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Selection for cold hardiness and late bolting for breeding winter beets

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Abstract

The yield of a regular spring sown sugar beet is limited by insufficient canopy in May and June. An autumn sown sugar beet (‘winter beet’) with early canopy closure is expected to have a yield increase of up to 26%. Winter hardiness and a system for bolting control are essential requirements for the breeding of winter beets. Therefore, we are evaluating the genetic variation for winter-hardiness and bolting behaviour within the *B. vulgaris* gene pool under field conditions. Furthermore, we will map QTLs for winter-hardiness and bolting behaviour and we will establish a system for bolting control by targeted suppression and expression of flowering time genes from sugar beet and *Arabidopsis thaliana*. This paper presents preliminary results for the genetic variation of winter-hardiness and bolting behaviour of 396 *Beta vulgaris* accessions and a concept for bolting control of beets.

Keywords
Beta vulgaris, bolting control, flowering genes, sugar beet, winter-hardiness

Introduction

The yield of spring sown sugar beet (*Beta vulgaris* subsp. *vulgaris* L.) beet is mainly restricted by limited light interception due to insufficient canopy in May and June when sun radiation already reaches high levels before canopy closure in July (JAGGARD and WERKER 1999). One strategy to overcome this asynchrony is an autumn sown beet whose leaf canopy has been developed early in spring and thus can optimally intercept high radiation levels in May and June. To grow an autumn sown sugar beet, however, two requirements have to be fulfilled: I) sufficient winter hardiness to survive the Central European winters and II) control of bolting and flowering, as the low winter temperatures will induce plant vernalization that promotes bolting of sugar beets in spring. Bolting and flowering are not desired during crop cultivation as this causes major beet and sugar yield losses. However, non-bolting plants are unable to flower and to produce seeds. Thus, it is necessary to establish a system for bolting control.

Material and Methods

To evaluate the genetic variation for winter-hardiness and bolting behavior under Central and Eastern European conditions, a representative set of 396 *B. vulgaris* accessions, including 278 members of the *Beta* core collection (FRESE 2000), was tested at four locations in two replications in two years, 2008/09 and 2009/10. The locations were Minsk and Nesvizh (Belarus) and Kiel and Dransfeld (Germany). The tested panel comprises 100 sugar beets, 90 red table beets, 62 leaf beets, 61 fodder beets, 56 wild beets and 27 accessions with unknown background. In 2008, the experiment was sown in August in a randomized complete block design in 2.1 m long single rows with a row distance of 45 cm. Each row contained 15 plants of one accession. The survival rates (SR) of the plants were determined as the ratio of living plants after and before winter. To identify late bolting accessions within the panel, the bolting behaviour of surviving plants was monitored. Accessions with at least one plant bolting before the 6th May were classified as early bolters. A second class includes accessions which started to bolt between the 6th and 18th May. Accessions starting to bolt between the 18th May and the 7th June were classified as late bolting. The accessions bolting later than the 7th June were encompassed as the latest bolting class.

Results and Discussion

Selection for cold hardiness and late bolting

Winter conditions in 2008/09 differed among the test locations with temperatures ranging from -12°C in Kiel to -24°C in Göttingen. The proportion of accessions with at least one plant surviving ranged from 33% in Minsk to 89% in Kiel, with Göttingen showing the best differentiation within the 396 tested accessions. Therefore, further focus is given only on data of this environment. In Göttingen, SR for the tested accessions ranged from 0 to 100%, indicating polygenic inheritance of winter hardiness (*Figure 1*). Sugar beet is by far the most winter hardy cultivated form within the *B. vulgaris* gene pool. Of the tested sugar beets about 90% had SR higher than 90%. The lowest SR of all sugar beet accession was 58%.

Our results indicate sufficient genetic variation in the *B. vulgaris* gene pool for improving winter hardiness in sugar beet. For further improvement of winter hardiness in sugar beet, tolerant sugar beet accessions were crossed to each other. Also, crosses between susceptible and tolerant sugar beet accessions were performed for the development of mapping populations for the genetic dissection of winter hardiness. Identification of QTLs responsible for winter hardiness might offer the opportunity for winter independent selection via marker assisted selection.

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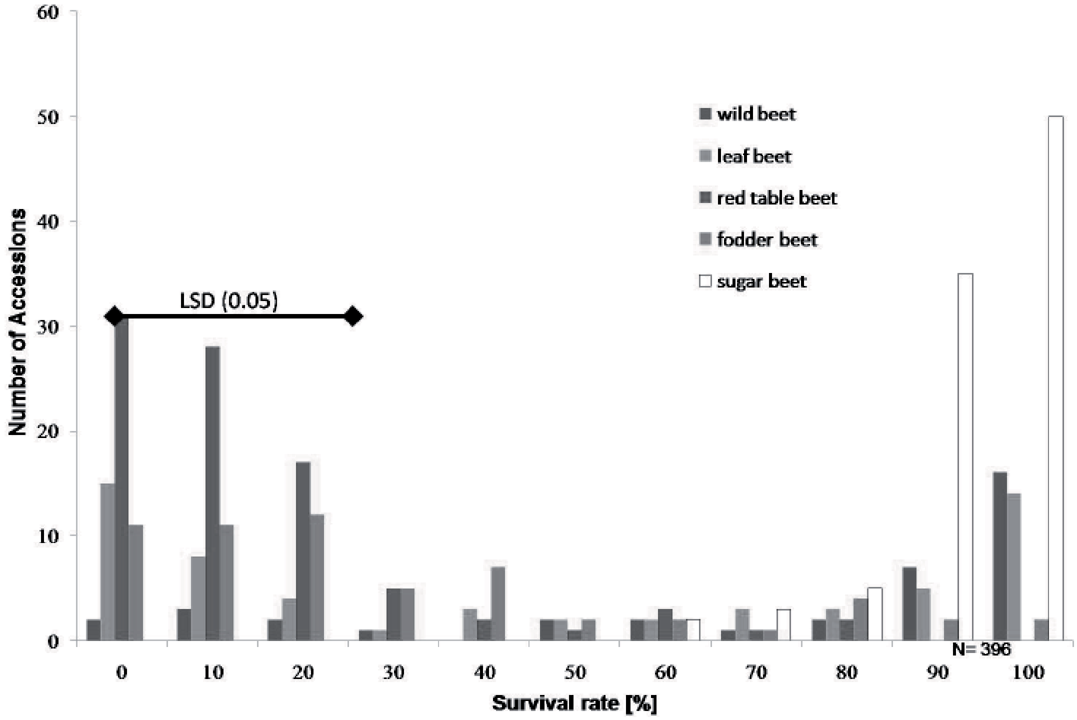

Figure 1: **Histogram of survival rates of 396 Beta vulgaris accessions after winter in Göttingen 2008/09. The survival rate is the ratio of plants after and before winter**

The panel of *B. vulgaris* accessions showed a variation in bolting time of more than a month, including early accessions bolting before the 6th of May to the latest bolting accessions which bolted after the 7th of June. Before the 6th of May about two thirds of the accessions started bolting. Comparing the different *B. vulgaris* types, Sugar beet accessions were among the earliest whereas leaf beets were among the latest bolting accessions. Of the sugar beet accessions 87% bolted earlier than the 6th of May. In contrast only 39% of the leaf beets were bolting at that date, whereas 30% of the leaf beets were bolting after the 18th of May. Therewith the leaf beets showed the widest variation for bolting time. Within the panel sugar beet displayed relatively low variation for bolting as only five percent were bolting later than the 18th of May.

Genotypes that do not bolt after winter are essential for the development of winter beets. As expected, there was no accession with complete bolting inhibition, however a large variation in bolting behaviour was observed. Early bolting of the majority of the sugar beet accessions can be explained by selection for early seed development and maturity during cultivar development, as early seed availability is desired by plant breeders for seed production. We performed crosses between late bolting accessions for enhanced bolting delay, as well as crosses of early and late bolting accessions to obtain mapping populations for QTL analysis of bolting behaviour. The large genetic variation within the species Beta creates an opportunity for the development of desired winter beets by crossing late bolting accessions among each other to accumulate late bolting alleles. However, creation of non bolting genotypes is only the first step towards a system of bolting control, as bolting is necessary for seed production. Therefore, controlled bolting is needed which can only be achieved by genetic modification.

A concept for bolting control of beets

Different concepts for flowering time and bolting control in crops have recently been published by JUNG and MÜLLER (2009). As an alternative to the above mentioned selection procedure genetic modification will be used. We will overexpress floral repressor genes of *A. thaliana* and *B. vulgaris* in sugar beet. Furthermore, we will express a hairpin RNAi-mediating construct to silence a floral promoter gene of sugar beet. In order to control the expression of the floral repressor genes and the silencing construct, we will use the FLP-FRT recombinase system of *Saccharomyces cerevisiae* (KILBY et al. 1995). This system will allow regular bolting and flowering in both parental hybrid components for hybrid seed production. One hybrid component line will contain a transgene for constitutive expression of an FLP recombinase. In contrast, the second component will contain a transgene consisting of the selectable marker gene *nptII* under control of a strong promoter and the non active bolting repressor construct without promoter. The *nptII* gene is flanked by FLP recognition site repeats (FRT sites). After crossing, both transgenes will be combined. The FLP recombinase will recognize the FRT sites flanking *nptII* and eliminate the *nptII* gene by recombination, thereby putting the floral repressor construct under the control of the strong promoter that had controlled the *nptII* gene before recombination. Thus, the floral repressor construct will be activated only in plants grown from hybrid seeds produced for field cultivation. For transformation experiments a number of flowering time candidate genes have been isolated

(MÜLLER et al. 2007). Using genes from *A. thaliana* and *B. vulgaris*, vectors have already been constructed to begin sugar beet transformation.

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