Functional Genomics of Floral Transition in Sugar Beet

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The timing of flowering is of pivotal importance in the life cycle of angiosperms and greatly impacts the agronomic value of numerous crop species. In sugar beet (*Beta vulgaris* L. ssp. *vulgaris*), bolting and flowering is undesirable because it drastically reduces root yield and interferes with harvest operations. Traditionally, sugar beet in moderate climates is sown in spring and harvested in fall. An extension of the growing season by sowing in fall (*i.e.*, one year before harvest) and cultivation over winter is expected to increase yield substantially and could expand the harvest period. However, winter cultivation in central Europe is not possible because vernalization would result in bolting and yield loss. To develop non-bolting winter beets, but also allow for induction of flowering for seed production, full control over the timing of floral transition is mandatory. This is difficult or impossible to achieve by traditional breeding, but may be accomplished by targeted genetic modification of floral transition genes and their induction requirements. We aim to transfer, through comparative genetic and genomic approaches, the extensive knowledge of flowering time control in model species to sugar beet, and to exploit this knowledge for the development of novel, high-yielding cultivars.

Floral transition is a major developmental switch that is tightly controlled by a network of proteins that perceive and integrate developmental and environmental signals to promote or inhibit the transition to flowering. In the model species *Arabidopsis thaliana*, many of the key genes have been identified and functionally characterized (reviewed in PUTTERILL et al., 2004; HE and AMASINO, 2005; BÄURLE and DEAN, 2006), and plant genome and EST sequencing projects are beginning to unveil the presence and evolutionary conservation of these genes across taxa (ALBERT et al., 2005; HECHT et al., 2005). For several genes equivalent or related functions in species as diverse as *A. thaliana* and *Oryza sativa* have been demonstrated, but inter-species comparisons are also starting to reveal flowering time genes that appear to have undergone functional divergence during the evolution of the respective lineages (PUTTERILL et al., 2004; LEE et al., 2005). In *B. vulgaris*, the genes and pathways that regulate floral transition are largely unknown. The tendency for early bolting (without a requirement for vernalization) is under the control of a single dominant gene termed *B* which is currently being cloned from its position on chromosome 2 (EL-MEZAWY et al., 2002; HOHMANN et al., 2003; GAAFAR et al., 2005; MÜLLER and JUNG, unpublished data). As a result of strong selection against early bolting, commercial sugar beet cultivars do not contain a functional *B* allele and behave as biennials. In the absence of *B*, induction and timing of flowering depends on vernalization and requires appropriate photoperiodic and developmental conditions, but to date there are no published reports on the identification and characterization of the regulatory genes involved. These genes, however, are prime candidates for targeted genetic approaches to suppress, or induce, flowering under controlled conditions.

To start identify floral transition gene candidates in sugar beet, we performed TBLASTN-based sequence similarity searches of the public sugar beet EST database (BvGI 1.0, www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=beet) with more than 20 flowering time control genes from *A. thaliana* (PUTTERILL et al., 2004; HE and AMASINO, 2005). Several ESTs were identified with high levels of homology that is not restricted to known conserved domains, incl. homologs of vernalization, photoperiod, and autonomous pathway genes (*e.g.*, *VIN3*, *GI*, *CO*, *FVE*, *FLK*). Subsequent identification of corresponding genomic sequences by BAC library screening, restriction fragment fingerprinting and partial sequencing of the genic region-of-interest in representative BACs confirmed sequence homology to the respective flowering time gene queries beyond the EST regions. Exon-intron structure was found to be largely conserved between homologs. Importantly, for a subset of genes, sequence and genomic DNA gel blot analysis also revealed the presence of multiple gene family members, thus underscoring the need for genome-wide approaches to make an informed selection of candidate genes for targeted genetic modification of flowering time. To identify the most suitable target genes for the development of winter cultivars, we aim to complement the homology-based approach by

a) genome-wide expression profiling
b) functional characterization by RNA interference and overexpression in transgenic plants
c) systematic screening for new allelic variants by TILLing, and
d) phenotyping for altered flowering time and bolting resistance.

In conclusion, our research is intended to provide, in the mid- to longer term, a new genetic tool kit for adaptation of flowering time to specific environmental conditions, with particular regard to winter cultivation of sugar beet. Genetic modification of flowering time control and vernalization requirement are also desirable in other cultivated species, e.g. cereals and grain legumes (for drought escape), fodder grasses (for suppression of flowering), and tree species (for acceleration of breeding and research), and - more generally - for the regional climatic adaptation of elite germplasm. Finally, in the long term, knowledge of floral transition in cultivated species may help to devise strategies to counteract the

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adverse effects of climatic change on flowering time.

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