Roberto Tuberosa · Andreas Graner
Emile Frison *Editors*

Genomics of Plant Genetic Resources

Volume 2. Crop Productivity, Food Security and Nutritional Quality

Springer

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Foreword

Plant genetic resources constitute the feedstock for the biotechnology and genetic engineering enterprises. Year 2013 marks the 60th anniversary of the discovery of the double helix structure of the DNA molecule. This discovery led to the birth of the new genetics based on genomics. The new genetics is helping to revolutionize plant breeding through both marker-assisted selection and recombinant DNA technology. It is in this context that this informative two-volume book entitled "Genomics of Plant Genetic Resources" edited by Prof. Roberto Tuberosa, Prof. Andrea Graner and Dr. Emile Frison is very timely and welcome.

The book deals with managing plant genetic resources, developing genomics platforms and approaches to investigate plant genetic resources, genome sequencing and crop domestication and mining allelic diversity. The different chapters written by eminent authorities shed much light on problems relating to both theoretical and applied genomics. We owe a deep debt of gratitude to the Editors for this labor of love in the cause of conservation and sustainable use of plant genetic resources. This book shows the pathway for achieving an ever-green revolution in agriculture based on enhancement of productivity in perpetuity without associated ecological harm.

M. S. Swaminathan

Foreword

Who would have believed only two decades ago that plant scientists would have access to nearly the complete genetic code of numerous plant species, including major crop species. The idea of having ready access to whole-genome sequences encompassing 140 million bases seemed like science fiction, let alone having available even larger genomes such as rice at 430 Mb or maize at 2500 Mb. And then proceeding to identify variation at the DNA level well beyond what was anticipated, such as the 2.6 SNPs (Single Nucleotide Polymorphisms) per kb in rice. Also produced at an unprecedented rate were literally hundreds of thousands of insert strains, allowing the association of sequences and traits. Who would have believed only a decade ago that we would be capable of analyzing the expression of genes across the whole genome and matching that profile with traits of interest. And now the area of metabolomics is allowing even more meaningful explanations of the biochemical and genetic pathways underlying important traits.

This book brings all of these advances in genomics to the forefront and prepares the plant scientist for the decade ahead. Important technologies are discussed such as association mapping, simulation modeling, and development of appropriate populations including advanced backcrosses and introgression-lines for incorporating traits into useful genetic materials. Such approaches are facilitating the identification of traits that are not obvious simply from observing the plant phenotype, and they provide ways to extract new and useful traits from wild related species. Comparing the genomic information across broadly-related species has generated important evolutionary information. In addition, the common occurrence of duplicated segments recognized in such studies may lead to information fundamental to plant performance.

Methods for the identification of genes underlying traits are improving every day. The association between allelic variation in a candidate gene and a trait is leading to a much greater understanding of the genetic control of traits. Numerous transcription factors and even non-coding sequences are being implicated as the basis of important genetic variation. Forward and reverse genetics are both found to be very useful in making these gene-trait associations.

The tremendous expansion of genomic analytical approaches along with efforts to reduce the cost, together with appropriate statistical designs and analyses, is making it easier and easier to use the ever-increasing sequence information to identify useful genes and gene families. This body of knowledge in plant genomics and its myriad of applications are nicely reflected in this book.

Ronald L. Phillips

Preface

This two-volume book collects 48 manuscripts that present a timely state-of-the-art view on how genomics of plant genetic resources contributes to improve our capacity to characterize and harness natural and artificially induced variation in order to select better cultivars while providing consumers with high-quality and nutritious food. In the past decade, the appreciation of the value of biodiversity has grown steadily, mainly due to the increased awareness of the pivotal role of plant genetic resources for securing the future supply of plant-derived products in the quantity required to meet the burgeoning needs of mankind. The remarkable progress made possible with the deployment of genomics and sequencing platforms has considerably accelerated the pace of gene discovery, the identification of novel, valuable alleles at target loci and their exploitation in breeding programs via marker-assisted selection or other molecular means. Clearly, a better understanding of the genetic make-up and functional variability underpinning the productivity of crops and their adaptation to abiotic and biotic constraints offers unprecedented opportunities for highly targeted approaches while shedding light on the molecular functions that govern such variability.

Meeting the challenges posed by climate change and the future needs of mankind for plant-derived products will require a quantum leap in productivity of the handful of species that provide the staple for our diet and existence. This quantum leap will only be possible through a more effective integration of genomics research with extant breeding programs. As we anticipate a further reduction in the cost of genotyping/sequencing, the exploitation of still largely untapped samples of wild germplasm stored in gene banks will become instrumental for the success of breeding programs. Importantly, the new selection paradigm ushered in by genomics greatly facilitates mining the genetic richness present in orphan crops and underutilized species, previously less readily accessible via conventional approaches.

The unifying picture that emerges from this book unequivocally shows the pivotal role played by genomics to characterize germplasm collections, mine genebanks, elucidate gene function, identify agronomically superior alleles and, ultimately, release improved cultivars. For each of these objectives, the book presents compelling case studies and examples; additional case studies are provided by the references of each chapter.

We hope that this book will provide a helpful reference to students, young researchers, crop specialists and breeders interested in a more effective characterization and utilization of plant genetic resources. In particular, we hope that reading of this book will encourage students and young scientists to pursue a career focused on the study of plant genetic resources and join forces with those already engaged in this challenging and equally fascinating field of science.

We wish to thank all the authors for their timely contributions that have made this book possible. We also thank all those who have contributed to the editing of this book. Last but not least, we wish to thank the policy makers and funding agencies that provide the funds required to collect, conserve, characterize and harness the allelic richness of plant genetic resources.

Roberto Tuberosa
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Part I
Harnessing Plant Genetic Diversity for Enhancing Crop Production and Its Sustainability

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

Siegbert Melzer, Andreas E. Müller and Christian Jung

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

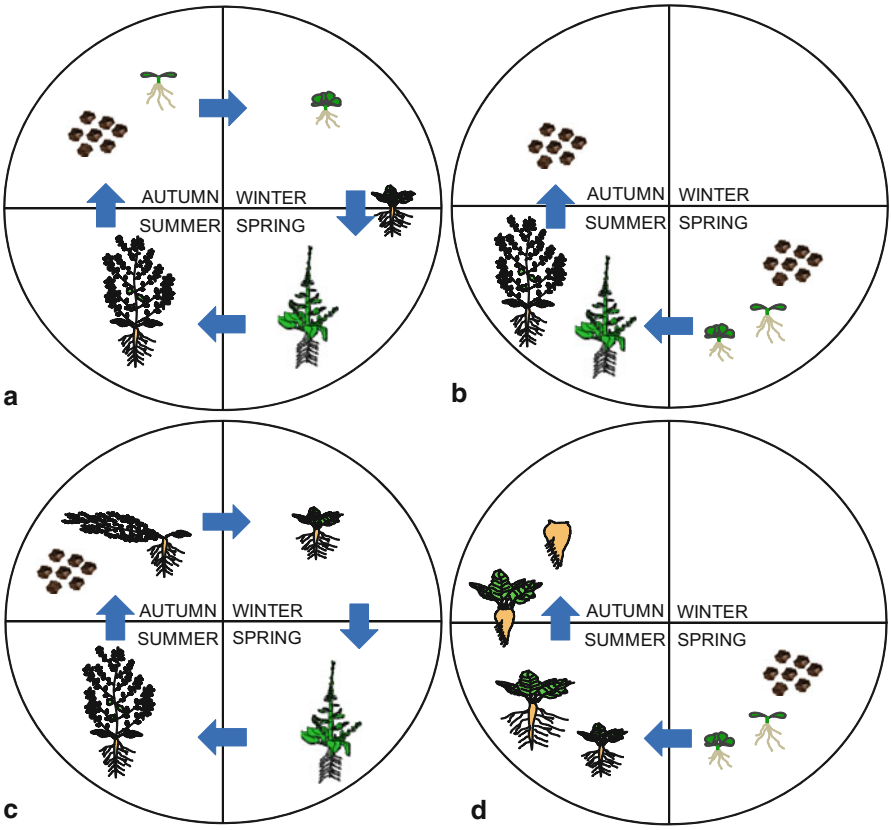

Fig. 1.2 The phenological development of sugar beet and its wild relative *Beta vulgaris* ssp. *maritima*. **a** Life cycle of biennial wild beets. **b** Life cycle of annual wild beets. **c** Life cycle of perennial wild beets. **d** Sugar beet field production ('spring beet').

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