PROJECT DETAILS

- **Title**: Enhancing yield and biomass in canola by modifying carbohydrate metabolism
- **Funders:** Agriculture and Agri-Food Canada, Canola Council of Canada, Alberta Canola, SaskCanola and Manitoba Canola Growers
- **Research program:** Canadian Agricultural Partnership
- **Principal investigator:** Michael Emes
- **Collaborators/additional investigators:** Ian Tetlow
- **Year completed:** 2021

Final report

Introduction

Carbohydrates such as starch provide the stored energy reserves of plants. The goal of the plant is to ensure seed production and we previously developed a novel technology which caused a remarkable boost in seed yield in Arabidopsis by modifying starch metabolism. When the Arabidopsis endogenous leaf starch branching enzymes (SBEs) were replaced with maize endosperm homologues ZmSBEI or ZmSBEIIb, the Arabidopsis plants demonstrated significant increases in starch biosynthesis and a dramatic increase in seed production that led to a 250% increase in total seed oil produced per plant (Liu et al., 2016). The increase in seed production was associated with an increase in the numbers of flowers and siliques per plant, while the fatty acid profile of the seed oil remained unaffected.

Canola (*Brassica napus*) is genetically close to Arabidopsis with highly conserved gene functions between the two species. The homologous SBEs in canola are assembled on both A and C genomes and they have high identities to those in Arabidopsis. This provided a feasible strategy to apply the above technology to canola. Canola is allotetraploid with a more complicated genetic background and, since no SBE knockout mutants are so far publicly available, our strategy for replication of this effect in canola has been divided into two stages:

(1) deletion of endogenous *BnaSBE*s by gene editing.

(2) expression of maize SBEI or SBEIIb.

Gene editing using the CRISPR/Cas9 system has been applied to edit the endogenous SBEs and we have successfully produced homozygous mutant lines targeting six SBE genes. The *sbe* sextuple mutant has been analysed with respect to biochemistry and plant development. Characterization of positive lines expressing maize SBEI or SBEIIb in the *sbe* sextuple mutant background has now been accomplished. Milestones and deliverables achieved in this period:

(1) evaluated the T1 generation of canola lines expressing ZmSBEI in the sextuple *sbe* mutant; these lines show an 8-20% increase in total seed yield, which is very promising given that they are a mix of heterozygotes and homozygotes;

(2) identification of homozygous lines for further evaluation and field trials;

(3) characterization of the "thick" main stem phenotype in the *sbe* sextuple mutant when compared to wild type plant;

(4) submission of a manuscript to the journal Plant Physiology.

Details of (1-3) are presented in the following sections.

Results

(1) Effects of expression of ZmSBEI in the *sbe* sextuple mutant

Having shown that the number of flowers in the lines expressing ZmSBEI in the *sbe* sextuple mutant was increased up to 2-fold compared to the WT, we then analyzed the number of siliques and total seed weight. Even at this early stage, with many more transgenic plants in the pipeline, line LW17S-1 displayed increased silique number (10-30%) and increased total seed weight (8-20%) (Figure 1). Three more independent lines are at the early stage of T1 generation and await phenotype evaluation. These data are highly promising and consistent with the results of ZmSBEI expression in the *sbe* quadruple mutant background.

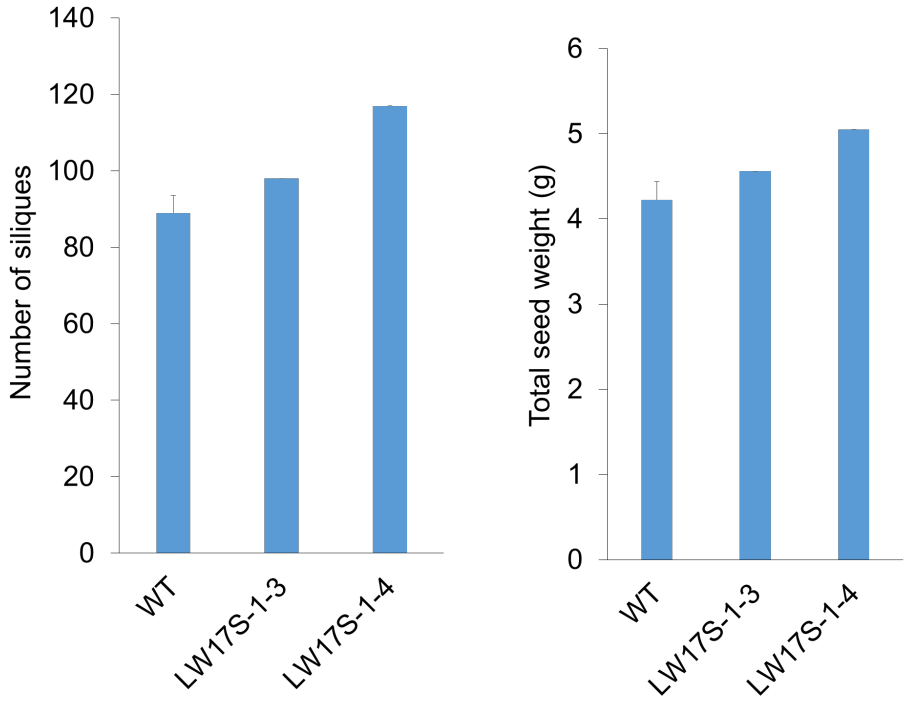

Figure 1. Overexpression of ZmSBEI in the *sbe* sextuple mutant background increases silique number and total seed weight. Comparison between two T1 lines and WT plant. Values for WT are means ± SE (standard errors) derived from 3 plants; values for lines LW17S-1-3 and LW17S-1-4 are from single plants as they are the progeny of the T0 generation.

(2) Identification of homozygous lines expressing ZmSBEI in the *sbe* sextuple mutant

Since the previously selected T0 lines expressing ZmSBEI in the *sbe* sextuple mutant were shown to contain a single T-DNA insertion, those T1 plants are expected to be homozygous when their progeny are all positive and do not segregate. Thus, homozygous lines have now been identified from three, independent, T0 lines, and their phenotypes will be further evaluated in greenhouse and field.

(3) Characterization of the thick main stem phenotype in the *sbe* sextuple mutant

While phenotyping the *sbe* sextuple mutant and WT plants, we noticed that at the maturation stage, the main stem in the sextuple mutant was evidently thicker than WT, by 21-50% when measuring the perimeter of internodes (Figure 2A, B). The cross-section areas in each internode was calculated using their respective perimeters, showing remarkably increased areas in the sextuple mutant by 80-125% (Figure 2C). For illustration, cross-sections of the second internode are shown in Figure 2D. There is clearly an increase in stem diameter and vascular bundle area in the sextuple mutant compared to WT. We also found that the sextuple mutant developed on average three more internodes than WT plants (Figure 2A, E). The additional sextuple

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mutant, LW13Q-22-167-8, which contains different mutations in *BnaA10_SBE2.1* genes from the current line LW13Q-22-59-36, also displayed this phenotype consistent with the hypothesis that the thick stem is associated with *BnaSBE* mutations. The seed number per silique in the sextuple mutant was approximately 10% less than that in WT (Figure 2E), resulting in slightly reduced total seed weight (10%) in the sextuple mutant (Figure 2F). Importantly, initial observations suggest that transgenic lines expressing *ZmSBEI* in the sextuple mutant produce a similarly thickened stem as well as increased yield compared to WT, suggesting a "double benefit" from these genetic changes.

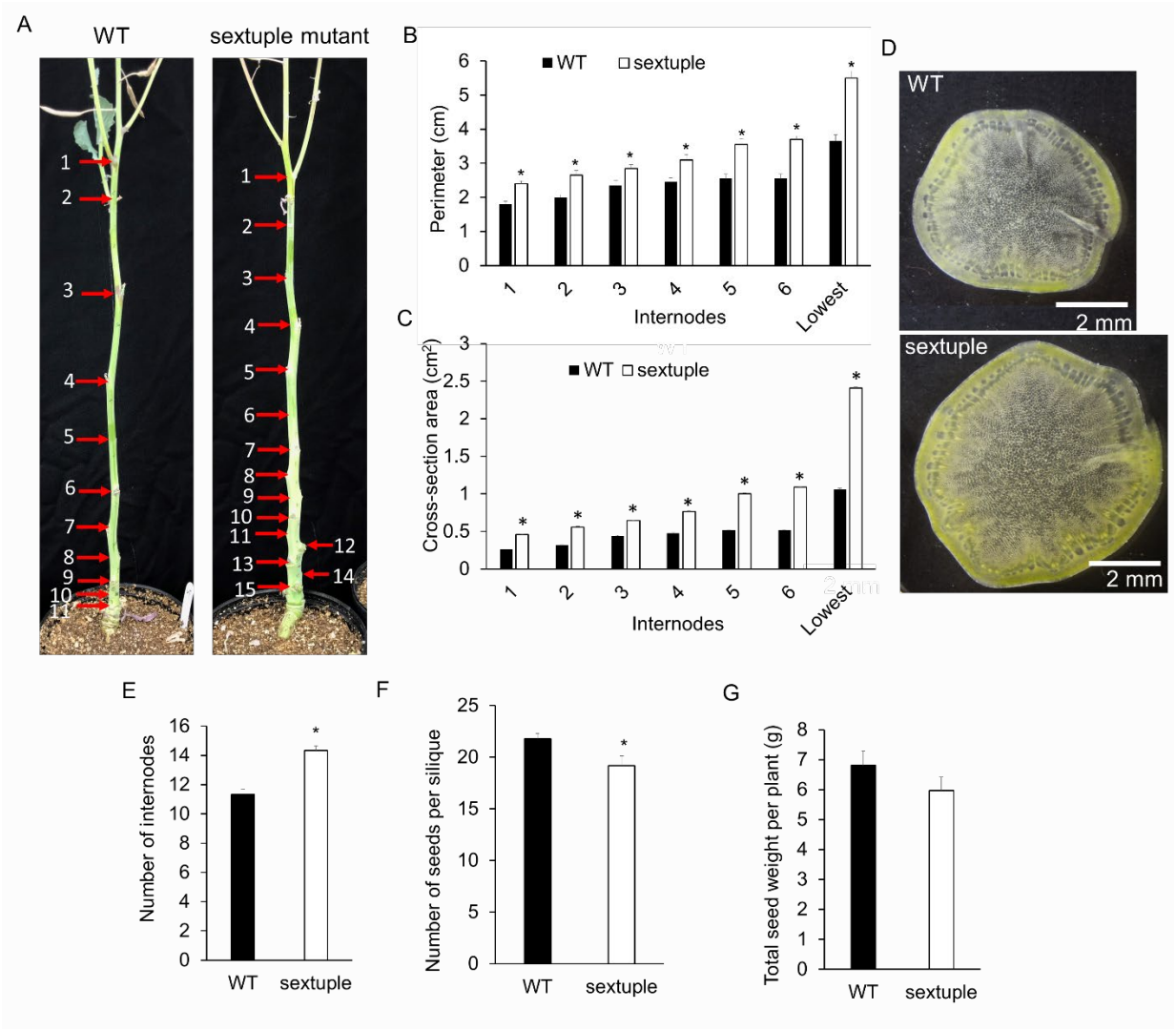

Figure 2. The *sbe* sextuple mutant has a markedly thicker main stem than WT.

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(A) Representative images illustrating thick stem and more internodes on the main stem in the sextuple mutant. Arrows indicate the positions of each nodes and numbers indicate the positions of nodes from top to bottom.

(B) Perimeters of internodes 1 to 6 counted from the top, and the lowest internode. Data represent mean and SE of six plants.

(C) Comparison of cross-sectional areas of internodes 1 to 6, counted from the top, and the lowest internode. Data represent mean and SE of six plants.

(D) Images of cross-sections at the second internode in WT and sextuple mutant. Bars = 2 mm.

(E) Number of internodes on the main stem. Data represent mean and SE of six plants.

(F) Number of seeds per silique. Data represent mean and SE of a total of 60 siliques from three plants.

(G) Total seed weight per plant. Data represent mean and SE of eight plants. Asterisks indicate statistically significant according to Student's t-test.

Future directions

We will continue to analyze the transformants expressing *ZmSBEI* or *ZmSBEIIb* in the *sbe* sextuple mutant background. Previous reports have shown that high temperature at flowering causes a deterioration in stem mechanical properties in canola, resulting in increased risk of crop lodging and accompanied yield loss (Wu et al., 2021, J Agro Crop Sci 207: 74-87). Given that the *sbe* sextuple mutant seeds maintained wildtype levels of seed oil synthesis (previous report), we will test whether the thickened stems can minimize yield loss under high temperature/drought stress in controlled environmental conditions. More importantly, our preliminary data has shown that the lines expressing ZmSBEI/ZmSBEIIb also possess the thick stem phenotype. Thus, once we have a number of homozygous lines expressing *ZmSBEI* or *ZmSBEIIb* in the *sbe* sextuple mutant background, we will test whether the higher yield performance of these transgenic lines, compared to WT, is further enhanced during abiotic stress prior to testing in field conditions. Given the anticipated changes arising from global climate change this technology may provide additional advantages. Once we have completed the current analyses, we will look to secure further funding to undertake these tests in greenhouse and field trials.

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