Genetic Identification of a Novel Locus (LB2) Regulates Bolting Time in Beta vulgaris

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Abstract

Bolting tendency in the facultative perennial species Beta vulgaris, which exhibits large intraspecific variation in bolting tendency, is a complex character governed by various environmental cues and multiple genetic factors. Significant variations in bolting time among annual Beta vulgaris ssp. maritima accessions were found. Three F2 populations derived from crosses between six annual beet accessions were analyzed for bolting tendency. Two populations (Bm38 and Bm49) exhibited phenotypic segregation ratios of 3:1 early-bolting and late-bolting which is expected for dominant-recessive inheritance of a monogenic trait. Phenotypic segregation ratio of the third population Bm49 was 15:1, early-bolting: late-bolting, which is expected for digenic dominant-recessive inheritance suggests the presence of a yet unidentified locus (LB2) which affects bolting time in annual Beta vulgaris.

Keywords

Beta Vulgaris; Sugar Beet; Bolting; Flowering; Floral Transition

Introduction

Sugar beet (Beta vulgaris L. ssp. vulgaris) is the only sucrose storing crop species that can be grown commercially in a wide variety of temperate climates. It accounts for about 30% of world sugar production and are important in Europe, North America, and increasingly in Asia, South America and North Africa. Cultivation of sugar beet in the tropical and subtropical regions, which are mostly developed countries, is a substantial goal (Abou-Elwafa et al., 2006; Abou-Elwafa et al., 2013).

Cultivated sugar beet is a biennial crop exhibits large intraspecific variation in vernalization requirement and life span, and includes annual accessions as well as long-lived, iteroparous perennials (Letchert, 1994; Hautekèete et al., 2002). In order to initiate bolting, sugar beet requires a combination of exposure to low temperatures between 2°C and 10º C (vernalization), followed by long-day conditions (Lexander 1980). In general, bolting time is accelerated and the number of bolters is increased as a result of vernalization (Sadeghian and Johansson 1993; Crosthwaite and Jenkins 1993; Abou-Elwafa et al., 2006). Significant variations have been observed among wild beet populations of the subspecies Beta vulgaris ssp. maritima in terms of life cycle and bolting behavior. These variations are latitude-dependent, and vary from perennial populations with vernalization requirement in northern areas to annual populations in the Mediterranean region (van Dijk et al., 1997). Variation in bolting time among wild beet populations was found to be mainly due to differences in their vernalization requirement. In contrast to biennial cultivated beets which have an obligate requirement for vernalization, annual wild beets bolt without prior vernalization. However, for sugar production early-bolting (bolting in the first growing season) is an undesirable because it drastically reduces root yield and interferes with mechanical harvesting, and breeders have successfully selected for the biennial habit (Jaggard and Werker, 1999; Rinaldi and Vonella, 2006).

Bolting tendency in sugar beet was suggested to be under the control of genes affected by vernalization and photoperiod and detected predominantly additive and dominant genetic effects, but also epistasis (Sadeghian et al., 1993). Owen et al. (1940) hypothesized the presence of a locus responsible for easy-bolting tendency in biennial beets, termed B, which the authors suggested to be allelic to B in annual beets. The annual growth habit in B. vulgaris was shown to be controlled by a major dominant gene, referred to as the bolting gene B, which promotes the initiation of bolting in long days without vernalization (Munerati, 1931; Abegg, 1936). The B gene was genetically mapped by different mapping approaches to chromosome II of sugar beet (Boundry et al., 1994; El-Mezawy et al., 2002). Recently, a candidate of the B gene (BoBTC1) was recently cloned and functionally characterized (Pin et al., 2012). Furthermore, three
genes were identified to regulate bolting behavior in sugar beet; i) the locus B2 which was mapped to chromosome VI of sugar beet and was shown to act epistatically to the B gene (Büttner et al., 2010), ii) a locus acting independently from the B gene in regulating bolting time termed B3 (Büttner et al., 2010), and iii) B4 locus which is genetically linked to the B gene on chromosome II and acting independently from the B gene in bolting regulation (Abou-Elwafa et al., 2012). Shavrukow (2000) suggested that late-bolting in sugar beet is regulated by a gene termed lb which is linked to the monogermity gene on chromosome II of Beta vulgaris. The use of the facultative long-day plant Arabidopsis thaliana as a model has led to the identification of several putative B. vulgaris orthologs of flowering time genes in Arabidopsis, including BvFL1 (an Arabidopsis FLC homolog), BvCOL1, BvFLK and BvFVE were identified and functionally characterized to be involved in the regulation of flowering time in sugar beet (Reeves et al., 2007; Chia et al., 2008; Abou-Elwafa et al., 2010).

In the current study we used a forward genetic approach to further elucidate the genetic basis of the late-bolting phenotype in B. vulgaris. Annual B. vulgaris ssp. maritima genotypes which exhibited variations in bolting behavior were crossed together to produce F2 populations segregating for bolting behavior which is essential for synchronization of bolting and flowering for hybrid seed production purposes. We hypothesized that the late-bolting phenotype is either: i) controlled by a single dominant gene, and a 3:1 phenotypic segregation ratio for bolting behavior (early-bolting vs. late-bolting) would be expected or ii) is genetically regulated by two unlinked loci in epistatic interaction. The expected phenotypic segregation for bolting behavior would also be 3:1, or iii) is a polygenic trait, and a deviation from the 3:1 phenotypic segregation ratio would be expected.

Materials and Methods

Plant Material

Annual B. vulgaris ssp. maritima accessions were collected from the Mediterranean coasts, and phenotyped for bolting time. Annual genotypes exhibited significant variations in bolting time were crossed together (Table 1; Figure 1). All crosses were conducted by bag isolation in the greenhouse. Cross progenies were identified phenotypically by hypocotyl color. F1 plants were propagated in the greenhouse, and selfed to produce F2 seed (Table 1). Three sibling F2 populations were used in the current study; i) population Bm07 consists of 93 individuals, ii) population Bm38 consists of 91 individuals and iii) population Bm49 which consists of 88 individuals.

### Table 1 Annual genotypes used for generation of F2 populations

<table>
<thead>
<tr>
<th>Annual pollinator</th>
<th>Annual seed parent</th>
<th>F1 plant selfed</th>
<th>F2 population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bm11-01</td>
<td>Bm11-21</td>
<td>BmF1-07</td>
<td>Bm07</td>
</tr>
<tr>
<td>Bm11-03</td>
<td>Bm11-34</td>
<td>BmF1-38</td>
<td>Bm38</td>
</tr>
<tr>
<td>Bm11-07</td>
<td>Bm11-37</td>
<td>BmF1-49</td>
<td>Bm49</td>
</tr>
</tbody>
</table>

FIG. 1 PHENOTYPING OF ANNUAL B. vulgaris ssp. maritima accessions. Six annual accessions were phenotyped for the onset of bolting under greenhouse conditions. Asterisks indicate significant differences among accessions as calculated by analysis of variance.

**Phenotypic Screening**

The annual accessions Bm11-01, Bm11-03, Bm11-07, Bm11-21, Bm11-34 and Bm11-37 and F2 populations were phenotyped for the onset of bolting. Nineteen to twenty two plants from each accession were phenotyped. All experiments were carried out in a glasshouse with 21-25°C day and 15-18°C night temperatures, under long day conditions (16/8 h) with supplementary lighting using 400-w incandescent units. Seeds were sown on April 10, 2012, in 9 × 9 × 9 cm pots. The average light intensity at night above the soil was approximately 23917 lux. Plants were phenotyped every two to three days for onset of bolting (BBCH scale code 51; (Meier 2001)) until September 15, 2012.

**Statistical Analysis**

The statistical analysis (t-tests) and Chi square (χ²) analysis was performed using SAS 9.1 (SAS Institute, Cary, NC, USA).
Results

Phenotypic Segregation for Late-Bolting

For each of the three crosses, 88 to 93 F2 plants were phenotyped for late-bolting behavior under long day conditions (early-bolting or late-bolting; Suppl. Tab. 1). All populations segregated for late-bolting behavior and contained both early-bolting and late-bolting individuals. In each population a distinct separation between the early-bolting individuals and the late-bolting ones was observed (Table 2; Figure 2). In the two populations Bm38 and Bm49, the phenotypic segregation ratios did not deviate significantly from the 3:1 segregation ratio of early-bolting and late-bolting plants expected for dominant-recessive inheritance of a monogenic trait, as tested by $\chi^2$ analysis (Table 2). For the third population (Bm07), the null hypothesis of a 3:1 ratio was rejected at either $\alpha=0.05$ or $\alpha=0.01$, respectively. Meanwhile, segregation of early-bolting and late-bolting plants in the population Bm07 did not deviate significantly from a ratio of 15:1 (Table 2), which is expected for digenic dominant-recessive inheritance of the trait when only the double recessive genotype is late-bolting.

<table>
<thead>
<tr>
<th>F2 population</th>
<th>Number of plants</th>
<th>EB $^1$</th>
<th>LB $^2$</th>
<th>$\chi^2$ test for $H_0=3:1$ (EB vs. LB)$^3$</th>
<th>$\chi^2$ test for $H_0=15:1$ (EB vs. LB)$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bm07</td>
<td>93</td>
<td>84</td>
<td>9</td>
<td>11.64**</td>
<td>1.86</td>
</tr>
<tr>
<td>Bm38</td>
<td>91</td>
<td>73</td>
<td>18</td>
<td>1.08</td>
<td>28.43**</td>
</tr>
<tr>
<td>Bm49</td>
<td>88</td>
<td>71</td>
<td>17</td>
<td>1.51</td>
<td>25.65**</td>
</tr>
</tbody>
</table>

$^1$ EB: early bolting plants  
$^2$ LB: Late bolting plants  
$^3$ $H_0$: null hypothesis for monogenic, dominant-recessive trait  
$^4$ $H_0$: null hypothesis for digenic, dominant-recessive trait  

** $\alpha=0.01$

Variation in Bolting Time Among Annuals and F2 Populations

The six annual B. vulgaris ssp. maritima accessions used in generating the three F2 populations were phenotyped for bolting time. Significant differences in number of days to bolting among annual accessions were found ($P<0.05$; Figure 1). Accessions Bm11-01, Bm11-34 and Bm11-37 revealed a significant delay in bolting time compared to the remaining accessions.

The three F2 populations were phenotyped for bolting time (Figure 2; Suppl. Tab. 3). In general, population Bm38 and population Bm49 plants started to bolt earlier than population Bm07. The majority of early bolting plants of populations Bm38 and Bm49 started to bolt much earlier (at 6-9 weeks after sowing), while the late-bolting plants bolted much later (at 12-14 weeks after sowing; Figure 2). In population Bm07 the early bolting plants were approximately normally distributed with considerable delay in bolting time either within the early bolting or the late bolting subpopulations (Table 3; Figure 2). To test the significance of differences in bolting time between the early bolting and the late-bolting individuals within each population, T-test was performed for number of days to bolting between the two phenotypic classes (early bolting and late-bolting). In all of the three populations, highly significant differences in annual bolting time between the two phenotypic classes were observed as indicated by T-test. The mean of days to bolting for the late-bolting subpopulation was significantly higher than that of the early bolting subpopulation (Table 3).

Discussion

The genetic control of late-bolting phenotype were analyzed in three sibling F2 populations derived from a cross between four annual beet accession differ significantly in their bolting tendency. The major findings described in this study are: i) contrary to what had been thought, the lb locus is not the only locus underlying late-bolting phenotype in B. vulgaris, and ii) the discovery of a novel late-bolting locus (LB2) which regulates late-bolting behavior independently from the previously identified lb gene.

Two F2 populations, Bm38 and Bm49, behaved...
similarly in segregation for bolting time. The non-significant deviation of the pattern of phenotypic segregation of early bolting and late-bolting plants in populations Bm38 and Bm49 from 3:1 suggested that, according to our hypotheses (see Introduction), late-bolting behavior in both populations is either monogenically inherited (hypothesis i), or coregulated by two loci in epistatic interaction (hypothesis ii). In contrast to populations Bm38 and Bm49, the segregation data for population Bm07 indicating that the late-bolting phenotype control involves (at least) one additional late-bolting locus (LB2). The fact that the segregation ratio does not deviate significantly from 15:1 suggests that this locus is not linked to the lb gene and acts independently of lb, but like lb is also inherited in a dominant recessive manner.

In all populations, plants of within population could be classified into two distinct groups in terms of number of days to bolting (early-bolting and late-bolting plants). In general, late-bolting plants bolted substantially and highly significantly later than individuals of the early-bolting group (Table 3). Two lines of evidence emphasize that the late-bolting phenotype in BM07 is controls by two independent late-bolting genes. Firstly, in contrast to Bm38 and Bm49 bolting plants occurred in large excess of what would be expected for monogenic inheritance of this trait, and the observed segregation ratio matched more closely the expectation for digenic inheritance. Secondly, the early-bolting plants in population Bm07 bolted essentially late within a wide range of 41 days (from 45 to 86 days after sowing with an average of 68.89 days) (Table 3; Suppl. Tab.). Similarly, the late bolting plants of Bm07 bolted substantially later than those of either the two other populations (with an average of 108.22 days). However, we also cannot exclude the possibility that the unexpectedly high phenotypic segregation ratio and the extremely late-bolting phenotype in this population are due to several quantitative loci (BvBTC1, B2, B3 and B4: Pin et al., 2012, Büttner et al., 2010; Abou-Elwaafa et al., 2012).

**Conclusion**

Our results add more complexity to the genetic control of late-bolting and floral transition in *B. vulgaris*. It was thought that the late bolting phenotype of *B. vulgaris* is genetically controlled by a single gene termed lb which was genetically mapped to chromosome II of *B. vulgaris* (Shavrukow 2000). A preliminary analysis of annual *B. vulgaris* ssp. maritima accessions for late-bolting suggests the presence of a unidentified locus (LB2) which is unlinked to the known late-bolting locus located on chromosome II (lb) and affects bolting time in annual *Beta vulgaris*. These genes may also prove to be candidate genes for the facilitation of induction and synchronization of bolting and flowering for hybrid seed production. A genetic map construction of population Bm49 is essential for QTL analysis to confirm the multigene inheritance of the late bolting phenotype and to locate the genetic position of the identified LB2 locus.

**REFERENCES**


