *Genetics* 148:885–892.

Lee, J.H., Cho, Y.S., Yoon, H.S., Suh, M.C., Moon, J., Lee, I., Weigel, D., Yun, C.H., Kim, J.K. (2005) Conservation and divergence of *FCA* function between Arabidopsis and rice. *Plant Mol. Biol.* 58:823-838.

Matsumura, H., Reich, S., Ito, A., Saitoh, H., Kamoun, S., Winter, P., Kahl, G., Reuter, M., Krüger, D.H., Terauchi, R. (2003) Gene expression analysis of plant host-pathogen interactions by SuperSAGE. *Proc. Nat. Acad. Sci. U.S.A.* 100:15718-15723.

Matsumura, H., Bin Nasir, K.H., Yoshida, K., Ito, A., Kahl, G., Krüger, D.H., Terauchi, R. (2006) SuperSAGE array: the direct use of 26-base-pair transcript tags in oligonucleotide arrays. *Nature Methods* 3:469-474.

Matsumura, H., Ito, A., Saitoh, H., Winter, P., Kahl, G., Reuter, M., Krüger, D.H., Terauchi, R.(2004) SuperSAGE. *Cellular Microbiology* 7:11-18.

Matsumura, H., Krüger, D.H., Kahl, G., Terauchi, R. (2008) SuperSAGE: A modern platform for genome-wide quantitative transcript profiling. *Current Pharmaceutical Biotechnology* 9:368-374.

Mutasa-Göttgens, E.S., Joshi, A., Holmes, H.F., Hedden, P., Göttgens, B. (2012) A new RNASeq-based reference transcriptome for sugar beet and its application in transcriptome-scale analysis of vernalization and gibberellin responses. *BMC Genomics* 13:99.

Pestsova, E., Meinhard, J., Menze, A., Fischer, U., Windhovel, A., Westhoff. P. (2008) Transcript profiles uncover temporal and stress-induced changes of metabolic pathways in germinating sugar beet seeds. *BMC Plant Biol.* 8:122.

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

Pin, A., Zhang, W., Vogt, S.H., Dally, N., Büttner, B., Schulze-Buxloh, G., Jelly, N.S., Chia, T.Y.P, Mutasa-Göttgens, E.S., Dohm, J.C., Himmelbauer, H., Weisshaar, B., Kraus, J., Gielen, J.J.L., Lommel, M., Weyens, G., Wahl, B., Schechert, A., Nilsson, O., Jung, C., Kraft, T., Müller, A.E. (2012) The role of a pseudo-response regulator gene in life cycle adaptation and domestication of beet. *Curr. Biol.* 22:1095-1101.

Putterill, J., Laurie, R., Macknight, R. (2004) It's time to flower: the genetic control of flowering time. *BioEssays* 26:363-373.

Reeves, P.A., He, Y., Schmitz, R.J., Amasino, R.M., Panella, L.M., Richards, C.M. (2007) Evolutionary conservation of the *FLOWERING LOCUS C*-mediated vernalization response: evidence from the sugar beet (*Beta vulgaris*). *Genetics* 176:295-307.

Robertson, N., Oveisi-Fordorei, M., Zuyderduyn, S.D., Varhol, R.J., Fjell, C., Marra, M., Jones, S., Siddiqui, A (2007) DiscoverySpace: an interactive data analysis application. *Genome Biol.* 8:R6.

Shitsukawa, N., Ikari, C., Shimada, S., Kitagawa, S., Sakamoto, K., Saito, H., Ryuto, H., Fukunishi, N., Abe, T., Takumi, S., Nasuda, S., Murai, K. (2007) The einkorn wheat (*Triticum monococcum*) mutant, maintained vegetative phase, is caused by a deletion in the *VRN1* gene. *Genes & Genetic Systems* 82:167-170.

Stout, M. (1945) Translocation of the reproductive stimulus in sugar beet. *Botanical Gazette* 107:86-95.

Trap-Gentil, M.V., Hebrard, C., Lafon-Placette, C., Delaunay, A., Hagege, D., Joseph, C., Brignolas, F., Lefèbvre, M., Barnes, S., Maury, S. (2011) Time course and amplitude of DNA methylation in the shoot apical meristem are critical points for bolting induction in sugar beet and bolting tolerance between genotypes. *J. Exp. Bot.* 62:2585-2597.

Trevaskis, B., Bagnall, D.J., Ellis, M.H., Peacock, W.J., Dennis, E.S. (2003) MADS box genes control vernalization-induced flowering in cereals. *Proc. Natl. Acad. Sci. U.S.A.* 100:13099-13104.

Figure legends

Figure 1. Floral transition genes in *Arabidopsis thaliana* and homologs in *Beta vulgaris.* Shown are the vernalization, photoperiod and autonomous pathways and floral integrator genes (modified from Jung and Müller, 2009). Gene names in bold letters indicate homology between flowering time regulators in *A. thaliana* and ESTs in *B. vulgaris* (at *E* values $<10^{-30}$). The green boxes mark genes for which orthologous gene sequences in sugar beet are available from published work as indicated (1, Reeves *et al.*, 2007; 2, Chia *et al.*, 2008; 3, Abou-Elwafa *et al.*, 2011; 4, Pin *et al.*, 2010).

Figure 2. Genome-wide expression profiling of vernalization response by SuperSAGE. SuperSAGE libraries were generated from cDNA prepared from young, immature leaves of a double-haploid sugar beet accession. Leaves were harvested at various time points (T_0, T_1, T_{1V} etc.) during a vernalization time-course experiment. Between $1.7x10^6$ and $2.2x10^6$ tags per library were sequenced. SD, short day (8 h light / 16 h dark) conditions.

Figure 3. Identification of vernalization-responsive candidates for bolting control genes in sugar beet. The graph shows the criteria for identification of tags which are significantly up-regulated in several library comparisons (as determined using DiscoverySpace and a 99% confidence level; Robertson *et al.*, 2007), and were used for the Venn diagram shown in Figure 4a.

Figure 4. Identification of vernalization-responsive candidates for bolting control genes in sugar beet. Shown in the Venn diagrams in a) and b) are the numbers of tags which are up- (a) or down-regulated (b) at $p \leq 0.01$ in vernalized samples compared to non-vernalized controls (as determined using DiscoverySpace4.0; Robertson *et al.*, 2007).

Figure 5. Annotation of vernalization-responsive SuperSAGE tags. Tags were assigned to cDNA contigs generated in the current project and annotated using BLAST2GO (http://www.blast2go.org).

Figure 6. Transcript accumulation of *BvBTC1*, *BvFT1* and *BvFT2* in *BvBTC1 RNAi* plants in long days (16 h light) at ZT6 before, at the end of, and after vernalization. Shown are the means of biological replicates (three clones per transgenic event). Error bars, mean ± SEM.

Figure 7. Diurnal expression profiles in *BvBTC1 RNAi* and non-transgenic biennial control plants in long days four weeks after vernalization. Shown are the means of biological replicates (three clones per transgenic event). Error bars, mean ± SEM.

Figure 8. Proposed model for a regulatory role of *BvBTC1* in biennial beets. The recessive allele in biennials is not sufficiently sensitive to long days (LD) or encodes a protein which is less active than in annuals and thus cannot transduce the inductive long-day signal in the first growing season. The cold-induced up-regulation of *BvBTC1* during winter and/or an enhancing activity of additional vernalization response factors restores the functionality of the gene and enables *BvBTC1* to stably repress *BvFT1* and activate *BvFT2*. Lines between genes do not imply direct interactions. Weak regulatory effects are indicated by grey lines. This model was published in Pin *et al.* (2012).

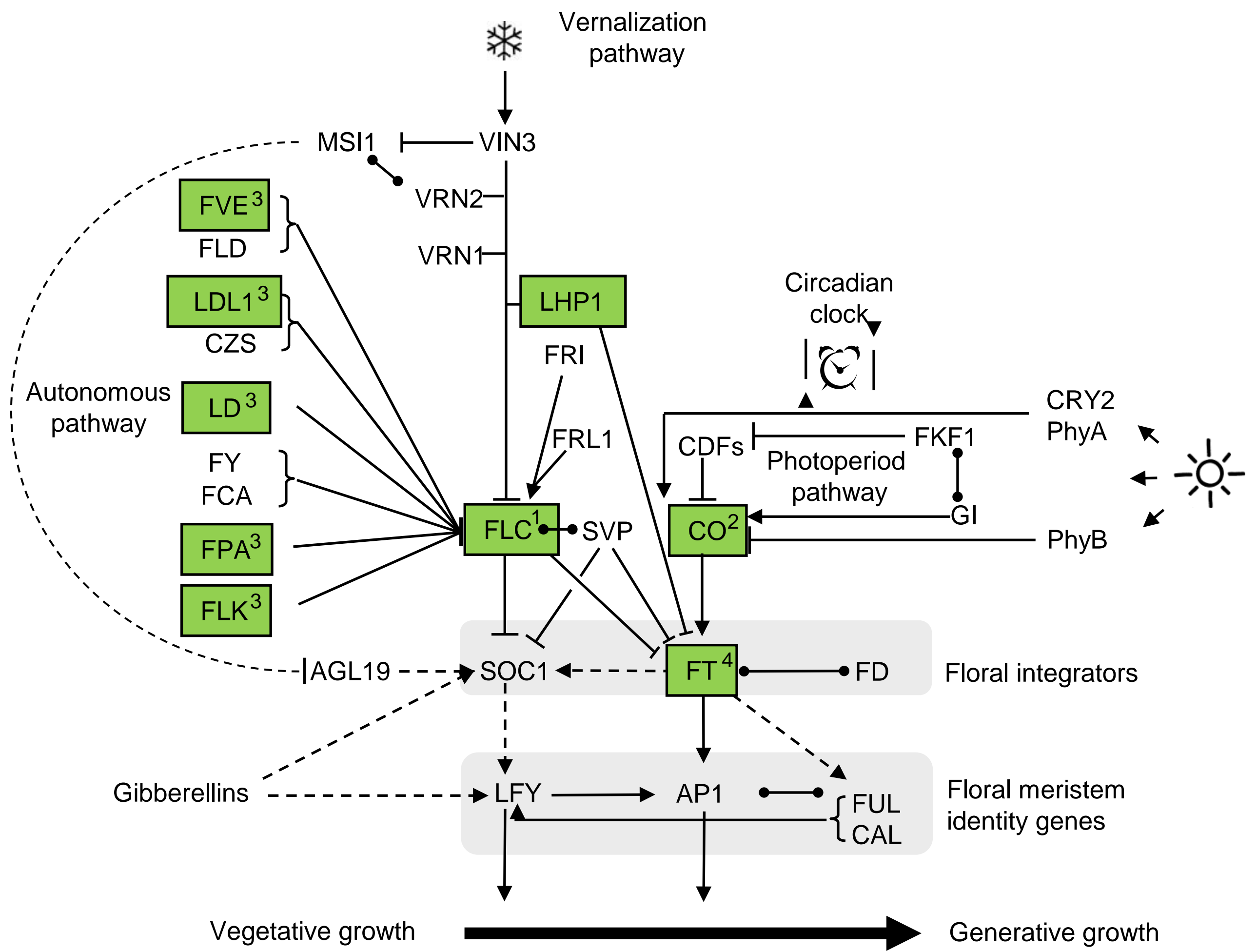


Figure 1

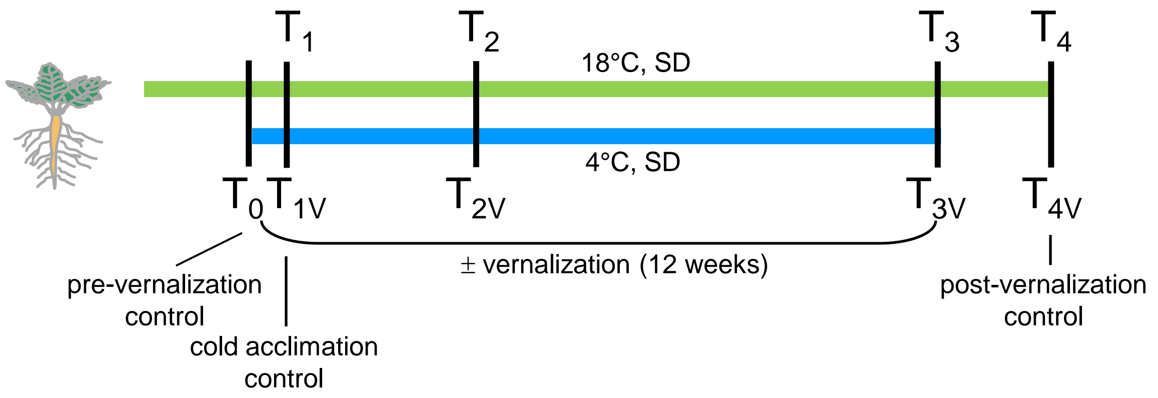


Figure 2

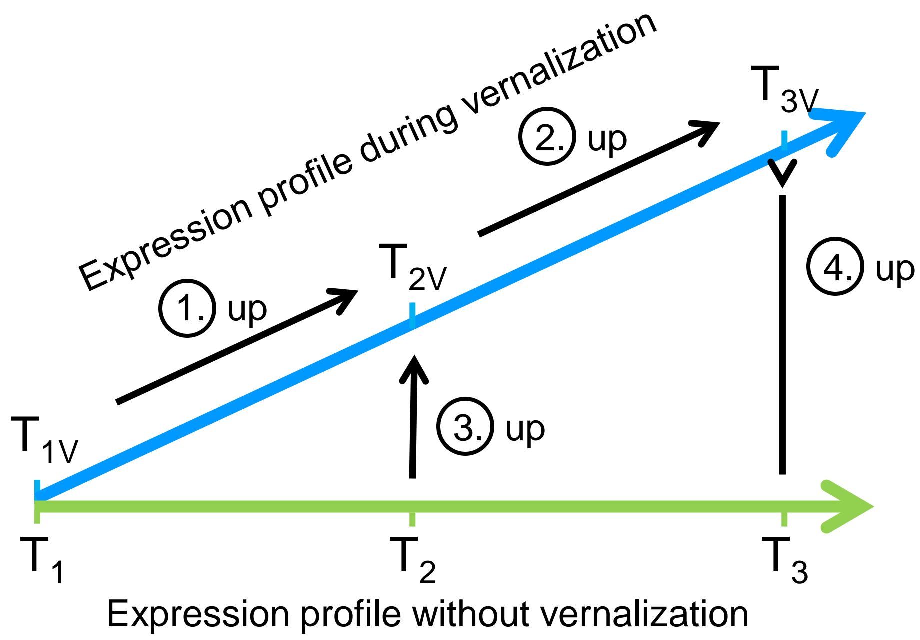
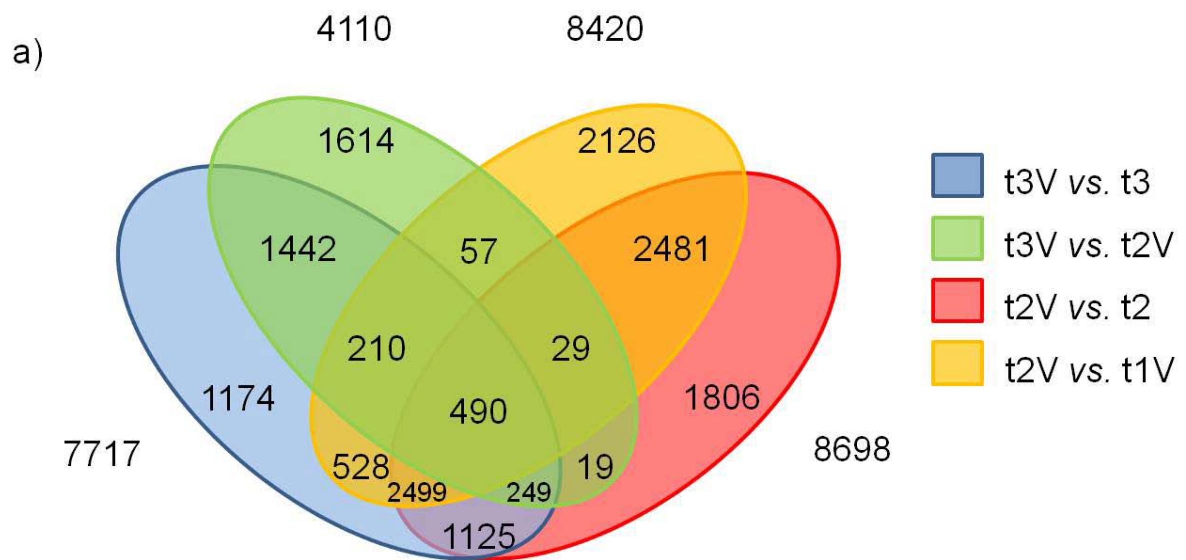


Figure 3



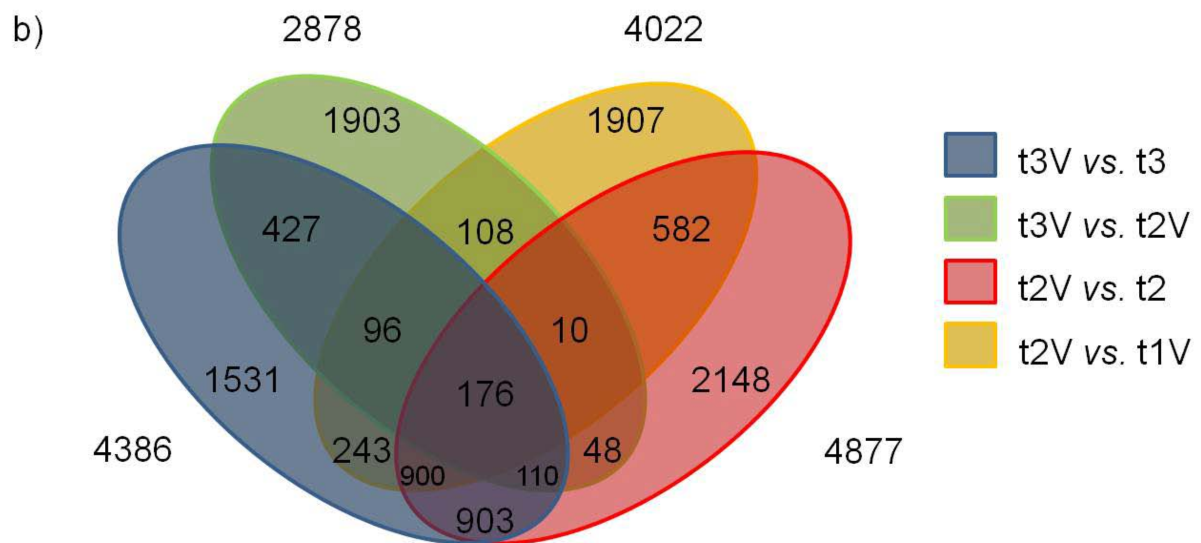


Figure 4

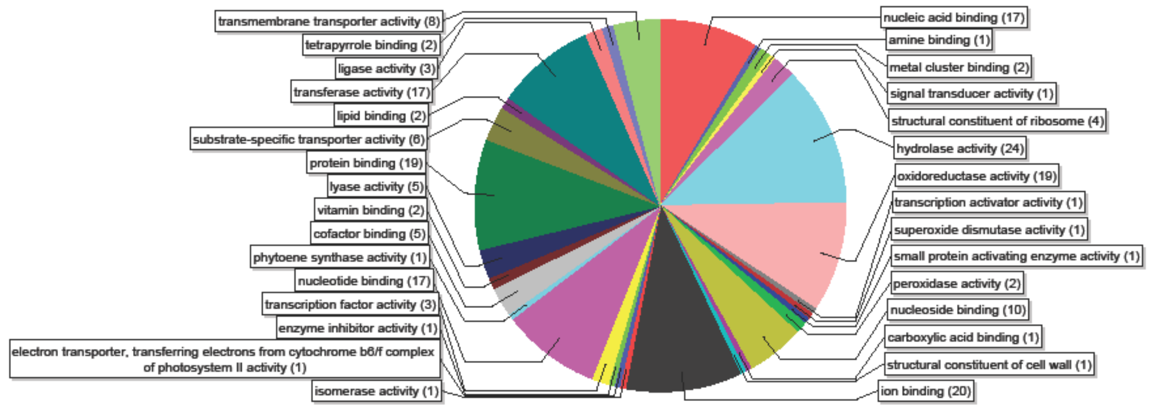


Figure 5

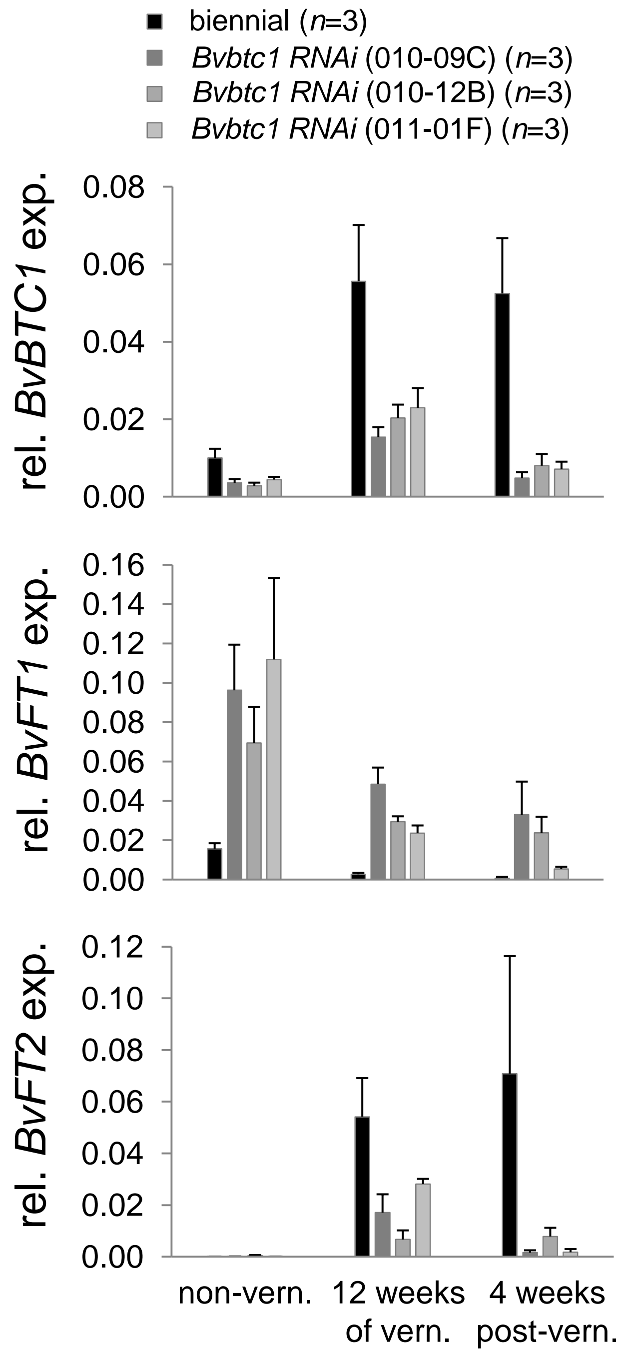


Figure 6

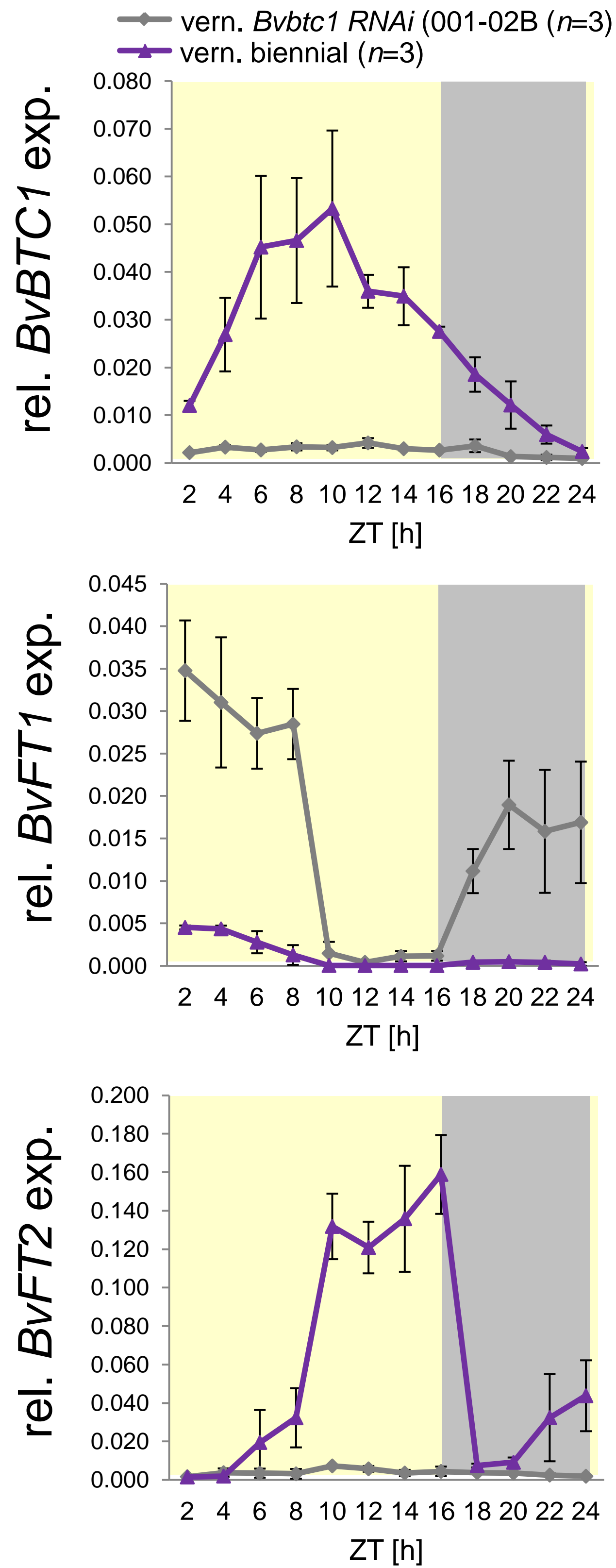


Figure 7

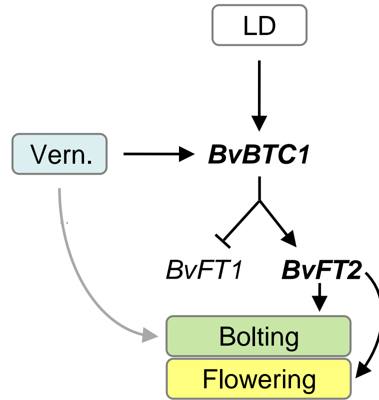


Figure 8

2. der wichtigsten Positionen des zahlenmäßigen Nachweises

A technician and a Ph.D. student were employed on the project for 36 months and 39 months, respectively. Costs of consumables occurred as shown below.

PCR, Real-time RT-qPCR and primers	9542.25 €
RNA extraction and cDNA synthesis	8403.83 €
Lab plastic ware	8304.85 €
Sequencing[1]	6000.00 €
Miscellaneous (enzymes, chemicals, DNA purification kits etc.)	7515.80 €
Sum	39766.73 €

[1] Included in the costs of material for sequencing are also the costs for sequencing of candidate sequence variants identified by TILLing, in accordance with the project proposal.

Travel expenses occurred for the project meetings, annual GABI status seminars, project presentations at national and international meetings, and collection of samples at our industrial project partners.

3. der Notwendigkeit und Angemessenheit der geleisteten Arbeit

The work carried out in the project was strictly result-oriented and necessary to reach the project goals. When new developments became apparent, work packages were adjusted in agreement with the *Projektträger Jülich* (PtJ).

4. des voraussichtlichen Nutzens, insbesondere der Verwertbarkeit des Ergebnisses im Sinne des fortgeschriebenen Verwertungsplans

Suppression of cold-induced flowering by targeted genetic modification of floral transition genes is expected to allow a significant expansion of the growing season and thus maximal growth of the storage root. When introduced into breeding programs for winter beets, the expected gains in yield may also contribute to further increase the potential of sugar beet as a source of bioethanol or biogas. The material generated in the current project is directly available to the participating breeders and will continue to be evaluated beyond the project period. Although in Europe GMOs are unlikely to enter the market in the foreseeable future, plant material and knowledge gained from the current project can be used for crop improvement strategies targeting other markets worldwide or alternative approaches currently under development.

5. des während der Durchführung des Vorhabens dem ZE bekannt gewordenen Fortschritts auf dem Gebiet des Vorhabens bei anderen Stellen

Several patent applications on the use of various genes for suppression of bolting were published by other parties during the running period of GABI-GENOFLOR, including patent applications on *BvFL1*, *BvBTC1* (referred to as *BvPRR7* in the respective patent applications), *BvFT1* and a *VIN3*-like gene. A major scientific publication described the identification and functional characterization of the *FT*-like genes *BvFT1* and *BvFT2* in beet (Pin *et al.*, 2010). Furthermore, one publication described epigenetic regulation of *BvFL1* and a *VIN3*-like gene in beet during vernalization (Trap-Gentil *et al.*, 2011). Genome-wide transcript profiling in sugar beet was reported in two publications (Pestsova *et al.*, 2008; Mutasa-Göttgens *et al.*, 2012).

6. der erfolgten oder geplanten Veröffentlichungen des Ergebnisses nach Nr. 6

Publications or intended publications

Pin, P. A., Zhang, W., Vogt, S. H., Dally, N., Büttner, B., Schulze-Buxloh, G., Jelly, N. S., Chia, T. Y. P., Mutasa-Göttgens, E. S., Dohm, J. C., Himmelbauer, H., Weisshaar, B., Kraus, J., Gielen, J. J. L., Lommel, M., Weyens, G., Wahl, B., Schechert, A., Nilsson, O., Jung, C., Kraft, T., Müller, A. E. (2012) The Role of a Pseudo-Response Regulator Gene in Life Cycle Adaptation and Domestication of Beet. *Current Biology* 22:1095-1101

Vogt, S. H., Rohardt, B., Rotter, B., Winter, P., Wolf, M., Wahl, B., Schechert, A., Weyens, G., Lefèbvre, M., Jung, C., Müller, A. E. (2012) Functional Genomics of Floral Transition for Targeted Genetic Modification of

Flowering Time in Sugar Beet. In: GABI – The German Plant Genome Research Program, Progress Report 2008 – 2011.

Müller, A. E, Schulze-Buxloh, G., Vogt, S. H., Wolf, M., Schechert, A., Stich, B., Jung, C. (submitted) Genetic Landscape of Flowering Time Control in Biennial Beets.

Vogt, S. H., Rotter, B., Winter, P., Müller, A. E. (in preparation) Genome-wide Transcript Profiling of Vernalization Response in a Biennial Species.

Vogt, S. H., Weyens, G., Lefèbvre, M., Wolf, M., Wahl, B., Schechert, A., Jung, C., Müller, A. E. (in preparation) Functional Analysis of Vernalization Response Genes in Sugar Beet.

Oral presentations

Müller, A. E. 'Life cycle control in flowering plants: A view from the distance (*Beta vulgaris*).' Invited talk, Colloquium of the Center for Biotechnology, Bielefeld University, May 7, 2012, Bielefeld, Germany.

Müller, A. E. 'The Genetic Control of Bolting in Beet.' Keynote Lecture, First Annual Meeting of the DFG Priority Program 'Flowering Time Control: From Natural Variation to Crop Improvement', February 22-24, 2012, Kiel, Germany.

Vogt, S., Rohardt, B., Rotter, B., Winter, P., Wolf, M., Schechert, A., Weyens, G., Lefèbvre, M., Jung, C., Müller, A. E. 'GABI-GENOFLOR: Functional Genomics of Floral Transition for Targeted Genetic Modification of Flowering Time in Sugar Beet.' 11th Status Seminar *Plant Biotechnology for the Future*, March 15-17, 2011, Potsdam, Germany.

Pin, P. A., Zhang, W., Büttner, B., Schulze-Buxloh, G., Chia, T., Mutasa-Göttgens, E., Gielen, J. J. L., Nilsson, O., Kraft, T., Jung, C., Müller, A. E. 'The Genetic Control of Early Bolting in Sugar Beet.' Plant & Animal Genome XIX Conference, January 15-19, 2011, San Diego, California.

Vogt, S., Büttner, B., Schulze-Buxloh, G., Abou-Elwafa, S., Zhang, W., Jung, C., Müller, A. E. 'Functional Genomics of Floral Transition in Sugar Beet.' 10th Gatersleben Research Conference (GRXC), November 22-24, 2010, Quedlinburg and Gatersleben, Germany.

Vogt, S., Zhang, W., Schulze-Buxloh, G., Büttner, B., Abou-Elwafa, S., Dietrich, M., Wolf, M., Schechert, A., Winter, P., Jung, C., Müller, A. E. 'GABI-GENOFLOR: Functional Genomics of Floral Transition for Targeted Genetic Modification of Flowering Time in Sugar Beet.' 9th GABI Status Seminar, March 3-5, 2009, Potsdam, Germany.

Zhang, W., Büttner, B., Schulze-Buxloh, G., Abou-Elwafa, S., Vogt, S., Chia, T., Mutasa-Gottgens, E., Winter, P., Jung, C., Müller, A. E. 'Functional Genomics of Floral Transition in Sugar Beet.' Keystone Symposium on Plant Sensing, Response and Adaptation to the Environment, January 11-16, 2009, Big Sky, Montana.

Zhang, W., Büttner, B., Schulze-Buxloh, G., Abou-Elwafa, S., Hohmann, U., Chia, T., Mutasa-Gottgens, E., Jung, C., Müller, A. E. 'Functional Genomics of Floral Transition in Sugar Beet.' Control of Flowering Time and Applications for Plant Breeding, September 22-24, 2008, Salzau, Germany.

Koch, G., Winter, P., Kahl, G., Jung, C., Müller, A. E. 'GABI-GENOFLOR: Functional Genomics of Floral Transition for Targeted Genetic Modification of Flowering Time in Sugar Beet.' 8th GABI Status Seminar, March 4-6, 2008, Potsdam, Germany.

Posters and other conference contributions

Vogt, S., Rohardt, B., Rotter, B., Winter, P., Wolf, M., Schechert, A., Weyens, G., Lefèbvre, M., Jung, C., Müller, A. E. 'GABI-GENOFLOR: Functional Genomics of Floral Transition for Targeted Genetic Modification of Flowering Time in Sugar Beet.' (Poster) 11th Status Seminar Plant Biotechnology for the Future, March 15-17, 2011, Potsdam, Germany.

Vogt, S., Schulze-Buxloh, G., Zhang, W., Dietrich, M., Rotter, B., Winter, P., Wolf, M., Schechert, A., Weyens, G, Lefèbvre, M., Jung, C., Müller, A. E. 'GABI-GENOFLOR: Functional Genomics of Floral

Transition for Targeted Genetic Modification of Flowering Time in Sugar Beet.' (Poster) 10th GABI Status Seminar, March 9-11, 2010, Potsdam, Germany.

Frerichmann, S.L.M., Kopisch-Obuch, F.J., Müller, A. E., Jung, C. 'Detection of mutant phenotypes in sugar beet by TILLING.' (Oral presentation) 10th GABI Status Seminar, March 9-11, 2010, Potsdam, Germany.

Tränkner, C., Kirchhoff, M., Kopisch-Obuch, F., Müller A. E., Jung, C. (2010) 'Innovative Ansätze zur genetischen Veränderung von Nutzpflanzen am Beispiel der Winterzuckerrübe.' *Schriftenreihe der Agrar- und Ernährungswissenschaftlichen Fakultät der CAU Kiel* 116:45-54.

Müller, A. E., Zhang, W., Büttner, B., Schulze-Buxloh, G., Abou-Elwafa, S., Frerichmann, S., Chia, T., Mutasa-Göttgens, E., Dohm, J., Himmelbauer, H., Jung, C. 'Identification of Flowering Time Regulators for Breeding Sugar Beet with Altered Vernalization Requirement' (Poster) 9th International Congress of Plant Molecular Biology, October 25-30, 2009, St. Louis, Missouri.

Frerichmann, S.L.M., Kopisch-Obuch, F.J., Schulze-Buxloh, G., Müller, A. E., Hohmann U., Jung, C. 'Detection of mutant phenotypes in sugar beet by TILLING.' (Poster) 9th GABI Status Seminar, March 3-5, 2009, Potsdam, Germany.