Chapter 1
Genetics and Genomics of Flowering Time Regulation in Sugar Beet

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Abstract Leaves from beets have been used since ancient times for nutrition and the swollen roots were one of the first sweeteners in the Middle Ages that could be stored through the winter. Breeding of beets to increase sugar content began only in the 18th century, after it was uncovered that the nature of the sweet taste of sugar cane and that of beet roots relies on the same sugar molecule. The major breakthrough in breeding sugar beet was the selection of beet progenies that, unlike their wild ancestors, did not flower in the first year of growth, correlating to a high root and thus sugar yield. This was the birth of the sugar beet that became a major crop in Europe and later on worldwide. Genetics has shown that the switch from annual to biennial beets relies mainly on one gene: the 'bolting gene' *B*. However, research from model plants has shown that the regulation of flowering is complex and involves many regulatory pathways, which perceive, transduce and integrate both endogenous and environmental cues for the fine tuning of flowering. Therefore, broad approaches to study flowering time in beets have been initiated, including both forward and reverse genetic studies to elucidate the molecular nature of *B* as well as other components of what is likely an intricate regulatory network also in beet. This chapter will give a short history of beet use and breeding as well as strategies and results from recent and current efforts to understand the regulation of flowering time in sugar beet.

1.1 The Sugar Beet Crop and Its Cultivated and Wild Relatives

Sugar beet (*B. vulgaris* L. ssp. *vulgaris* Sugar Beet Group) is the only sucrose-storing species of moderate climates. It belongs to the genus *Beta* that is now grouped in the Amaranthaceae (formerly Chenopodiaceae) subfamily Chenopodiaceae (The Angiosperm Phylogeny Group 2009).

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Species of the genus *Beta* show great morphological variation of leaves and roots, including colors that vary from red to yellow due to the production of two betalains, betacyanine (red-violett) or betaxanthine (yellow). Domesticated forms of *B. vulgaris* have been used since antiquity. Leaf beets (syn. mangold, swiss chard) (*B. vulgaris* L. ssp. *vulgaris* Leaf Beet Group) form fleshy leaves and have a long tradition as a vegetable. Red table beets (beetroot) (*B. vulgaris* L. ssp. *vulgaris* Garden Beet Group) form a thickened root and hypocotyl with an intense dark red color. They have a high content of free folic acid (vitamin B12), and are used as a vegetable and for production of natural colors for food additives. Fodder beets (*B. vulgaris* L. ssp. *vulgaris* Fodder Beet Group) form a thickened root and hypocotyl and are traditionally used as animal feed, mainly for dairy cattle.

Sugar beets form a thickened root, which like other cultivated forms store substantial amounts of sucrose. While sucrose concentration in fodder beets ranges between 4 and 10 %, sugar beet roots may contain more than 20 % sucrose. Under central European growing conditions the sucrose concentration typically is 17–18 % (Biancardi et al. 2005).

The development of sugar beet as a cultivated species began in 1747 when the German chemist A. S. Marggraf detected cane sugar within the roots of garden beets, frequently grown as a vegetable at that time. Cultivation started on a very small scale at the end of the 18th century in Germany when F. C. Achard grew 'sugar beet' near Berlin. The first beet root processing sugar factory was constructed in 1801 in Silesia, a Prussian province at that time. That year is regarded as the beginning of sugar beet cultivation.

The sucrose content of beet roots at the beginning of beet cultivation was estimated to be around 4 %. By mass selection, sucrose content was raised to 16 % by the end of the 19th century. At that time, the market share of sugar beet sucrose was 62 %, the rest coming from sugar cane. After World War I, it dropped dramatically to 23 %. In 2010, the world beet harvest reached 227 Mt fresh weight giving rise to 32.3 Mt of sugar that is 19 % of the total world production (166.8 Mt of sugar (http://www.zuckerverbaende.de/zuckermarkt/zahlen-und-fakten/weltzuckermarkt/erzeugung-verbrauch.html). Sugar beets are grown in many countries of the Northern hemisphere. The total sugar beet area harvested was 4.3 Mha (2009). Major producers were the Russian Federation (770,000 ha), the United States of America (465,000 ha), Germany (384,000 ha), and France (374,000 ha) http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/E

The species of the genus *Beta* are divided into two sections (Kadereit et al. 2006) (Fig. 1.1). All cultivated forms belong to the same subspecies *B. vulgaris* ssp. *vulgaris*. Together with their wild progenitor *B. vulgaris* ssp. *maritima* (L.) Arcang, they belong to section I (Beta). *B. vulgaris* ssp. *maritima* and the related wild species ssp. *adanensis* (*Pamuk*) *Ford-Lloyd & Will., B. patula* Ait. and *B. macrocarpa* Guss. grow around the Mediterranean and the coasts of northwest Europe up to Scandinavia and between the Capverdian Islands and Bangladesh. There are only wild species in the section II (Corollinae). Apart from diploid species, tetraploids, pentaploids and hexaploids also exist within this section. Those species grow on the hilly and mountainous regions in Turkey and adjacent countries (Fig. 1.1).

Sections of the genus *Beta*	Chromosome numbers	Life history	Natural habitats
I. SectioBeta:			from southwest Norway to Capverdian Islands, from Bangla Desh to Canary Islands
B. vulgaris L.			
ssp. *vulgaris*	18	biennials (cultivated beet)	
• Cultivar group Leaf beet			
• Cultivar group Gardenbeet			
• Cultivar group Fodder beet			
• Cultivar group Sugar beet			
ssp.*maritima* (L.) Arcang.	18	annuals, biennials, iteroparous perennials	
ssp. *adanensis* (Pamukc. ex Aellen) Ford-Lloyd and J. T. Williams	18	annuals (strictly semelparous)	
B. patula Ait.	18	annuals	
B. macrocarpa Guss.	18, 36	annuals	
II. Sectio Corollinae:			hilly and mountainous regions in Turkey and adjacent countries
Base species *B. corolliflora* Zosimovic ex Buttler	36		
B. macrorhiza Steven	18		
B. lomatogona Fisch et Meyer	18		
B. nana Boisier et Heldreich	18		mountain heights of Greece
Hybrid species			
B. trigyna Waldstein et Kitabel	45, 54		
B. x *intermedia* Bunge			

Fig. 1.1 The species of the genus *Beta* (as revised by Kadereit et al. 2006)

The systematics within this genus have been disputed for a long time until a new taxonomy was proposed in 2006 (Kadereit et al. 2006) which became official in June 2009. The species *B. nana*, which formerly belonged to section III, was moved to section II and the former section IV (Procumbentes) (Lange et al. 1999) became the new genus *Patellifolia* (Kadereit et al. 2006) (Fig. 1.1).

1.2 Sugar Beet Breeding and Genetics

For 200 years, sugar beet has been cultivated for sucrose production. The main breeding aims were high sucrose yield in combination with quality traits such as low Na/K content and low α-amino acid content to reduce the amount of molasses. Mass selection for sucrose content was extremely successful in the early period of beet breeding because the dry matter, whose content was easily measurable, mainly consists of sucrose. Today sucrose is measured directly by refractrometry and α-amino acid and Na/K content are determined separately. Mass selection has been replaced by single plant selection in the 1930s and today hybrids are exclusively used in the major beet-growing areas. But also a number of other traits have been substantially improved during the past 50 years. The introduction of monogermic seeds laid the foundation for beet production at an industrial scale. The successful breeding for

rhizomania resistance was a breakthrough for the cultivation of sugar beet in many growing areas of the Northern hemisphere where soils are often contaminated with the beet necrotic yellow vein virus (BNYVV).

Recently, sugar beet became an interesting alternative as a renewable energy resource in central Europe. Biomass production in this part of the world heavily relies on maize and alternatives are urgently needed. Sugar beet has the highest dry matter production capacity under central European growth conditions. The beet with its high sucrose content is also suitable for loading fermenters to produce methane. Thus, breeding biomass beets, which do not necessarily have to exhibit the quality traits of 'sucrose beets', has become an interesting option. One means to increase biomass yields is to grow winter beets. Those beets are sown before winter (preferentially in August). They overwinter in the field and develop their shoot mass early in spring. Harvest time is expected to be earlier than for conventionally grown 'spring' beets. The yielding potential of winter beet has been estimated to be $\sim 20\,\%$ higher than that of conventional beet (Hoffmann and Kluge-Severin 2011), but requires a strict bolting control (see Sect. 1.9).

The haploid chromosome number of *Beta* species is 9 ($x = 9$). Sugar beet is a diploid species with $2n = 2x = 18$ chromosomes and a haploid genome size of 758 Mb (Arumuganathan and Earle 1991). Triploids and tetraploids exist, which have been frequently used in beet breeding. Thus, the sugar beet crop is a rare example of a seed-propagated triploid crop species. Several molecular marker-based genetic maps have been published (Barzen et al. 1992; Pillen et al. 1992; Schondelmaier and Jung 1997; Schumacher et al. 1997; Grimmer et al. 2007; McGrath et al. 2007; Schneider et al. 2007) and used for mapping major genes and polygenes of agronomic importance. Unfortunately, routine procedures for doubled haploid production such as microspore or anther culture are so far lacking. Doubled haploids can only be produced by costly and time-consuming gynogenesis. Therefore, F_2 or advanced inbred populations have been used for mapping. Apart from bolting time genes, which will be discussed in Sects. 1.6–1.8, major genes for nematode resistance (Cai et al. 1997; Kleine et al. 1998), rhizomania resistance (Barzen et al. 1992; Barzen et al. 1997; Lein et al. 2007a) and monocarpic seeds (Barzen et al. 1992) have been mapped. Also, a number of QTLs have been placed on the beet chromosomes such as *Cercospora* leaf spot resistance (Nilsson et al. 1999; Schäfer-Pregl et al. 1999; Setiawan et al. 2000), *Rhizoctonia* root rot resistance (Lein et al. 2007b), fertility restorer genes (Hjerdin-Panagopoulos et al. 2002) and quality traits (Schneider et al. 2002). The efficiency of association mapping in sugar beet was recently demonstrated by mapping a number of quantitative traits (e.g. sucrose content) in a panel of 460 elite sugar beet lines (Würschum et al. 2011). Other resources for studying the beet genome have been established in the past years and will be discussed in Sect. 1.4.

1.3 Phenology of *Beta Species*

The development of sugar beet after sowing in spring is characterized by secondary root growth and the formation of a large leaf rosette in the first year. During the vegetative phase, sugar beets develop a large harvestable organ, which is mainly

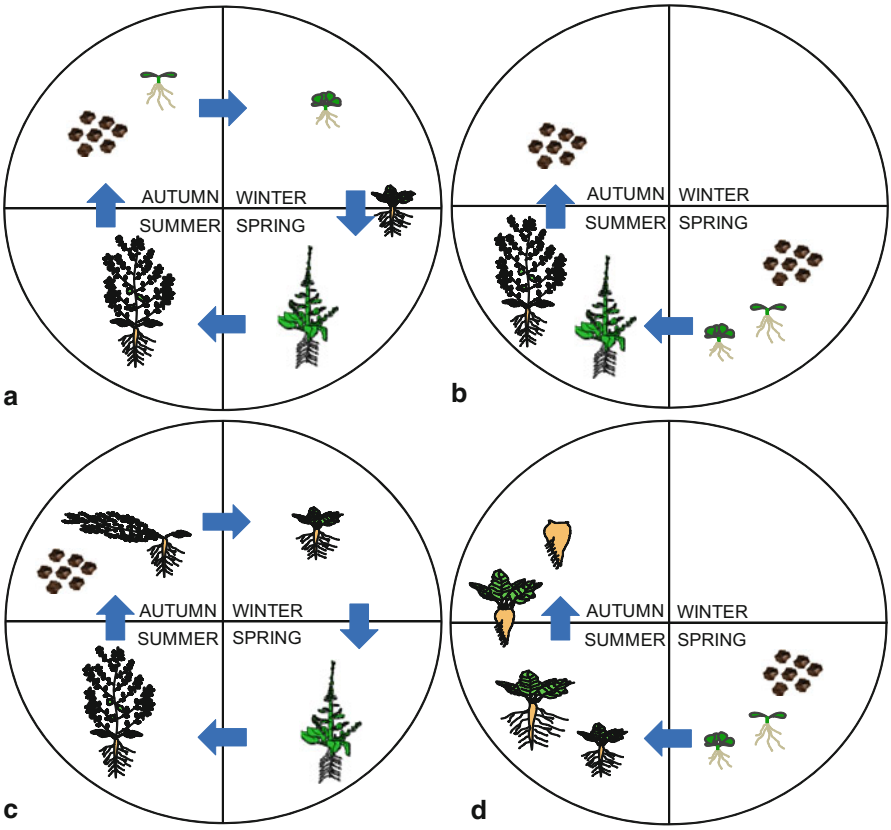

Fig. 1.2 The phenological development of sugar beet and its wild relative *Beta vulgaris* ssp. *maritima*. **a** Life cycle of biennial wild beets. **b** Life cycle of annual wild beets. **c** Life cycle of perennial wild beets. **d** Sugar beet field production ('spring beet').

formed by the root and contains only small portions of epicotyl and hypocotyl. The beet root results from secondary thickening with up to 12 successive concentric rings of cambia (Bell et al. 1996). Each cambium forms a cylindrical ring of xylem and phloem tissue and parenchyma cells in between two rings (Bhambie et al. 2000). The number of rings is much smaller in fodder beet and red beet (3–5 rings).

Sugar beets enter the generative phase only after exposure to cold temperatures typical for winter periods under central European conditions. The first visible event is the elongation of the shoot, referred to as 'bolting', usually followed by flower formation (Fig. 1.2a). A plant can have more than one flowering shoot, which are panicles and carry numerous hermaphrodite flowers (up to 10,000) that are formed in the axils of bracts. In wild beets, 2–4 flowers are merged and develop a multigerm seed ball. *B. vulgaris* is an allogamous species due to a gametophytic self-incompatibility system controlled by two series of sterility alleles (*S1-Sn*, *Z1-Zn*). Thus, self-pollination is avoided leading to highly heterozygous and heterogeneous wild populations. However, a self-fertility locus with a self-fertility allele *SF* exists, which is frequently used for selfing sugar beets to produce inbred lines (Biancardi et al. 2005).

Fig. 1.3 Cultivated beets and the related wild species *Beta vulgaris* ssp. *maritima*. **a** Ready-to-harvest beets, bolting (right) and non-bolting, with and without leaves (left). **b** Beet volunteers in a beet production field. **c** Flowering beets for seed production. **d** Annual wild beet *Beta vulgaris* ssp. *maritima*

Wild beets from the Mediterranean area are annuals, flower early without vernalization and finish their life cycle within the first year (Fig. 1.2b). By contrast, wild beets growing in the northern regions are biennials with a marked requirement for cold temperatures for flowering (Fig. 1.2a). Furthermore, long-lived, iteroparous perennials exist in the subspecies *maritima*, which produce offspring in successive cycles (Hautekeete et al. 2002) (Fig. 1.2c). However, all *Beta* species are strict long-day (LD) plants.

The onset of bolting is of greatest importance for the cultivation of sugar beet as well as of root and leaf beet. High root yield is only guaranteed if beets do not flower (Fig. 1.2d). The storage root of bolting and flowering beets is much smaller, thus sucrose yield is drastically reduced after bolting (Bürcky 1986, Fig. 1.3a) and bolting during beet production must be completely avoided. Consequently, breeders have

selected against early bolting since the beginning of beet breeding. This has been quite successful because early bolters can be easily identified and eradicated during mass selection. However, when seed production was moved to southern Europe, where annual wild beets are abundant there was an increased risk of cross pollination between wild beets and male sterile sugar beet seed parents in seed production fields. Since early bolting is controlled by a single dominant allele (see Sect. 1.5) heterozygous beets resulting from cross pollination would bolt early, creating a need for rigorous elimination of wild beets and strict isolation of seed production fields. Today, molecular markers (Gaafar et al. 2005, see Sect. 1.5) are employed for testing seed lots for the presence of early bolters. However, even biennial beets can have a tendency towards early bolting under certain environmental conditions such as exposure to cold temperatures in spring (Fig. 1.3b). Therefore, sowing time is delayed in some areas with a risk of cold temperatures late in spring (Milford and Burks 2010). On the other hand, for seed production beets must bolt and flower readily after winter (Fig. 1.3c). Therefore, breeders have selected for early flowering after winter, and completely bolting-resistant beets (that will never bolt) are not found among cultivated beets.

1.4 Genomic Resources for Beet

Genetic mapping has been used in model and crop plants to map and clone many flowering time genes in recent years (Turck et al. 2008). In contrast to model species, no collection of defined flowering time mutants is available for sugar beet. However, phenotypic variation for flowering time is easily observable among natural accessions and in structured populations derived from crosses between annual wild beets, beet cultivars and/or breeding lines. QTL analyses have been performed and linkage maps are available, but efforts to construct high density molecular marker maps thus far are rare (Lange et al. 2010). Therefore, cloning of flowering time genes from beet by mapping procedures is still challenging and time consuming (McGrath et al. 2007).

Other resources for studying the beet genome have been established in the past years. Large insert libraries exist for several beet genotypes which representatively cover the whole beet genome (Hohmann et al. 2003; Hagihara et al. 2005; Schulte et al. 2006; Lange et al. 2008). A sugar beet EST database can be found at Michigan State University (http://genomics.msu.edu/sugarbeet/index.html) and approximately 30,000 *B. vulgaris* ESTs are listed in GenBank (http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucest&cmd=DetailsSearch&term=(beta+vulgaris)+AND+%22Beta+vulgaris%22%5Bporgn%5D&save_search=true). From The GenBank EST database, 315 ESTs have been placed on a sugar beet map (Schneider et al. 2007) and 2,752 were used to produce macroarrays for expression analyses (Pestsova et al. 2008). An Agilent 15 K oligonucleotide microarray has been established, which was used for mapping 392 BAC-end derived sequences and 119 ESTs (Lange et al. 2010). A beet genome mapping and sequencing consortium has started working in 2004 with the aim to physically map (GABI—The German

Plant Genome Research Program Progress Report 2004–2007; http://www.gabi.de/client/media/3/gabi_progrep_ii_web.pdf) and sequence the whole beet genome using second generation sequencing technology (http://www.gabi.de/projekte-alle-projekte-neue-seite-144.php). Approximately 67,000 genomic survey sequence fragments including BAC end sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/nucgss/?term=%22Beta%20vulgaris%22) and the preliminary annotation yielded approximately 28,000 gene models (Weisshaar et al. 2011). To characterize sugar beet seed vigor, a proteomic analysis was performed and 759 proteins with specific root, cotyledon and perisperm expression profiles were identified (Catusse et al. 2008). Finally, at least two transcriptomics projects are underway to generate genome-wide expression profiles and/or transcript sequences for specific developmental processes (vernalization) or tissues (shoot apex) that are relevant for the study of flowering time control in beet (http://www.gabi.de/projekte-alle-projekte-neue-seite-171.php; http://www.ncbi.nlm.nih.gov/bioproject/73561).

1.5 Genetics of Bolting Time in Beet

While beets in natural environments require long days for bolting to occur, there is considerable intraspecific variation in vernalization requirement, which follows a latitudinal cline. Wild beets from the southern part of the species distribution area (the Mediterranean) bolt in the first year without experiencing prolonged periods of cold temperatures and generally behave as annuals, but may live and flower for up to three consecutive years (Van Dijk 2007). Beets from northern latitudes including the Atlantic and North Sea coasts have a longer lifespan of approximately 4–17 years or more (Hautekeete et al. 2002; Van Dijk 2007) and commonly require vernalization, but there is quantitative variation among natural populations of different geographic origins in the extent of cold exposure required (Van Dijk et al. 1997; Boudry et al. 2002). Owen et al. (1940) coined the term 'photothermal induction' to describe the inductive effects of low temperatures and long photoperiods on bolting in *B. vulgaris* and showed that, although not required in their natural habitats, exposure to cold also promotes and accelerates bolting in annuals. As a result of strict selection during the breeding process against the annual character, which is associated with poor root yield and interferes with harvest operations, sugar beet and other cultivated beet forms require vernalization to bolt and, for seed production, are grown as biennials.

Genetically, the annual growth habit is under the control of a major dominant gene that has long been referred to as the 'bolting gene' or '*B*' (Abegg 1936). Plants which are derived from crosses between homozygous annual (*BB*) and biennial beets (*bb*) and are heterozygous at the *B* locus (*Bb*) behave as annuals under favorable conditions but may bolt several days later than homozygous annuals (Munerati 1931; Abegg 1936; Mutasa-Göttgens et al. 2010). Heterozygotes may also fail to bolt in the first year under suboptimal photothermal conditions as they are present e.g. in late spring, summer or autumn sowings (Owen 1954; Boudry et al. 1994; Abe et al. 1997). In

addition to appropriate environmental conditions, the manifestation of the annual character is also influenced by additional, modifying genes (Abe et al. 1997; Büttner et al. 2010; Abou-Elwafa et al. 2012). Furthermore, Owen et al. (1940) defined a locus for easy-bolting tendency (*B*') in a biennial beet accession which does not bolt without prior vernalization under field conditions, but bolts easily and early without vernalization under relatively low temperatures and long photoperiods in the greenhouse. On the basis of linkage data between the *B* locus and the *R* locus for hypocotyl color, and between *B*' and *R*, the authors concluded that *B*' is allelic to *B*.

Following the original observation by Rimpau (1876, 1880) that the annual habit is dominantly inherited in beet, and later work by Munerati (1931), who on the basis of phenotypic data for large F2 populations segregating for annuality suggested further that this trait follows a monogenic mode of inheritance, Abegg (1936) was able to show that the *B* gene (or 'factor' in the language of his time) in Munerati's annual accessions was linked to the hypocotyl color factor '*R*'. Abegg calculated a cross-over value of 15.5 % between *B* and *R*, and by considering the previously identified linkage relationship between *R* and *Y*, another locus affecting pigmentation, defined the first linkage group with three morphological markers in beet. The *Y-R-B* linkage group together with additional markers was later assigned to chromosome II according to the standard nomenclature for beet chromosomes suggested by Schondelmaier and Jung (1997). Using a backcross population derived from a biennial parent and a different annual accession than had been analyzed in Munerati's and Abegg's early studies, Boudry et al. (1994) confirmed linkage of a locus for annuality, which was presumed to be *B*, to *R* in their population, and were able to identify several restriction fragment length polymorphism (RFLP) markers more closely linked to *B*. The *B* locus was further fine-mapped by anonymous fragment length polymorphism (AFLP) mapping to a 0.37 cM interval of chromosome II using another unrelated mapping population (El-Mezawy et al. 2002). BAC library screening and bulked segregant analysis identified, respectively, several sequence-based markers which flank the *B* locus on either side (Hohmann et al. 2003; Gaafar et al. 2005) or completely co-segregate with *B* (Büttner et al. 2010).

Besides the major bolting locus *B*, two recent studies identified additional, previously unknown loci, which contribute to annual bolting in wild beets (Büttner et al. 2010; Abou-Elwafa et al. 2012). A screen for bolting mutants derived from ethyl methanesulfonate (EMS) mutagenesis of the same annual beet accession that was used for fine-mapping of *B* (El-Mezawy et al. 2002) identified five M3 families which bolted only after vernalization and thus behaved as biennials (Hohmann et al. 2005). Somewhat surprisingly at the time, in two out of several F2 mapping populations derived from crosses between these biennial genotypes and an annual wild beet accession, the annual bolting phenotype did not co-segregate with the *B* locus, but instead was mapped to a new locus on chromosome IX which was named *B2* (Büttner et al. 2010). Because all plants in these populations carried the dominant allele for annuality at the *B* locus, but approximately one quarter of plants failed to bolt without vernalization, the authors concluded that *B2* acts epistatically with *B* to co-regulate vernalization-independent bolting. The genetic and phenotypic data further indicated that *B2*, similar to *B*, harbors a major gene which is inherited in a

dominant-recessive fashion. Co-segregation analyses of the remaining populations segregating for annuality revealed that the natural accession used as annual parent carries at least one additional locus which also promotes bolting, but in contrast to *B2* appears to act independently of *B*. A QTL analysis of annual bolting in two populations showed that the *B* locus and the newly identified locus, termed *B4*, contributed equally to phenotypic variation in bolting behavior, and that *B4* also exhibited a dominant gene action (Abou-Elwafa et al. 2012). The *B4* locus is genetically linked to the *B* locus and was mapped to chromosome II at a genetic distance of 11 cM from *B*.

1.6 Flowering Time Genes and Their Regulation in Beet

1.6.1 Beet Homologs of the FLC Gene and Putative Regulators

Vernalization, a prolonged exposure of plants to cold temperatures over winter, is a prerequisite for many plants to flower in the following spring or summer. For Arabidopsis and other Brassicaceae, it has been shown that the MADS box gene *FLC* is the main regulator of the vernalization response. *FLC* acts as a flowering time repressor showing a characteristic expression pattern: before vernalization *FLC* mRNA accumulates to high levels, but during vernalization expression declines and remains low post-vernalization. In winter-annual Arabidopsis accessions, *FLC* is repressed by an epigenetic mechanism and is only de-repressed in the next generation. There are five paralogs of *FLC* (*MAF1–MAF5*) that are also reported to be regulated by vernalization in Arabidopsis, but these show only a mild response compared to *FLC* (Ratcliffe et al. 2003).

In cereals, wheat and barley winter varieties also exhibit a clear vernalization requirement for flowering. However, the vernalization response involves different major players, often without clear homologs in dicot species, indicating that the regulation of vernalization response evolved independently in dicots and monocots (Kim et al. 2009). One exception is *VRN3*, an *FT* ortholog from Arabidopsis, as both *FT* and *VRN3* integrate signals from various regulatory pathways and promote flowering.

Recently, in beet a homolog of *FLC* that was named *BvFL1* has been identified in EST libraries by using a phylogenetic approach (Reeves et al. 2007). The authors showed that four splice variants of *BvFL1* RNA were present in beets, which they constitutively expressed in an Arabidopsis *flc3* null mutant. All splice variants caused later flowering (Reeves et al. 2007) but to a much lesser extent than transgenic plants overexpressing the endogenous Arabidopsis *FLC* gene (Michaels and Amasino 1999). Nevertheless, *BvFL1* also acts as a repressor for flowering in transgenic Arabidopsis and two of the four splice variants are also down-regulated in beet leaves in response to a vernalization treatment of 90 days. However, after vernalization, expression of these splice variants was not stably repressed and the expression recovered to pre-vernalization levels. In addition, *BvFL1* is expressed at equal levels in annual and vernalization-requiring biennial beets, suggesting that the difference

in vernalization requirement cannot be attributed to differences in the abundance of *BvFL1* transcripts (Reeves et al. 2007). Therefore, it seems unlikely that *BvFL1* is the primary target for the vernalization response in biennial sugar beets.

In Arabidopsis, *FLC* is also regulated by a number of genes assigned to the 'autonomous pathway' of flowering time control (Simpson 2004). Beet homologs of several pathway members, namely *BvFLK*, *BvFVE1*, *BvLD* and *BvLDL1*, have recently been identified by Abou-Elwafa et al. (2011). It was shown that *BvFLK* overexpression leads to earlier flowering and can complement an Arabidopsis *flk* mutant. *BvFLK* also repressed the endogenous *FLC* gene in transgenic Arabidopsis, suggesting that gene function is at least conserved to some extent between Arabidopsis and beets. However, the authors also found indications for evolutionary divergence of autonomous pathway gene homologs in Arabidopsis and beets. Overexpression of *BvFVE1* in an Arabidopsis *fve* mutant did not rescue the late flowering mutant phenotype. Furthermore, in apparent contrast to its homolog in Arabidopsis, *BvFVE1* is under circadian clock control. Since beet carries a second closely related *FVE* homolog (*BvFVE2*), it is conceivable that *BvFVE1* and *BvFVE2* underwent sub-functionalization and that *BvFVE2* is a functional *FVE* ortholog (Abou-Elwafa et al. 2011).

1.6.2 Photoperiodic Pathway and CO Homologs

While the key regulators of vernalization requirement and response differ between distantly related species such as *A. thaliana* and cereals, a core component of the photoperiodic regulation of flowering appears to be largely conserved among angiosperms. The central regulator of the photoperiod pathway in Arabidopsis is the CONSTANS, CONSTANS-LIKE and TIMING OF CAB EXPRESSION 1 (CCT) domain transcription factor gene *CONSTANS* (*CO*), which promotes flowering in response to LD conditions (Suarez-Lopez et al. 2001). CO activity is diurnally regulated both at the transcriptional level and post-translationally, and is highest at the end of the light phase in long days when high levels of *CO* transcription and high CO protein stability coincide (Turck et al. 2008; Srikanth and Schmid 2011). The concurrent effects of both exogenous and endogenous factors on *CO* activity at critical times of the day, which involve circadian clock-regulation of transcriptional *CO* regulators and light-regulated stabilization of the protein, suggest that regulation of *CO* can account for much of the molecular basis of the 'external' and 'internal coincidence' models proposed by Bünning (1936), Pittendrigh and Minis (1964) and Pittendrigh (1972) for the induction of flowering (and other biological processes) by photoperiod (Turck et al. 2008; Srikanth and Schmid 2011). Once stably expressed under inductive LD conditions, CO transcriptionally activates *FT* in the leaf vasculature. Although the exact mode of this activation is not well understood, several co-regulatory proteins which interact with CO and contribute to the regulation of *FT* have been identified (Wenkel et al. 2006; Song et al. 2012).

CO homologs have been identified in numerous dicotyledonous and monocotyledonous species, including species which flower in response to different photoperiodic conditions such as short-day and day-neutral plants (reviewed in Turck et al. 2008). For several of these species, including both LD and short-day (SD) plants among monocots, a functional role of *CO* homologs in photoperiodic regulation of flowering has been demonstrated. However, the mode of action of *CO* genes differs to some extent between species and may be modified by various interactions with co-regulatory genes and/or light-induced changes of the protein, as has been suggested for rice (Turck et al. 2008; Ishikawa et al. 2011). Furthermore, there is also increasing evidence for *CO*-independent photoperiodic regulation in monocots including rice, where a regulatory mechanism involving the species-specific transcriptional regulators *Grain number, plant height and heading date 7* (*Ghd7*) and *Early heading date 1* (*Ehd1*) enables expression of the *FT* homolog *Heading date 3a* (*Hd3a*) under SD conditions irrespective of *Heading date 1* (*Hd1*), the rice ortholog of *CO* (Doi et al. 2004; Itoh et al. 2010). In barley, the major determinant of LD response was identified as *Photoperiod-H1* (*Ppd-H1*), which also carries a CCT domain but is otherwise unrelated to *CO* (Turner et al. 2005), whereas the function of *CO* homologs in barley is less understood and allelic variants have not been identified. A recent study of transgenic plants over-expressing *HvCO1*, the closest barley homolog of *CO* and the putative ortholog of *Hd1* in rice (Griffiths et al. 2003), showed that *HvCO1* indeed also promotes flowering in barley, in a process involving activation of the *FT* homolog *HvFT1* (Campoli et al. 2011). Interestingly, natural variation at the *Ppd-H1* locus affected flowering time irrespective of high transgenic expression of *HvCO1*, leading the authors to suggest that *Ppd-H1* may 'bypass' the regulatory *CO-FT* interaction (Campoli et al. 2011) and raising the possibility that, while *HvCO1* is a functional regulator of flowering time, the photoperiod response in barley may also involve an *HvCO1*-independent pathway.

Like in other species, a family of *CO*-like genes has also been identified in beet (Chia et al. 2008). However, none of the closest *CO* homologs in beet identified thus far (*BvCOL1* and *BvCOL2*) appear to be true orthologs of *CO*, but instead are more closely related to *CO-LIKE 1* (*COL1*) and *COL2* in Arabidopsis. Consistently, the diurnal expression profile of *BvCOL1* more closely resembled the profiles of *COL1* and *COL2*, and showed that *BvCOL1*, in contrast to *CO*, was not or only very weakly expressed at the end of the light phase in LDs (Chia et al. 2008). Nevertheless, over-expression of *BvCOL1* in Arabidopsis rescued the late-flowering phenotype of the loss-of-function *co-2* mutant and activated *FT* expression, suggesting at least a certain degree of functional conservation of the *BvCOL1* gene product (Chia et al. 2008). Perhaps noteworthy, the over-expression of *HvCO1* failed to complement the same mutant, which was suggested to result from sequence variation at conserved positions in a B-Box-type zinc finger domain (B-Box2) (Campoli et al. 2011), whereas this domain is highly conserved between *CO* in Arabidopsis and *BvCOL1*. Like the *B* and *B4* loci, *BvCOL1* was mapped to chromosome II, but at large genetic distances of approximately 22–24 cM upstream of *B* and 35–38 cM upstream of *B4* (Chia et al. 2008; Abou-Elwafa et al. 2012).

1.6.3 Two Copies of FT Homologs with Different Function in Beet

FT is a member of a protein family with structural similarities to mammalian phosphatidylethanolamine-binding protein (PEBP) domains (Kardailsky et al. 1999; Kobayashi et al. 1999) and a hitherto unknown biochemical function. In Arabidopsis, the PEBP protein family consists of three phylogenetically distinct groups represented by FT, TFL1 and MFT. FT and TSF are components of the long-sought florigen signals that promote flowering in Arabidopsis under LDs, but also integrate signals from other flowering time pathways, whereas TFL1 acts antagonistically to prevent flowering. It has been shown in other species (including both LD and SD plants) that expression of *FT* orthologs rises in response to inductive photoperiods, and that constitutive expression induces early flowering whereas mutations in *FT* orthologs delay flowering. As had been expected for the long elusive florigen, the FT protein moves from the leaves to the apex where it establishes flowering (Turck et al. 2008).

In sugar beet, Pin et al. (2010) identified two paralogous *FT* genes. Surprisingly, these genes, which were termed *BvFT1* and *BvFT2*, have antagonistic functions. While *BvFT1* acts as a repressor, *BvFT2* promotes flowering. After vernalization, biennial sugar beets are competent to flower, but the vernalized plants still require long days for floral induction. Diurnal expression studies in annual, biennial and vernalized biennial plants in different photoperiods showed that under non-inductive SD conditions *BvFT2* expression was hardly detectable, whereas *BvFT1* showed a distinct morning expression peak. Without vernalization, only annual beets bolt in LDs which was found to be coincident with very low *BvFT1* expression, whereas *BvFT2* expression peaked after 12 hours of illumination in an 18 hour photoperiod. However, unvernalized biennial beets exhibited a very different LD expression profile. Here, *BvFT2* was not expressed, whereas *BvFT1* showed a peak of expression in the morning not dissimilar to that also observed in non-inductive SD. During vernalization, *BvFT1* was down-regulated and *BvFT2* was up-regulated, indicating that *BvFT2* may be repressed by *BvFT1*. Moreover, when vernalized biennial plants were transferred to SD conditions, which lead to de-vernalization and suppression of bolting, *BvFT1* expression was induced and *BvFT2* was repressed. Finally, the observed correlation of *BvFT2* expression with the initiation of flowering in both annual and biennial beet and a complementation analysis in Arabidopsis *ft* mutants suggested that *BvFT2* is the functional *FT* ortholog in sugar beet.

In transgenic approaches overexpressing *BvFT2* under the control of the 35S promoter or down-regulating *BvFT2* expression by RNA interference (RNAi), it was demonstrated that *BvFT2* is essential for flowering in sugar beet. 35S::*BvFT2* biennial plants flowered prematurely in tissue culture without vernalization and annual *BvFT2* RNAi plants failed to bolt and continued to produce leaves in LD for more than 400 days, which was correlated with strongly reduced *BvFT2* transcript levels. Since in these transformants *BvFT1* expression was not altered, the results indicate that the modulation of flowering time is directly regulated by *BvFT2* and

not indirectly via modulation of *BvFT1* expression by *BvFT2*. Transgenic annual beets constitutively expressing *BvFT1* also failed to bolt and showed a strong down-regulation of *BvFT2*, indicating that *BvFT1* represses *BvFT2* expression. Vernalized transgenic biennial beets over-expressing *BvFT1* showed a non-bolting phenotype for more than 6 months under inductive LD and a strong down-regulation of *BvFT2*. Together, these results indicate that *BvFT1* suppresses flowering under non-inductive conditions, which at least in part may be mediated by repression of *BvFT2* expression. *BvFT1* is down-regulated during vernalization, which eliminates the repressive effect of *BvFT1* on *BvFT2* and allows sugar beet plants to respond to inductive LD conditions (Pin et al. 2010). Interestingly, *Chenopodium rubrum*, a SD plant and close relative of *B. vulgaris*, also carries two close homologs of *FT* that show different expression profiles, suggesting different roles of the encoded proteins (Cháb et al. 2008). However, a functional study in *Chenopodium* has not been performed yet. It would be highly interesting to compare the functional roles of the *FT* genes in these closely related species and their possible contributions to the differentiation of these species into SD and LD plants.

Since *BvFT1* and *BvFT2* are paralogs and show a high degree of sequence similarity, it was interesting to evaluate the encoded proteins with respect to activating or repressing functions. From previous studies in Arabidopsis, it was known that a swap of the fourth exon from *FT* (i.e. a promoter of flowering) to *TFL* (i.e. a repressor) converted FT to TFL function and vice versa (Ahn et al. 2006). Strikingly, this could also be achieved by swapping just a single amino acid of an external loop of exon 4 (Hanzawa et al. 2005). The external loops encoded by exon 4 of *BvFT1* and *BvFT2* only differ by three amino acids out of 14. Exchanging exon 4 or only the external loop regions between *BvFT1* and *BvFT2* also resulted in opposite protein functions, indicating that the functional domains for the repression or promotion of flowering through PEBP proteins are well conserved in different eudicot clades (Pin et al. 2010).

1.6.4 GA Metabolism and Early Bolting

It has been shown that treatments with gibberellins (GAs) can induce bolting and flowering especially in LD plants that form rosettes under non-inductive conditions (Zeevaart 1983). In some LD plants, the amount of endogenous GAs is increased when these plants are transferred into inductive environments (Metzger 1995), but GA-deficient and GA-insensitive mutants of Arabidopsis have also provided strong evidence that GAs are required for floral induction in SD and play only a minor role in LD (Wilson et al. 1992). In Arabidopsis, the response to GA with regard to flowering time regulation is integrated by *LFY* and *SOC1*. *SOC1* is activated by GAs and overexpression of *SOC1* can rescue the non-flowering phenotype of *ga1-3* mutants in SD, whereas *soc1* mutants show a reduced sensitivity to GAs. The expression of *LFY* is directly regulated by the GA-activated GAMYB protein, while SOC1 also activates *LFY* by directly binding to its promoter. Therefore, GAs regulate *LFY* transcription by both *SOC1*-dependent and -independent pathways (Lee and Lee 2010).

Previous studies in sugar beet have shown that GAs play a role during reproductive growth (Lenton et al. 1975), and that GA contents after vernalization and during bolting increases significantly (Sorce et al. 2002). Exogenously applied GAs can stimulate cell growth in a genotype specific manner (Sadeghian et al. 1993b), but endogenous levels of GAs are not limiting for bolting and the application of exogenous GAs failed to induce bolting and flowering in non-vernalized sugar beets (Mutasa-Göttgens et al. 2009). Mutasa-Göttgens et al. (2010) studied the interaction of GAs, the bolting gene *B* and vernalization under controlled physiological conditions by analyzing the effect of GA_4 addition in inductive LDs and non-inductive SDs in sugar beet populations segregating for the *B* allele and grown with or without vernalization. Under SD conditions, GA_4 promoted bolt initiation independently of the *B* allele, but stem elongation did not occur without prior vernalization. Moreover, vernalization and addition of GA_4 could not substitute for LD conditions, indicating that long days are absolutely required for flowering in sugar beets. Finally, the authors showed that exogenous application of GA_4 had no significant effect on bolting and flowering in LD, suggesting that GAs are not a limiting factor for bolting and flowering under these conditions.

1.7 A Model for Bolting Time Regulation in Beet

While the nature and regulatory roles of the major bolting loci in beet that were detected genetically await identification and further characterization, the presence of *FT* genes in beet as well as of homologs of photoperiod, vernalization and autonomous pathway genes suggest that important components of flowering time regulation are also conserved in beet. However, the pioneering study by Pin et al. (2010) on the antagonistic action of the *FT* genes in beet and the finding that neither *B* nor the other two mapped bolting loci, *B2* and *B4*, correspond to any of the closest beet homologs of the major flowering time regulators *FT*, *CO* and *FLC*, indicate that in beet different or additional components may have evolved as central regulators of floral transition. According to the current data, *BvFT2* is a functional ortholog of *FT* in Arabidopsis and may also in beet act as a central 'executive' component of florigen which under favorable conditions transduces the promotive signals perceived by the plant in LD or under vernalizing conditions (Pin et al. 2010). However, upstream of *BvFT2*, the *FT* paralog *BvFT1*, which does not have a counterpart in Arabidopsis, acts as a repressor of *BvFT2* in SD (in both annuals and biennials) and, in biennials, in the absence of vernalization regardless of day-length. Similar to *FLC* in Arabidopsis, *BvFT1* expression in biennials is gradually down-regulated during vernalization, resulting in the release of *BvFT2* repression and enabling its activation in the long days that follow winter. In contrast to *FLC*, however, *BvFT1* is also down-regulated in LD, but only in annuals the LD signal suffices for stable repression of *BvFT1* throughout the course of the day and activation of *BvFT2* (Pin et al. 2010). Because annual beets, carrying the dominant *B* or *B4* alleles, bolt readily in long days, whereas biennial beets require vernalization as an additional stimulus for bolting to occur, it is conceivable

that the recessive alleles may be impaired in sensing the inductive LD signal, and that *B* and/or *B4* are part of a (*CO*-dependent or independent) photoperiod pathway in beet which mediates the effect of day-length on the two *FT* genes. Because *B2* acts epistatically to *B*, it may constitute another component of the same floral induction pathway. Large-scale mapping and sequencing projects are underway to identify the underlying genes (Müller, Jung and colleagues, unpublished) and are expected to help elucidating the molecular basis of the long-postulated interaction of photoperiod and vernalization response mechanisms in beet (Owen et al. 1940). However, the currently available data for beet suggest that not only monocots and dicots have evolved different molecular mechanisms to adapt the timing of reproduction to the climatic conditions of their natural habitats, but that different strategies have also evolved within the dicot clade (Jarillo and Pieiro 2011).

1.8 Exploiting Natural Variation for Bolting Time

All cultivated beets are non-bolting under non-inductive environmental conditions, typically after sowing in (late) spring. Beet varieties grown as winter beets in some regions of southern Europe, where mild winters are predominant, have a high bolting resistance. On the other hand, plants with an extremely high bolting resistance would create problems in sugar beet seed production and have thus been avoided by breeders (Sadeghian and Johansson 1993).

Although undesirable, beets can have a tendency for early bolting under central European growing conditions and differences can be found among breeding lines (Sadeghian et al. 1993a). Longer periods of cold temperatures (5–8 °C) in spring can result in a significant increase of bolting beets in the field (Jolliffe and Arthur 1993) (Fig. 1.3b). Several studies have also measured the genetic effects underlying early bolting in biennial beets grown under flowering inductive conditions, e.g. by sowing early in spring (Jolliffe and Arthur 1993; Sadeghian and Johansson 1993). Under these conditions the frequency of bolters in July increased to 75 % (Jolliffe and Arthur 1993). Early bolting in biennial cultivated beets was found to be a quantitative character and high additive genetic effects were calculated (Sadeghian and Johansson 1993). In most studies bolting resistance was dominant over bolting susceptibility (Jolliffe and Arthur 1993).

Rich phenotypic variation for bolting time has been found in recent studies from our institute. In the course of a project to breed winter beets (see Sect. 1.9) a worldwide collection of cultivated and wild *B. vulgaris* (Fig. 1.3d) accessions was grown over winter at different locations across Europe. While annual flowering was abundant among wild beets, all cultivated types including chards and red table beets were biennial. Interestingly, all sugar beets bolted early after winter whereas wild beets showed a larger variation for bolting behavior even after winter (unpublished data). This demonstrates the potential to breed beets with altered bolting characters from the primary gene pool of *B. vulgaris*. With the sequences of major flowering time regulators at hand, it has also become possible to analyze phenotypic variation in

bolting time for associations with sequence variation in candidate genes, and a corresponding project has been initiated. Finally, we are also using TILLING (Targeting Induced Local Lesion in Genomes; McCallum et al. 2000) to detect new sequence variation in flowering time regulators. In brief, candidate gene sequences are amplified by PCR and subjected to endonuclease digests to detect mismatches between a reference wild type sequence and variant alleles, which arose either as a result of natural variation or after EMS-induced mutagenesis. While the frequency of induced mutations was found to be very low in two independent EMS-mutagenized beet populations, a number of 'natural' sequence variants have been found which in some cases could be correlated with altered bolting behaviour (Frerichman et al., in preparation).

1.9 Strategies to Breed Winter Beets by Manipulating Major Bolting Time Regulators

Sugar beets are sown in spring under central European growth conditions (temperate climates). Winter beets, i.e. beets sown before winter, are only grown on a limited scale in some parts of southern Europe. There has been increasing evidence in the past years that winter beets cultivated under temperate climates could be superior in yield to traditionally grown beets (Hoffmann and Kluge-Severin 2010). Under these conditions beets would be sown in August and stay in the field over winter. Thus, winter hardiness is a prerequisite for winter beet cultivation, and experimental data indicate that there is sufficient genetic variation for winter hardiness within the primary gene pool of *B. vulgaris*. A total of 396 cultivated and wild *B. vulgaris* accessions were grown at five different locations across central Europe. As expected for a character which has never been targeted by breeding, survival rates varied widely across the test locations and ranged from 0.7 to 86.3 % (Kirchhoff et al. 2012). The sugar beet accessions showed the highest winter hardiness and had a survival rate of 39.7 % across all environments, suggesting that there is sufficient genetic variation for breeding winter beets.

Winter beet field trials have been performed on a limited scale in Europe. Due to the lack of bolting resistant beets traditional spring beet cultivars were used, which, when sown before winter, started bolting in spring and set flowers a few weeks later. Thus, their root yield was considerably reduced, and bolting beets have a lower sugar but a higher marc content which makes them unsuitable for sugar recovery (Hoffmann and Kluge-Severin 2011). Because root mass is highly correlated with the season and duration of radiation absorption, the yield potential of winter beets was estimated by the absorbed light and the radiation use efficiency. An increase of up to 26 % was estimated independently by two different research groups (Jaggard and Werker 1999; Hoffmann and Kluge-Severin 2010) which makes autumn sown beets an interesting alternative. Moreover, winter beets will reach harvest time earlier. It has been speculated that winter beets will reach the same yield as spring beets 6–8 weeks earlier (Hoffmann and Kluge-Severin 2010). This alone could be a strong

advantage over spring beet cultivation because sugar factories would be able to make better use of their capacities if the beet harvest period will be extended. However, a substantial increase in beet yield requires a higher sink capacity (Hoffmann and Kluge-Severin 2010), and it remains to be seen whether this will be the case in winter beets. Furthermore, for the yield potential to be realized in the field, high disease pressure before winter has to be taken into account, which requires new plant protection management strategies.

The breeding of beets which are bolting-resistant after winter is a specific challenge for beet breeders. No genetic variation for this character is expected among the elite germplasm because breeders have selected for relatively early bolting and rapid flowering after winter, as otherwise seed production would not be economical. Field trials at our institute confirmed that most beet cultivars bolt readily after winter. However, we also found some variation in *B. vulgaris* for bolting behaviour, with some accessions bolting late or comprising high ratios of non-bolting plants after winter. Some accessions repeatedly did not flower until the end of summer, which is the desired phenotype of a winter beet (unpublished data). The genetic causes underlying this phenotype are presently being investigated. It remains to be seen whether the causative alleles can be useful in winter beet breeding because flowering is necessary for seed production.

Our increasing understanding of flowering time regulators in beet (see Sect. 1.6) offers a chance for genetic modification of bolting and flowering time. In principle, they rely on either repression of floral promoters or over-expression of floral repressors. While disruption of gene function can be obtained by mutagenesis (e.g. TILLING) the only realistic option currently available for gene over-expression is genetic modification by transformation. Thus, the resulting plants will be transgenic which causes some legal constraints. Examples of floral promoters as targets for mutagenesis or RNAi are *BvFT2*, as demonstrated by Pin et al. (2010) using the RNAi approach, or the bolting gene *B*, whereas the repressor gene *BvFT1* or homologs of floral repressors in other species are potential candidates for gene over-expression. For *BvFT1*, Pin et al. (2010) demonstrated that constitutive expression from a strong promoter can indeed delay bolting after vernalization for at least 6 months.

Reverting the non-bolting phenotype to induce bolting and flowering for seed production is another challenge (Fig. 1.4a). Different strategies have been suggested (Jung and Müller 2009). The expression of a transgene can be controlled if it is under transcriptional regulation of an inducible promoter, e.g. ethanol- or acetaldehyde-inducible promoters (Fig. 1.4b). Alternatively, a floral repressor such as *BvFT1* is brought under the transcriptional regulation of a constitutive or any other promoter only after cleavage of a spacer fragment separating both sequences (hybrid/recombinase approach). For site-directed cleavage, recombinase target sites flanking the spacer are introduced into a construct (parent 1). The second parent is transformed with a recombinase gene. Both parents would be expected to flower normally after vernalization, and hybrid seeds can be produced as usual. The hybrids, however, would not bolt after vernalization because the spacer fragment is removed as a result of recombinase activity, thus allowing the floral repressor gene to be transcribed from the promoter. Transgenic plants carrying both constructs have

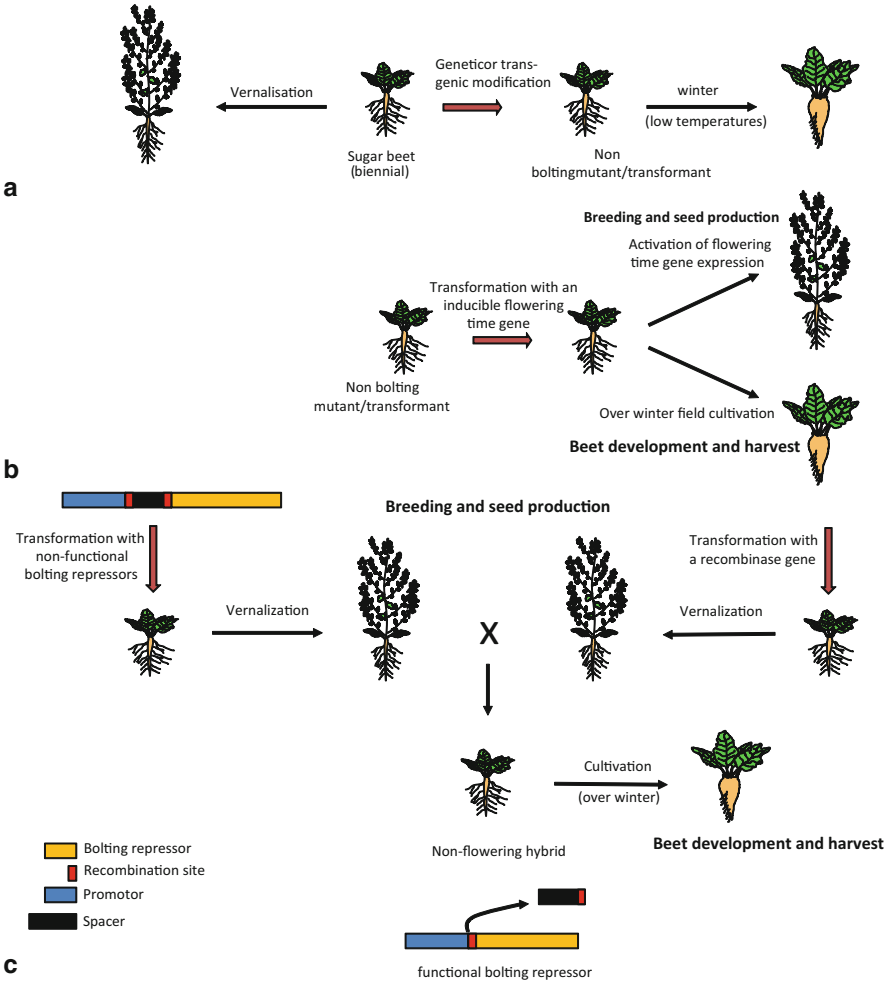

Fig. 1.4 Strategies to breed winter beets, which are bolting resistant after winter. **a** Selection of non-bolting beets after winter. **b** Bolting induction after transformation of non-bolting beets with an inducible flowering time control gene (after Jung and Müller 2009). **c** A transgenic hybrid approach to breed non-bolting F_1 hybrids by activating a flowering time repressor. Parental lines containing either a FLP recombinase or different inactive repressors for flowering time control could be used. After a genetic cross the FLP recombinase activates the repressor in F1 hybrids by depleting a spacer between promoter and coding sequence

been obtained and are presently under investigation (Fig. 1.4c). A third alternative could be the use of two late flowering parents whose phenotypes are caused by different gene actions. Depending on the nature and genetic interactions of these genes, the hybrid may not flower, or at least flower later than the parents, due to additive effects of the parental alleles. If bolting can be delayed until the time of harvest, this

approach could be an interesting alternative because it avoids, in principal, a need for genetic modification. Late bolting accessions which have been already identified from the primary gene pool of *B. vulgaris* (unpublished data) can be used as donors of late flowering alleles. A caveat of this approach is that breeding with late flowering lines is slow as a result of prolonged generation cycles. In general, hybrid approaches as described above seem a realistic option for winter beet breeding because all beet cultivars presently on the market are hybrids. Genetic modification of flowering time genes to breed non-bolting beets would be a rare (and to date unique) example of transgenic plants with improved yield potential.

Note Added in Proof Since the writing of this chapter the identification of the *B* locus by map-based cloning was described (Pin et al. 2012). In this report it was shown that the pseudo-response regulator gene *BOLTING TIME CONTROL 1* (*BvBTC1*) at the *B* locus is required for bolting in the absence of vernalization. Surprisingly, the recessive *Bvbtc1* allele in biennials facilitates bolting, too, but does so only after vernalization. Induction of bolting by *BvBTC1* or *Bvbtc1* is mediated through the regulation of *BvFT1* and *BvFT2* in both non-vernalized (annual) and vernalized (biennial) beets. According to the proposed model, expression of the dominant and fully functional *BvBTC1* allele in annual beets is enhanced in the late afternoon hours of long days, which leads to repression of *BvFT1* and activation of *BvFT2*, and in turn bolting and flowering. Biennial sugar beet cultivars carry a partial loss-of-function allele which is insufficiently active in response to long days without prior vernalization. The prolonged exposure to cold over winter enhances *Bvbtc1* activity, and possibly that of co-regulatory genes, thus restoring the plant's responsiveness to long days in the following spring. Under climate chamber conditions, down-regulation of *Bvbtc1* by RNAi resulted in suppression of bolting after vernalization, suggesting that this gene can be part of strategies towards the development of winter beets. The central role of *BvBTC1* and the *BvFT1/BvFT2* module in the regulation of vernalization requirement and photoperiod response also distinguishes beet from the well-studied flowering time control systems in Arabidopsis and cereals and illustrates the evolution of diverse floral regulatory mechanisms among angiosperms.

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