

1 **RESEARCH ARTICLE**

2 **Vernalization alters sugar beet (*Beta vulgaris*) sink and source**  
3 **identities and reverses phloem translocation from taproots to shoots**

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18 **Short title:** Sugar beet sink source reversion

19 **One-sentence summary:** Cold treatment transforms sugar beet (*Beta vulgaris*) taproots from  
20 sucrose-storing sink organs to sucrose-mobilizing source organs prior to bolting.

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24 **ABSTRACT**

25 During vegetative growth, biennial sugar beets maintain a steep gradient between the shoot (source)  
26 and the sucrose-storing taproot (sink). To shift from vegetative to generative growth, they require  
27 a chilling phase, called vernalization. Here, we studied sugar beet sink-source dynamics upon cold  
28 temperature-induced vernalization and revealed a pre-flowering taproot sink to source reversal.  
29 This transition is induced by transcriptomic and functional reprogramming of sugar beet tissue,  
30 resulting in a reversal of flux direction in long distance transport system, the phloem. As a key  
31 process for this transition, vacuolar sucrose importers and exporters, BvTST2;1 and BvSUT4, are  
32 oppositely regulated, leading to re-mobilization of sugars from taproot storage vacuoles.  
33 Concomitant changes in the expression of floral regulator genes suggest that the now deciphered  
34 processes are a prerequisite for bolting. Our data may thus serve dissecting metabolic and  
35 developmental triggers for bolting, which are potential targets for genome editing or breeding  
36 approaches.

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## 40 **Introduction**

41 Plants modulate not only the shape and size of their organs, but also physiological and molecular  
42 properties in these structures during development and as a response to environmental stimuli. In  
43 general, sink organs in plants depend on the import of carbohydrates, mainly sucrose, from source  
44 organs. However, previous sink organs may differentiate into ‘sources’, which then, in turn,  
45 provide mobilized storage products to newly emerging sinks.

46 The relative strengths of sinks and sources can be adjusted by the activity of sucrose synthesizing  
47 and degrading enzymes (Herbers and Sonnewald, 1998), and by alteration of the activities of  
48 phloem located sucrose loaders (Imlau et al., 1999; Gottwald et al., 2000; Srivastava et al., 2008;  
49 Chen et al., 2012). As a consequence, both, sucrose metabolizing enzymes and transporters  
50 represent targets relevant for breeding strategies aiming at yield increase of crops (Ludewig and  
51 Sonnewald, 2016; Sonnewald and Fernie, 2018).

52 Sucrose is the primary sugar transported in the phloem from source to sink organs. After unloading  
53 at the sinks, sucrose can be used as energy precursor, and as building block for growth and storage  
54 compound biosynthesis. Non-green storage organs like tubers or taproots must maintain a steep  
55 source to sink gradient. To do so, imported sucrose is rapidly converted into relatively inert storage  
56 compounds like starch or is compartmentalized intracellularly into large cell vacuoles. As given,  
57 sink and source identities of plant organs are dynamic and corresponding transitions are initiated  
58 after onset of endogenous developmental signals (Turgeon, 1989) or in response to specific  
59 environmental stimuli (Roitsch, 1999). Thus, dynamic regulation of genes and enzymes involved  
60 in carbohydrate metabolism and import of sugars into the phloem of mobilizing storage organs are  
61 key for source establishment of former sinks (Viola et al., 2007; Liu et al., 2015; O’Neill et al.,  
62 2013; Boussiengui-Boussiengui et al., 2016).

63 Sugar beet (*Beta vulgaris*), the major crop species providing industrial sucrose in the temperate  
64 zones of Europe and North America, exhibits a biennial lifecycle and forms a large taproot during  
65 the first year of its development. This taproot represents a reversible sink, which contains up to  
66 20% of its fresh weight as sucrose. The vacuolar sucrose loader, named TONOPLAST SUGAR

67 TRANSPORTER2;1 (*BvTST2;1*), has been identified to be a key element for sugar accumulation  
68 in this storage organ (Jung et al., 2015). During the second year the taproot provides previously  
69 stored sucrose as precursor for the formation of a markedly large inflorescence.

70 The emergence of the sugar beet inflorescence strictly depends on a previous phase of cold  
71 temperatures, which induces molecular reprogramming known as vernalization. This vernalization-  
72 dependent bolting leads to significant loss of taproot sugar and biomass, and therefore yield. This  
73 loss of yield contributes to the fact that sugar beets are solely cultivated as an annual crop.  
74 Accordingly, sugar beet is sown in spring and harvested in the following late autumn. A prolonged  
75 cultivation period (particularly autumn to autumn) and thus, identification of bolting-resistant  
76 varieties have therefore become primary goals in sugar beet breeding over the last decades  
77 (Hoffmann and Kluge-Severin, 2011; Hoffmann and Kenter, 2018).

78 Two major early-bolting loci, *B* and *B2* have been identified in the sugar beet genome in recent  
79 years, encoding the pseudo response regulator gene *BOLTING TIME CONTROL 1*, *BTC1* (Pin et  
80 al., 2012) and the *DOUBLE B-BOX TYPE ZINC FINGER* protein *BvBBX19* (Dally et al., 2014),  
81 respectively. In annual beets, expression of both genes leads to repression of the floral repressor  
82 gene *FT1*, and subsequent induction of the floral inducer gene *FT2* and vernalization-independent  
83 flowering upon long-days (Pin et al., 2010; Dally et al., 2014). Biennial beets are homozygous for  
84 the recessive *btc1* and *bbx19* alleles, which encode non-functional proteins unable to repress the  
85 inhibitory function of *FT1* (Pfeiffer et al., 2014). Accordingly, biennial sugar beets require  
86 vernalization for *BTC1*- and *BBX19*-independent *FT1* repression and flowering (Pin et al., 2010).  
87 Obviously, floral induction and sink-source transition must be tightly interconnected in sugar beet.  
88 A coordinated network of floral inducers and repressors initiates the transition to bolting after  
89 vernalization, but adjustment of the metabolic set-up appears equally important for the  
90 morphological and physiological restructuring of taproots prior to formation of inflorescences.  
91 However, little information is available on the molecular physiological processes in sugar beet at  
92 the early time points of vernalization.

93 In this work, we therefore sought to understand how chilling temperatures, representing a *condition*  
94 *sine qua non* for vernalization, might influence sugar metabolism, photosynthesis, phloem  
95 translocation, and therefore source and sink identities of shoots and taproots. We combined  
96 comprehensive transcriptome and proteome analyses with recording of organ growth  
97 characteristics, photosynthetic parameters and metabolite quantification. In summary, our analyses

98 revealed an unexpected cold-dependent reversal of sink and source identities of taproots and shoots,  
99 respectively, prior to bolting at the very early stages of vernalization.

100 Despite inactivation of photosynthesis in the cold, shoot biomass increased at the expense of  
101 taproot sucrose. We recorded a substantial export of taproot sugar in the cold, which correlates  
102 with altered activities of sugar ex- and importers and with a markedly altered expression of genes  
103 involved in either sucrose synthesis or degradation. We speculate that this so far hidden metabolic  
104 reprogramming is a prerequisite for initiation of bolting as corresponding flux redirection transports  
105 sugars from the taproot to the shoot. However, this process might also contribute to the pronounced  
106 frost sensitivity of sugar beet. Thus, our findings provide a molecular-physiological explanation to  
107 the well-known problem of sugar beet cultivation (loss of yield due to the biennial lifecycle) and  
108 provide new targets to achieve bolting resistance and winter hardiness in this crop species.

## 109 **Results**

### 110 **Cold exposure causes rapid loss of shoot and root water, but not of shoot biomass production**

111 To resolve cold-dependent growth dynamics of sugar beet source and sink organs, we monitored  
112 shoot and taproot weights of plants from three different hybrid genotypes (GT1, GT2, and GT3),  
113 (initially grown under control conditions [20°C], then acclimated for one week at 12°C) for 19 days  
114 after transfer to cold (4°C) conditions (**Figure 1**). Shoot dry weight (DW), but not fresh weight  
115 (FW) continued to increase during the exposure of the plants to 4°C. Consequently, shoot water  
116 content gradually decreased by almost half at the end of the recorded time (**Figure 1A**).  
117 Simultaneously, FW but also DW of taproots decreased together with the taproot water content  
118 during the cold exposure period (**Figure 1A, B**). These results showed that growth of taproots was  
119 more affected than that of shoots in the cold and suggested differential physiological and metabolic  
120 responses of the shoot and root tissues to cold exposure.

### 121 **Sugar levels behave differently in shoots and taproots in the cold**

122 Accumulation of soluble sugars in shoots is a common response to low temperatures and part of  
123 cold acclimation process of many plant species (Steponkus, 1971; Wolfe and Bryant, 1999; Strand  
124 et al., 1997). Also, in our cold-dependent growth analysis, leaf material (obtained from the very  
125 same sugar beet plants as was used for biomass and water content calculation (**Figure 1A**))  
126 exhibited a clear increase in the levels of glucose and fructose (and to a lesser extent of the  
127 disaccharide sucrose) after transfer to 4°C (**Figure 1C**). In contrast to soluble sugars, leaf starch

128 contents in all three genotypes decreased rapidly after transfer to 4°C, reaching 20 to 33% of the  
129 value present prior to transfer (**Figure 1C, rightmost panel**).

130 In taproot tissue, sugar accumulation dynamics differed markedly from those in shoots. Glucose  
131 and fructose levels slightly increased in the cold, but reached only between 10 to 20 percent of the  
132 monosaccharide concentrations of leaves. Prior to transfer to 4°C, taproot sucrose levels exceeded  
133 those of monosaccharides 30- to 100-fold. Taproot starch levels of all genotypes were extremely  
134 low and did hardly change during cold treatment (**Figure 1D**). The three genotypes analyzed,  
135 however, exhibited different sugar and starch accumulation dynamics in the cold. While GT2 and  
136 GT3 taproot sucrose levels clearly decreased in the cold, GT1 sucrose levels fluctuated only  
137 marginally. Interestingly, the steep drop in sucrose concentration in taproots of GT3 (by about 400  
138  $\mu\text{mol/g DW}$ ) and to a lesser extend of GT2 (by about 200  $\mu\text{mol/g DW}$ ) was not accompanied by a  
139 proportionate increase of monosaccharides, as would be expected for an exclusive hydrolysis of  
140 sucrose. These massive losses of taproot sucrose rather suggested that this sugar was either (i)  
141 increasingly respired, (ii) converted into compounds other than the monosaccharides glucose and  
142 fructose, or (iii) exported from the taproot tissue into other organs. In the following, we aimed to  
143 elucidate the fate of sucrose with respect to these possibilities.

#### 144 **Cold exposure affects photosynthesis rate and carbon dioxide assimilation**

145 In cold tolerant plants like Arabidopsis, sugars accumulate in leaves in the cold when  
146 photosynthetic activity is maintained during reduced sucrose phloem loading and increased sugar  
147 import into vacuoles of leaf mesophyll cells (Strand et al., 1997; Wingenter et al., 2010;  
148 Pommerrenig et al., 2018). We analyzed the impact of cold on sugar beet photosynthesis with pulse  
149 amplitude modulated (PAM) fluorometry and CO<sub>2</sub> assimilation with gas exchange measurements  
150 (**Figure 2**). These measurements revealed that Photosystem II quantum yield (Y(II)), leaf CO<sub>2</sub>  
151 concentrations ( $C_i$ ), CO<sub>2</sub> assimilation rate ( $A$ ), and leaf transpiration rate ( $E$ ) were dependent on  
152 the ambient temperature and that plants exposed to cold responded with a decline in photosynthetic  
153 efficiency (**Figure 2**). All three genotypes showed a slight but significant reduction of Y(II) already  
154 after one week transfer to 12°C. Simultaneously, non-photochemical quenching Y(NPQ) increased,  
155 and non-regulated energy dissipation Y(NO) decreased at this temperature in the leaves of all three  
156 genotypes (**Figure 2A**). The higher Y(NPQ) quantum yield at 12 °C compared to 20°C indicated  
157 an increased flow of electrons towards the Mehler-Ascorbate peroxidase pathway (Asada et al.,  
158 1998) upon exposure to this temperature to undergo e.g. thermal energy dissipation at Photosystem

159 II reaction centers. After transfer to 4°C, Y(II) decreased further and did not recover over the time  
160 period tested. However, the decrease of Y(NPQ) quantum yield and the significant increase in  
161 Y(NO) quantum yield indicated that electrons underwent unregulated energy dissipation which  
162 might induce free radicals and membrane damage at this low temperature (**Figure 2A**).  
163 Measurements of CO<sub>2</sub> gas exchange showed that the reduced PSII activity, as determined by PAM  
164 fluorometry was accompanied by a drastic decline of the CO<sub>2</sub> assimilation rate (*A*) at 4°C but not  
165 at 12°C (**Figure 2B**). Transpiration rates (*E*) increased transiently in all three genotypes already at  
166 12°C but more severely at 4°C. The elevated transpiration coincided with a chilling-dependent  
167 increase in the leaf CO<sub>2</sub> concentration, indicating that despite increased stomata opening, activities  
168 of Calvin cycle enzymes were greatly reduced (**Figure 2B**).

169 To gain insight into global cold-dependent gene expression of sugar beet source and sink tissues,  
170 we performed RNA-seq analyses on leaf and taproot tissue of sugar beet plants from the above  
171 genotypes exposed to cold (4°C) or control (20°C) conditions. Samples were collected 14 days  
172 after transfer from 12°C to 4°C, i.e. when metabolic accumulation of sugars (**Figure 1**) and  
173 photosynthetic rate were maximally contrasting. The obtained RNA-seq reads were mapped to the  
174 sugar beet reference genome (Dohm et al., 2013). Transcriptome sequencing data has been  
175 deposited in the GenBank Sequence Read Archive (BioProject PRJNA602804).

176 Exposure to cold induced global rearrangement of gene expression in both shoot and taproot tissues  
177 (Supplemental Figure 1). We extracted transcript information on genes involved in photosynthesis.  
178 In a PC analysis based on expression values in leaf tissue of genes annotated as ‘photosynthesis’,  
179 ‘photosynthesis.lightreaction’, ‘photosynthesis.calvin cycle’, or ‘photosynthesis.photorespiration’  
180 by Mapman Ontology for sugar beet, the PC1 separated the temperature treatments in the three  
181 genotypes. PC1 explained 84.5%, PC2 7.1% of the variance in expression between 4°C and 20°C  
182 within the genotypes (**Figure 2C**). Independent genotypes were not clearly separated and  
183 accordingly, expression levels of photosynthesis-related genes behaved similarly in all three  
184 genotypes (**Figure 2C**, Supplemental Figure 1). At 20°C, about 9% of all transcript reads of each  
185 genotype could be assigned to ‘photosynthesis’ subgroups. After exposure to 4°C, this group was  
186 represented by only 3% of all reads, indicating a drastic downregulation of photosynthesis-related  
187 genes in the cold (**Figure 2D**). Downregulation of expression was for example observed for  
188 transcripts with homology to genes encoding RubisCO activase (BvRCA), RubisCo small subunit  
189 (BvRBCS), a Chlorophyll A/B binding protein (BvCABA), and Plastocyanin (BvPC) (**Figure 2E**,

190 **upper row**). Genes related to ROS processing on the other hand displayed differential regulation.  
191 Whereas genes encoding Glutathione reductases were upregulated in the cold, genes encoding  
192 Superoxide-dismutase or Ascorbate reductase were down- or not significantly regulated,  
193 respectively (**Figure 2E, bottom row**). In summary, the data demonstrated that sugar beet  
194 photosynthesis was extremely sensitive to chilling temperatures below 12°C and suggested that the  
195 (hardly occurring) assimilation of CO<sub>2</sub> does not completely account for the increase in biomass and  
196 sugar determined for leaves of cold-treated sugar beet (**Figure 1**).

### 197 **Cold temperatures alter major carbohydrate metabolism in shoots and taproots**

198 We investigated whether the reduction of taproot sucrose concentration in the cold could be  
199 explained with increased respiration and whether cold conditions would result in differential  
200 expression of genes involved in major carbohydrate metabolism (**Figure 3**). Respiration in taproot  
201 tissue was dependent on the examined part of the taproot, in that it decreased with increasing depths  
202 of the surrounding soil (**Figure 3A**). This position-dependent decrease in respiration (proportionate  
203 to the depth of soil surrounding the respective part of the taproot) was also observed at 4°C,  
204 however, in each part of the taproot, respiration was – in comparison to the corresponding control  
205 – generally lower when sugar beets had been exposed to 4°C (**Figure 3A**). This data suggested  
206 that, in the cold, carbohydrates in the taproot were used for glycolytic and oxidative catabolism to  
207 a lesser extent than under the 20°C control condition. In shoots, i.e. in source leaves of all  
208 genotypes, on the contrary, respiration increased in the cold (**Figure 3B**), indicating that the mature  
209 leaves, which hardly assimilate CO<sub>2</sub> at this temperature (**Figure 2**), had a high requirement for  
210 carbohydrate supply from other sources. One of these sources was probably starch, which  
211 decreased in leaves in the cold (**Figure 1**). PC and heat map analysis, loaded with expression values  
212 of genes assigned as “major CHO metabolism”, revealed organ- and temperature-dependent  
213 differences (**Figure 3C, Figure 3D**). The first principal component PC1 explained 66.9% of the  
214 expression differences between roots and shoots and the PC2 accounted for 17.9% of the  
215 differences in expression between 20°C and 4°C. Both organs showed clearer separation at 20°C  
216 in comparison to 4°C (**Figure 3C**). The heat map representation visualizes that expression levels  
217 of genes contributing to starch degradation and synthesis in leaves were up- (starch degradation)  
218 or downregulated (starch synthesis) by cold exposure, respectively. Despite extremely low starch  
219 levels in taproots (**Figure 1**), starch-related genes were also expressed and regulated in taproots  
220 (**Figure 3D**). This observation is in line with a report from Turesson et al (2014) who showed that

221 starch metabolic enzymes were active despite the lacking occurrence of starch in taproots  
222 (Turesson et al., 2014).

223 Expression levels of sucrose synthesis genes were upregulated in roots in the cold but unchanged  
224 in shoots. Sucrose degradation genes, however, were clearly downregulated in roots but slightly  
225 upregulated in shoots (**Figure 3D**). Sucrose Phosphate Synthase (SPS) and Sucrose Synthase  
226 (SUS) are key factors of sucrose biosynthesis and degradation and regulate carbohydrate  
227 partitioning between source and sink tissues (Voll et al., 2014; Sturm, 1996; Martin et al., 1993;  
228 Kovtun and Daie, 1995). A genome-wide search in the sugar beet genome (RefBeet 1.2, (Dohm et  
229 al., 2013)) identified two SPS and four SUS isoforms. Bayesian analysis identified both SPS  
230 isoforms as homologs of the Arabidopsis SPS 'A' subgroup (Voll et al., 2014) (Supplemental  
231 Figure 3). The two SPS isoforms showed differential organ-specific and cold-dependent  
232 expression. In shoots of all genotypes, expression of *SPSA1* was about 10-fold higher than in roots,  
233 when plants had been exposed to 20°C. Cold treatment upregulated its expression in roots up to  
234 sevenfold, but did not affect expression levels in the shoot. *SPSA2* expression at 20°C was low in  
235 shoots but high in roots of all three tested genotypes. The expression of this isoform was previously  
236 identified as taproot-specific, glucose-induced, and sucrose-repressed (Hesse et al., 1995). *SPSA2*  
237 expression was also unaltered or even downregulated (in case of GT2) in shoots upon cold  
238 treatment, but, as opposed to *SPSA1*, *SPSA2* expression was induced in taproots of all genotypes.  
239 On the protein level, revealed by MS-based analysis of the soluble proteome from the very same  
240 taproot tissues as was used for the transcriptome analysis, BvSPSA1 but not BvSPSA2 was slightly  
241 upregulated. SPS activity, however, was higher under 4°C in comparison to 20°C in both protein  
242 extracts from leaves and taproots (Supplemental Figure 3). Higher levels of UDP in taproots and  
243 Sucrose-6-Phosphate in shoots in the cold in comparison to control temperatures along with the  
244 elevated levels of the allosteric SPS activator G-6-P (Huber and Huber, 1992) supported a scenario  
245 in which SPS activity was elevated in both roots and shoots (Supplemental Figure 3).

246 The expression of the four sucrose synthase isoforms showed tissue and temperature-dependent  
247 differences. While *BvSUS1* and *BvSUS2* isoforms were strongly expressed in roots and their  
248 corresponding proteins highly abundant, *BvSUS3* and *BvSUS4* were hardly expressed and their  
249 corresponding proteins were not detected by MS in a soluble proteome fraction (**Figure 3E**,  
250 Supplemental Figure 4). Both *BvSUS1* and *BvSUS2* were ten (*BvSUS1*) to hundredfold (*BvSUS2*)  
251 higher expressed in roots in comparison to shoots. After the cold exposure period, mRNA levels



252 of both isoforms decreased about half in the roots. *BvSUS2* transcript levels in shoots increased ten  
253 to twentyfold, however, without reaching the high levels in taproots (**Figure 3E**). *BvSUS2*, but not  
254 *BvSUS1* was also reduced at the protein level indicating differential protein turnover dynamics of  
255 the two isoforms in the cold (Supplemental Figure 4).

256 To determine the cellular energy state of shoot and taproots, adenylate levels were measured  
257 (**Figure 3F**). ATP, ATP/ADP ratio, and energy charge ( $EC = [ATP] + 0.5 [ADP]/[ATP] + [ADP]$   
258  $+ [AMP]$ ) increased in shoots of all genotypes. This elevated energization of shoot tissue in the  
259 cold can be explained by the drastic decrease in ATP-consuming CO<sub>2</sub> assimilation (**Figure 2B**)  
260 and the increase of respiration in shoots (**Figure 3B**). On the other hand, energization of taproot  
261 tissue did not change in the cold. Although ATP levels also increased, ATP/ADP ratios of GT1 and  
262 GT2 taproots were unaltered or even decreased in GT3. Also EC of taproots did not increase in the  
263 cold but rather decreased in tendency in GT2 and GT3 taproots (**Figure 3F**). Taken together, these  
264 data indicate that developing taproots shifted in the cold from a sucrose consuming/storing towards  
265 a sucrose synthesizing tissue and that leaves adopted – at least in part – characteristics of sink  
266 tissues.

### 267 **Cold temperatures reverse phloem translocation of sucrose and esculin**

268 The above data indicated that cold-induced shoot sugar accumulation was not or only insufficiently  
269 fueled by carbon dioxide assimilation, or starch degradation, and suggested that carbon used as  
270 building block for shoot metabolites might be remobilized from taproot storage cells. To track the  
271 fate of taproot-based carbon after exposure to cold temperatures, we directly fed taproot tissue with  
272 radiolabeled <sup>14</sup>C-sucrose by injecting the substance from the exterior into the fleshy parenchymatic  
273 taproot tissue of plants grown under 20°C control conditions or cold-exposed plants (5 days at 12°C  
274 and then 7 days 4°C). The treated plants were then kept for one more week at control or cold  
275 temperatures and then dissected into individual leaves and taproots. The leaves or longitudinal thin  
276 sections of taproots were pressed and dried, and incorporated radioactivity was visualized using  
277 phosphor imaging plates and software (**Figure 4**, Supplemental Figure 6, Supplemental Figure 7).

278 This analysis surprisingly revealed that plants grown under the 4°C condition showed distribution  
279 of radioactivity in source leaves. Radioactivity in leaves of cold-treated plants was detected in leaf  
280 veins and intensity gradually decreased towards the leaf tip indicating transport via the phloem  
281 vessels (**Figure 4B**). In plants grown under control conditions, however, radioactivity could hardly

282 be detected in source leaves (**Figure 4C**). However, radioactivity was to some extent detectable in  
283 young sink leaves of control plants and extractable from combined shoot petioles (**Figure 4D**).  
284 This radioactivity may represent xylem transported sucrose or derivatives due to injury of  
285 punctuated vessels as a result of the invasive inoculation procedure. The drastic water loss in shoots  
286 upon cold (**Figure 1**) however indicated that at 4°C radiolabeled sucrose was not efficiently  
287 transported to prior source leaves via the xylem but rather via the phloem.

288 To test this hypothesis, we used a strategy less invasive to the organs/tissues examined later, and  
289 more realistically mirroring the actual transport of assimilates (including the prior “downward”  
290 transport. We loaded esculin, a phloem mobile coumarin glycoside (Knoblauch et al., 2015)  
291 recognized by several sucrose transporters, including the *Beta vulgaris* phloem loader BvSUT1  
292 (Nieberl et al., 2017) onto source leaves and assessed esculin transport routes directly via detection  
293 of esculin-derived fluorescence in thin sections of leaf petioles of source leaves from the very same  
294 plants, which had not been loaded with esculin, after transfer to cold or under control conditions.  
295 Here we observed that blue esculin fluorescence was solely detected in phloem of vascular bundles  
296 of source leaves from plants transferred to cold. However, the fluorescence was not only confined  
297 to the phloem region but also detected to some small extent in a bundle region interspersed with  
298 the yellow fluorescence of the lignified xylem vessels (**Figure 4**). At 20°C, esculin fluorescence  
299 was never detected in the phloem (**Figure 4**).

300 To follow sucrose flow directly from the site of inoculation in the taproots, we performed  
301 longitudinal thin sections of taproots inoculated with the radiolabeled sucrose and exposed the  
302 tissue to phosphor imaging plates (**Figure 4**, Supplemental Figure 6, Supplemental Figure 7).  
303 Radioactivity in taproots from plants exposed to 4°C was detectable and concentrated in veiny or  
304 spotty structures that resided between the site of inoculation and the taproot top (crown) tissue. At  
305 higher magnification, these structures could be identified as vascular bundles (Supplemental  
306 Figure 6). In taproots from plants grown under control conditions, no such distinct darkening of  
307 vascular structures could be observed, although some observed blackening of crown tissue  
308 indicated that radioactivity was also transported upwards into the direction of the  
309 shoot (Supplemental Figure 7). However, in most cases, radioactivity in 20°C taproots was either  
310 merely confined to parenchymatic regions near the site of inoculation or concentrated in thick  
311 strands that reached from the site of inoculation towards the emergence of lateral roots. These  
312 results indicated that radiolabeled sucrose and esculin – the latter first being translocated to the

313 base of the petiole of the loaded leaf and though (at least parts of) the taproot - were preferentially  
314 transported from taproots into shoots in the cold but not under control conditions and suggested  
315 that sucrose released from parenchymatic storage tissue was also transported in the same manner.

### 316 **Vacuolar sucrose importer and exporter genes and proteins show opposite cold-dependent** 317 **expression**

318 Next, we analyzed whether transport of sucrose from taproots to shoots in the cold could be  
319 mediated by differential activity of vacuolar sucrose importers and exporters. In Arabidopsis,  
320 vacuolar sucrose import and export are mediated by activity of TST1 and SUC4 transporters,  
321 respectively (Schulz et al., 2011; Schneider et al., 2012). In sugar beet, the TST1 homolog  
322 BvTST2;1 is responsible for vacuolar sucrose accumulation (Jung et al., 2015). TST2;1 expression  
323 in the taproots of all tested genotypes greatly exceeds that in leaf tissue substantiating its role as  
324 the sucrose loader of taproot parenchyma vacuoles (**Figure 5**). Interestingly, both mRNA and  
325 protein abundance decreased significantly in all genotypes in taproots after cold  
326 treatment (**Figure 5B**, Supplemental Figure 8).

327 Export of sucrose from the vacuole is presumably mediated by a SUC4/SUT4 family homolog. We  
328 identified Bv5\_124860\_zpft.t1 as the unambiguous homolog to the Arabidopsis SUC4 isoform and  
329 accordingly termed the corresponding transporter BvSUT4 (Supplemental Figure 10). N-terminal  
330 fusions of the BvSUT4 coding sequence with GFP transiently transformed into *Beta vulgaris* or  
331 Arabidopsis mesophyll protoplasts clearly indicated that BvSUT4 was a tonoplast located protein  
332 (**Figure 5D**). BvSUT4 mRNA showed lower abundance in older plants in comparison to younger  
333 ones (Supplemental Figure 9). In contrast, TST2;1 mRNA increased with progression of leaf  
334 development confirming the suggested oppositional activities of the TST2;1 and SUT4 transport  
335 proteins (Supplemental Figure 9). In the RNA-seq data from the cold-treated genotypes examined  
336 in this study, SUT4 mRNA levels increased significantly in taproots in the cold (**Figure 5C**). These  
337 data indicated that vacuolar taproot sucrose import was decreased and vacuolar taproot sucrose  
338 release increased under cold conditions and suggested that the opposite regulation of BvTST2;1  
339 and BvSUT4 in taproots was the underlying driving force for the accumulation and delivery of  
340 sugars in shoots.

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343 **Expression of floral regulator genes is adjusted in the cold**

344 The observed re-translocation of sucrose from taproots to shoots might represent a preparative  
345 metabolic and genetic rearrangement for initiation of flowering. We therefore extracted  
346 information on expression of flowering regulator genes and observed significant downregulation  
347 of the floral repressor *BvFT1* and upregulation of the floral activator *BvFT2* in the cold in leaves  
348 (**Figure 6**). These results agree with reports from Pin et al (2010), where cold treatment also  
349 induced *FT2* and repressed *FT1* expression (Pin et al., 2012). The genotypes analyzed here have  
350 biennial growth behavior thus *BTC1* and *BBX19* may not influence *FT1* expression. However,  
351 these two genes were reciprocally cold regulated. While *BTC1* was downregulated in the cold,  
352 *BBX19* was upregulated. In contrast to results from Pin et al. (2012), where vernalized biennials  
353 had increased *BTC1* mRNA levels in comparison to non-vernalized plants (Pin et al., 2012), *BTC1*  
354 was downregulated in the cold. However, in the mentioned study, expression was analyzed after  
355 and not during early stages of vernalization. We found that *BTC1* and *BBX19* were expressed in  
356 both, shoots and taproots, and expression of *BBX19* in taproots exceeded that in the shoot at 20°C  
357 almost threefold. However, potential targets of these encoded loss-of-function proteins, *FT1* and  
358 *FT2* were specifically and exclusively expressed in leaf tissue (**Figure 6**). In summary, these data  
359 showed that the vernalization process was already transmitted to the expression level of floral  
360 regulator genes and that transcriptional changes of related genes did occur in both, shoots and  
361 taproots.

## 362 **Discussion**

363 In this work we discovered a so far unknown switch of sink and source identities of taproots and  
364 shoots upon cold exposure of sugar beet plants. In contrast to sinks like seeds, culms or tubers,  
365 which adopt source identities after complete differentiation and subsequent separation from their  
366 nourishing source, the sink-source switch in sugar beet occurred in response to an environmental  
367 stimulus when both shoot and taproot tissues were still physiologically connected.

368 At 4°C, shoot CO<sub>2</sub> assimilation was drastically reduced but PSII activity stayed relatively high  
369 (**Figure 2**). This correlation indicates that enzymes of the Calvin-Benson cycle slowed down in the  
370 cold and could not utilize electrons liberated from the photosynthetic electron transport (PET)  
371 chain. During the decreased CO<sub>2</sub> fixation rates at cold conditions, high PET rates may have  
372 detrimental effects because they produce harmful reactive oxygen species (ROS) like super-oxide,  
373 hydrogen peroxide or hydroxyl anions (Suzuki and Mittler, 2006; Choudhury et al., 2017;  
374 Pommerrenig et al., 2018). During the cold exposure kinetic (**Figure 1 and Figure 2**), we recorded  
375 decreased CO<sub>2</sub> assimilation already at 12°C. As indicated by the increased Y(NPQ) percentage  
376 (**Figure 2A**), the Mehler-Ascorbate Pathway (Asada, 1999) might act as an additional quencher for  
377 PET-released electrons at this temperature. At 4°C, this scavenging pathway apparently also  
378 slowed down, as indicated by the further decrease in Y(II) but also Y(NPQ), and the concomitant  
379 increase in Y(NO). The significant increase in Y(NO) at 4°C is indicative for non-regulated energy  
380 dissipation, which can severely damage chloroplast membranes and plant cells in a cold- and high  
381 light dependent manner. Under those sustained challenging conditions, the cold response was  
382 apparently transduced to the level of gene expression where it led to an effective downregulation  
383 of transcripts of photosynthesis-related genes (**Figure 2D and 2E**). Induction of glutathione  
384 reductase genes supported a scenario in which leaves induced cellular counter measures against  
385 light-induced electron overflow at the photosystems and damages of chloroplasts (**Figure 2E**).

386 These data are in agreement with results from Arabidopsis, where photosynthesis as well as  
387 expression of *RBCS* and *CAB* genes were significantly reduced after shifting of 23°C-grown plants  
388 to 5°C, although photosynthesis recovered after prolonged exposure to cold (Strand et al., 1997).  
389 In summary, these metabolic and transcriptomic changes would eventually result in drastic  
390 decrease of CO<sub>2</sub> incorporation into sugars, which are required for growth and protection of cell  
391 vitality in the cold.

392 Despite inactivation of photosynthesis, however, sugars continued to accumulate in leaves and  
393 decreased in taproots in the cold (**Figure 1**). Decreasing sucrose levels in taproots and impaired  
394 respiration in root tissue indicated that sucrose was not used for energy metabolism during cold at  
395 the same rate as under control conditions in the taproot (**Figure 3**). Cold tolerant plants like  
396 *Arabidopsis* accumulate sugars in leaves in the cold by maintaining photosynthetic activity,  
397 reducing sucrose phloem loading, and increasing sugar import into leaf vacuoles (Wingenter et al.,  
398 2010; Nägele and Heyer, 2013). While *Arabidopsis* has the ability to overcome sugar repression of  
399 photosynthesis after prolonged exposure to cold (Huner et al., 1993; Strand et al., 1997), such  
400 mechanism apparently does not occur in sugar beet in the same manner. In contrast, the drastic  
401 decrease of photosynthetic activity in the shoot rather turned leaves into sink organs, which were  
402 supplied with sugar from taproots (**Figure 4**).

403 Under non-chilling temperatures, reversibility of taproot sink and remobilization of sugars from  
404 storage vacuoles might become essential when leaves have to re-grow after wounding of the shoot  
405 caused by e.g. feeding damage or when a new strong sink like the inflorescence is formed after  
406 winter. However, as indicated by the movement of radiolabeled sucrose and fluorescent esculin  
407 towards the shoot, and therefore to previous source leaves also early cold response triggered a  
408 remobilization of carbohydrates from taproot storage (**Figure 4**). While sucrose biosynthesis and  
409 hydrolysis were reciprocally regulated under warm and cold conditions (**Figure 3**), levels of taproot  
410 sucrose decreased upon cold treatment (**Figure 1**). In agreement with this process, we identified  
411 opposing regulation of the major vacuolar sucrose importer (BvTST2;1) and putative major  
412 exporter (BvSUT4) in the same tissue (**Figure 5**). BvTST2;1 expression and protein abundance  
413 was significantly downregulated, while, in contrast, BvSUT4 was upregulated in the cold. The role  
414 of BvSUT4 as an exporter of sucrose is supported by its general homology to sucrose transporters  
415 of the SUC/SUT family and by its homology to AtSUC4 (**Figure 5**), for which both sucrose export  
416 activity and vacuolar localization have been shown (Schulz et al., 2011; Schneider et al., 2012). It  
417 seems unlikely that sugars are released from vacuoles in the cold as monosaccharides via other  
418 transporters, e.g. by the already described BvIMP protein (Klemens et al., 2014). This is because  
419 vacuolar invertases - a prerequisite for vacuolar monosaccharide generation and thus export - are  
420 hardly active at the analyzed developmental stage (Giaquinta, 1979; Godt and Roitsch, 2006).

421 The previously explained findings are schematically explained in the following model (**Figure 7**).

422 It is surprising that flux transition occurred already pre-bolting i.e. before the formation of an  
423 inflorescence that would then act as new sink organ utilizing remobilized taproot sugars as building  
424 blocks. During the early phases of vernalization warmer temperatures or longer daylight, additional  
425 prerequisites for bolting (Mutasa-Göttgens et al., 2010; Ritz et al., 2010), do not yet signal onset  
426 of spring. However, simultaneously to the switching of identities, the cold exposure also led to  
427 adjustment of expression levels of floral regulator genes. *FT1* and *FT2*, the floral repressor and  
428 activator genes (Pin et al., 2010), respectively, showed reciprocal regulation in the cold in shoots  
429 (**Figure 6**). The expression of the flowering-related genes *BTC1* and *BBX19* in taproot tissue  
430 suggested that taproots might also be involved in the perception of vernalization. It is tempting to  
431 think into a direction where newly identified (Pfeiffer et al., 2014; Broccanello et al., 2015;  
432 Tränkner et al., 2017) or yet undiscovered bolting loci might harbor yet uncharacterized factors  
433 which might integrate both, bolting and required sink-source transition, similar to the recently  
434 described FT homolog *StSPS6A* (‘tuberigen’) in potato (Navarro et al., 2011; Abelenda et al.,  
435 2019).

436 Our study represents a comprehensive analysis of sugar beet taproot tissue during cold treatment  
437 and shows that cold temperatures induce a sink to source transition, which establishes accumulation  
438 of taproot-based carbohydrates in the shoot. For this, sugars have to be loaded into taproot phloem,  
439 transported from taproots to shoots, and unloaded in leaf tissue. Currently it is unknown whether  
440 taproot phloem loading in the cold involves an apoplastic step, whether the same phloem vessels  
441 are being used for root- and shoot-bound sugar trafficking, and how sugar unloading is established  
442 in former source leaves. Latter issue possibly involves a reprogramming of transporter activity that  
443 could mediate sugar efflux from the vasculature to the mesophyll involving both passive and active  
444 transport processes. Our transcriptomic and proteomic approach might reveal candidate factors and  
445 transporters involved in this unloading in the cold in the future.

446 The findings also have implications for agriculture and breeding, where attempts have been made  
447 to grow sugar beet over all seasons (Hoffmann and Kluge-Severin, 2011; Hoffmann and Kenter,  
448 2018), a scenario which will become more and more realistic by the generation and employment  
449 of bolting-resistant hybrid genotypes (Pin et al., 2010; Pfeiffer et al., 2014; Tränkner et al., 2016).  
450 In addition, biennial growth of sugar beet might become facilitated by the increasing occurrence of  
451 climate change-induced “warm” winters in e.g. middle and Northern Europe (Lavalle et al., 2009)  
452 that would allow cultivation of sugar beet under non-freezing, non-lethal low temperatures.

453 However, even under non-freezing, but prolonged above zero chilling conditions, the advantages  
454 of a longer vegetation period would be negated, at least to some extent, by the herein described  
455 trade-off of cold-induced taproot sugar loss. This phenomenon might also partially account for the  
456 observed reduced yield and higher marc to sugar ratio of autumn- or early spring-sown sugar beet  
457 plants (Hoffmann and Kluge-Severin, 2011).

458 In future, it will be highly valuable to analyze this observed sink-source transition of taproots in  
459 bolting resistant mutants without the activating function of FT2 (Pin et al., 2010) to reveal whether  
460 FT activity is required for triggering this transition. Equally relevant will be the generation of  
461 BvSUT4 mutant plants to study effects of lacking vacuolar sucrose efflux for floral induction and  
462 cold tolerance. Such modified plants would possibly exhibit a diminished taproot sucrose release  
463 and therefore a reduced building block supply for inflorescence formation. This potential impact  
464 on bolting makes BvSUT4 a highly relevant target for breeding approaches (Pfeiffer et al., 2014;  
465 Chiurugwi et al., 2013) aiming at bolting resistance and at withholding cold-induced sucrose loss  
466 from taproots.

## 467 **Materials and Methods**

### 468 **Plant Material and Growth conditions**

469 Three hybrid sugar beet genotypes (GT1, GT2, GT3; KWS SAAT SE, Germany) were used for  
470 this study. Plants were germinated and grown on standard soil substrate ED73 (Einheitserdwerke  
471 Patzer, Germany)/ 10% (v/v) sand mixture under a 10 h light/14 h dark regimen, 60% relative  
472 humidity, and  $110 \mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity. For growth- and sugar accumulation kinetics, plants  
473 were grown for 6 weeks at 20°C, transferred for 1 week at 12°C and then 3 weeks at 4°C. For  
474 RNA-seq and proteome analysis, plants were grown for 10 weeks at 20°C, transferred for 1 week  
475 at 12°C and then 2 weeks at 4°C. Control plants were kept at 20°C. For harvest, plants were  
476 dissected into shoot and taproot tissues. 4 pools out of three different plants were made for each  
477 tissue. Tissues were chopped with a kitchen knife, transferred to liquid nitrogen, and kept at -80°C  
478 until further processing.

### 479 **Chlorophyll Fluorescence Measurements**

480 Photosynthetic activity was measured using an Imaging-PAM *M-Series-System* (Heinz Walz,  
481 Effeltrich, Germany). Plants were placed in the dark for 12 min to deplete the energy of PSII.



482 Capacity of PSII was measured by saturation with 14 cycles of PAR 76 ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ )  
 483 light-pulses at 0s, 50s, and 70s. Recorded fluorescence was used for calculation of effective  
 484 quantum yield of PSII [ $Y(II) = (Fm'-F)/Fm'$ ], quantum yield of non-photochemical quenching  
 485 [ $Y(NPQ) = 1 - Y(II) - 1/(NPQ+1+qL(Fm/Fo-1))$ ] and of non-regulated energy dissipation [ $Y(NO)$   
 486  $= 1/(NPQ+1+qL(Fm/Fo-1))$ ]. Required factors were calculated by the formulas [ $NPQ = (Fm-$   
 487  $Fm')/Fm'$ ], [ $qN = (Fm-Fm')/(Fm-Fo')$ ], [ $Fo' = Fo / (Fv/Fm + Fo/Fm')$ ], [ $qP = (Fm'-F)/(Fm'-Fo')$ ]  
 488 and [ $qL = (Fm'-F)/(Fm'-Fo') \times Fo'/F = qP \times Fo'/F$ ].

### 489 Gas Exchange Measurements

490 A GFS-3000 system (Heinz Walz, Effeltrich, Germany) was employed to analyze gas exchange-  
 491 related parameters. A 2.5 cm<sup>2</sup> gas exchange cuvette was used to measure CO<sub>2</sub>-assimilation rate,  
 492 respiration, leaf CO<sub>2</sub> concentration, and transpiration of sugar beet source leaf. Leaf regions  
 493 including large central mid ribs were omitted. The conditions inside of the cuvette were set to the  
 494 same temperature, humidity and CO<sub>2</sub>-concentration the plants had been grown at. Measurement  
 495 sequence is listed in **Table 1**. The listed intervals were determined by a trial-experiment, in which  
 496 the time necessary for stabilization of the flow of CO<sub>2</sub> after transfer of the leaf section into the  
 497 cuvette and adoption to the changed light-intensities was measured. The measurement was started  
 498 after stabilization of the CO<sub>2</sub>-flow, which required about 5 minutes. Measurements were performed  
 499 with 4 plants in 3 technical (repeated measurements of the same plant) replicates over a time of  
 500 1 min for each condition to account for variation caused by observed natural leaf-fluctuation and  
 501 leaf area outside of the cuvette. The 30 second interval between the measurements was necessary  
 502 for the leaf to return to the stabilized value.

503 **Table 1:** Sequence for gas-exchange measurements

time [s]	Light-intensity	measurement
+0	PAR 0	
+220	PAR 0	photosynthetic activity
+30	PAR 0	photosynthetic activity
+30	PAR 0	photosynthetic activity
+460	PAR 125	respiration/transpiration (light)
+30	PAR 125	respiration/transpiration (light)

+30	PAR 125	respiration/transpiration (light)
+320	PAR 0	respiration/transpiration (dark)
+30	PAR 0	respiration/transpiration (dark)
+30	PAR 0	respiration/transpiration (dark)

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#### 504 **Respiration of sugar beet taproot tissue**

505 Respiration of taproots was measured by cutting out 0.5 cm<sup>2</sup> tissue cubes from central taproot  
506 regions and measuring CO<sub>2</sub> production in a whole-plant cuvette with a volume of 60 cm<sup>3</sup>. Values  
507 were normalized to tissue weight.

#### 508 **RNA extraction and sequencing**

509 RNA was isolated from three biological replicates per genotype, tissue (leaf and root, respectively)  
510 and treatment, respectively. About 100 mg frozen plant material were pulverized in a tissue lyser  
511 (Qiagen, Hilden, Germany) at 30 Hz for 90 sec. After grinding, samples were again transferred to  
512 liquid N<sub>2</sub>, supplemented with 1.5 ml QIAzol Lysis reagent (Qiagen, Hilden, Germany), vortexed  
513 three times for 30 sec, and centrifuged at 4 °C for 10 min at 12,000 g. Supernatants were transferred  
514 to fresh tubes, incubated at room temperature (RT) for 5 min, extracted with 300 µl chloroform,  
515 vortexed for 15 sec, and centrifuged at 4 °C for 15 min at 12,000 g. Aqueous supernatants were  
516 transferred to fresh tubes and RNA precipitated with 750 µl isopropanol for 10 min at RT and spun  
517 down at 4 °C for 10 min at 12,000 g. Precipitates were washed with 75% EtOH and the RNA pellets  
518 dried at 37 °C for 5-10 min prior to resuspension in 100 µl DEPC-H<sub>2</sub>O by gentle shaking at 37 °C  
519 for 5-10 min. To remove residual contaminants, RNA was further purified using the RNeasy KIT  
520 (Qiagen, Hilden, Germany). Per 100 µL RNA suspension, 350 µl RLT buffer (provided with the  
521 kit) were added and vortexed briefly. Then, 250 µl ethanol were added and the mixture was  
522 vortexed again. The RNA was spin-column purified and finally eluted from the column for a final  
523 volume of 50 µl (in DEPC-H<sub>2</sub>O) per sample. The RNA was quantified (NanoDrop 2000/2000c,  
524 Thermo Fisher) for each sample prior to further processing or storage at -80 °C. RNA quality was  
525 confirmed using an Agilent Technologies 2100 Bioanalyzer (Palo Alto, CA, USA). RNAs (2 µg  
526 per sample) were transcribed to cDNAs and sequenced using an Illumina, Inc. HiSeq 2000 system.  
527 Sequencing and assembly were provided as a custom service (GATC GmbH, Konstanz, Germany).  
528 The statistical analysis process included data normalization, graphical exploration of raw and  
529 normalized data, test for differential expression for each feature between the conditions and raw *p*-

530 value adjustment. The analysis was performed using the R software (Team, 2017), Bioconductor  
531 (Gentleman et al., 2004) packages including DESeq2 (Anders and Huber, 2010; Love et al., 2014)  
532 and the SARTools package developed at PF2 – Institute Pasteur.

### 533 **Phylogenetic analysis**

534 Multiple sequence alignments of amino acid sequences were performed using Clustal Omega  
535 (Sievers et al., 2011). Bayesian phylogenetic analysis was performed with MrBayes version 3.2  
536 (Ronquist et al., 2012). MrBayes always selected the best-fit models ‘Jones’ (Jones et al., 1992)  
537 and ‘WAG’ (Whelan and Goldman, 2001) for amino acid substitution analysis of SPS proteins and  
538 SUS proteins, respectively. MrBayes conducted two parallel Metropolis coupled Monte Carlo  
539 Markov chain analysis with four chains for 300,000 generations. Trees were sampled every 1,000  
540 generations. The analyses were run until the standard deviation of split frequencies were below  
541 0.005. Consensus trees were computed after burn-in of the first 25% of trees and visualized using  
542 FigTree version 1.4.3.

### 543 **PCA and heatmap analysis**

544 For RNAseq data the mean cpm values were used for the analysis. Data were visualized using  
545 ClustVis (Metsalu and Vilo, 2015).

### 546 **Analysis of soluble sugars and starch**

547 Leaves and taproots were harvested separately, frozen in liquid nitrogen, freeze-dried and stored at  
548 -80°C until use. Pulverized material was extracted twice with 1 ml 80% EtOH at 80°C for 1 h.  
549 Combined extracts were evaporated in a vacufuge concentrator (Eppendorf, Hamburg, Germany)  
550 and pellets were resolved in ddH<sub>2</sub>O. For starch isolation pellets were washed with 80% EtOH and  
551 1 ml ddH<sub>2</sub>O. 200 µl water were added to the pellet and the sample was autoclaved for 40 min at  
552 121°C. 200 µl enzyme-mix (5 U  $\alpha$ -Amylase; 5 U Amyloglucosidase in 200 mM Sodium-Acetate  
553 pH 4.8) were added to the pellet and starch was hydrolytically cleaved into glucose-units at 37°C  
554 for 4 h. The enzymatic digestion was stopped by heating the samples to 95°C for 10 min. After  
555 centrifugation (20,000 g; 10 min; 21°C) the supernatant could be used for starch quantification.  
556 Extracted sugars and hydrolytically cleaved starch were quantified using a NAD<sup>+</sup>-coupled  
557 enzymatic assay (Stitt et al., 1989).

### 558 **Analysis of phosphorylated metabolites**

559 The contents of phosphorylated intermediates (Glucose-6-Phosphate, Fructose-6-Phosphate,  
560 Sucrose-6-Phosphate, UDP-Glucose, UDP) were determined according to (Horst et al., 2010).

### 561 **Radiolabeled sucrose translocation assay**

562 Ten- to 12-week old sugar beet plants grown at 20°C under short day conditions (10 h light, 14 h  
563 darkness) were used for the analysis. Plants for cold-treatment were grown for 1 more week at  
564 12°C and then kept for 6 to 7 days at 4°C. Taproots from 4°C and 20°C plants were partially  
565 uncovered from surrounding soil substrate and a 1 mm hole punched with a biopsy stance into the  
566 upper half of the taproot (approximately 1 cm below the soil surface). The created pit was filled  
567 with 10 µl of 1 to 2 diluted radiolabeled sucrose (536 mCi/mmol) (Hartmann Analytic,  
568 Braunschweig, Germany) and coated with a drop of Vaseline. Plants were then kept for another 10  
569 days at 4°C or 20°C (control). At the end of the treatment, all source leaves of injected plants were  
570 detached and individually pressed between blotting paper. For detection of radioactivity in taproots,  
571 taproots were dug out, washed and cut in thin slices (approximately 0.5 mm thick) with a truffle  
572 slicer and pressed between blotting paper. Radioactivity was recorded with Phosphor-Image plates  
573 (exposed for 4 to 5 h to adaxial surface of pressed and dried leaves or to dried taproot slices) and  
574 plates were analyzed with a Cyclone Storage Phosphor Screen (Packard Bioscience, Meriden, CT,  
575 USA). For quantification of radioactivity in petioles, source leaf petioles from the same leaves used  
576 for phosphoimaging were cut off, ground, and pulverized. 5 to 10 mg powder were mixed with 2  
577 ml scintillation cocktail and counts per minute (cpm) recorded with a TRI-Carb 2810TR liquid  
578 scintillation analyzer (Perkin Elmer, Waltham, MA, USA).

### 579 ***In planta* esculin transport**

580 Ten-week old sugar beet plants grown at 20°C under short day conditions (10 h light, 14 h darkness)  
581 were used for the analysis. One source leaf per plant (usually from leaf stage 10 to 12) was abraded  
582 at the adaxial side with fine sandpaper (grade 800). About 500 µl of a 100 mM esculin  
583 sesquihydrate (Carl Roth, Karlsruhe, Germany) solution was distributed over the injured leaf  
584 surface with a plastic pipette. Treated leaves were coated with plastic foil, kept for 2 more days at  
585 20°C and then transferred to 4°C or kept at 20°C (control). After 5 to 7 days in the cold, not esculin-  
586 loaded source leaves were detached and sections of petioles were analyzed for esculin fluorescence  
587 with a Leica TCS SP5II confocal microscope (Leica, Mannheim, Germany) using a HCX PL APO  
588 lamda blue 20.0x0.70 IMM UV objective. Slices of taproots from the very same plants were

589 analyzed for esculin fluorescence to ensure that esculin was successfully translocated into taproots  
590 in both cold-treated and control plants. The emission bandwidths were 440 – 465 nm for detection  
591 of esculin fluorescence and 594 – 631 nm for lignin fluorescence.

### 592 **Soluble protein extraction**

593 Plants were harvested, washed, and separated in the cold into taproots and source leaves. Frozen  
594 leaf-tissue was pulverized with N<sub>2</sub>(l) using a Retsch mill (Retsch GmbH, Germany). 800 µl buffer  
595 E1 (50 mM HEPES-KOH pH 7.5, 10 mM MgCl<sub>2</sub>, 1 mM EDTA pH 7.5, 2 mM DTT, 1 mM PMSF,  
596 1 mM Pefabloc, 5 mM aminohexanoic acid, 0,1% (v/v) Triton X-100, 10% (v/v) glycerol) were  
597 transferred to 100 mg of pulverized tissue into 1.5 ml Eppendorf cups. Samples were vortexed and  
598 centrifuged for 3 min at 12.000g at 4°C. 500 µL of the supernatant were loaded onto a Sephadex  
599 NAP5 (G25) column (GE Health Care, United Kingdom), pre-equilibrated with buffer E1 w/o  
600 Triton X-100. Eluents were collected in precooled Eppendorf cups and stored at -20°C. Taproot  
601 tissues were treated as above with the following alterations: Taproots were blended with buffer E1  
602 at 4°C until a homogenous pulp was obtained. The pulp was roughly filtered through a kitchen  
603 sieve and centrifuged. 5 ml of the supernatant were dialyzed through a membrane with 12 kDa pore  
604 size for 48 h against 2 L ddH<sub>2</sub>O. Water was exchanged seven to eight times. Samples were collected  
605 in precooled Eppendorf cups and used for enzymatic tests or stored at -20°C. Liquid  
606 chromatography and tandem mass spectrometry was performed as described in (Jung et al., 2015).

### 607 **Isolation of taproot vacuoles and vacuolar proteins**

608 Vacuoles were isolated as described by (Jung et al., 2015).

### 609 **Sucrose Phosphate Synthase assay**

610 80 µg of soluble protein were added to 200 µl freshly prepared E<sub>max</sub> (50 mM HEPES-KOH pH 7.5,  
611 20 mM KCl, 4 mM MgCl<sub>2</sub>, 12 mM UDP-Glc, 10 mM Frc-6-P : Glc-6-P (1:4)) , E<sub>lim</sub> (50 mM  
612 HEPES-KOH pH 7.5, 20 mM KCl, 4 mM MgCl<sub>2</sub>, 4mM UDP-Glc, 2mM Frc-6-P : Glc-6-P (1:4), 5  
613 mM KH<sub>2</sub>PO<sub>4</sub>) and E<sub>blank</sub> (= E<sub>max</sub> w/o UDP-glucose and sugar-phosphates), respectively. Samples  
614 were incubated for 20 min at 25°C, followed by 5 min at 95°C to stop the reaction and centrifuged  
615 at 12.000 g at 4°C for 5min. 100 µL of the supernatant were pipetted to 100 µL 5 M KOH and  
616 incubated 10 min at 95°C. The solution was mixed with 800 µL anthrone (14.6 M H<sub>2</sub>SO<sub>4</sub>, 0,14%  
617 (w/v) anthrone) and absorbance immediately measured at 620 nm. A calibration-standard was  
618 made with 0-5 mmol sucrose.

## 619 **Subcellular localization of BvSUT4 in Arabidopsis and sugar beet mesophyll protoplasts**

620 The BvSUT4 CDS (Bv5\_124860\_zpft.t1= BVRB\_5g124860) was amplified from *B. vulgaris* leaf  
621 RNA with the gene specific primers BvSUT4-CACC-f (5'-CAC CAT GAC AGG CCA GGA CCA  
622 AAA TA-3') and BvSUT4-rev (5'-TAC ATG CAT CAC ATG AAC TCT GG-3'). The resulting  
623 open reading frame was cloned into pENTR/D-TOPO (Life Technologies, Darmstadt, Germany),  
624 sequenced and recombined into the Gateway-compatible destination vector pK7FWG,0 (Karimi et  
625 al., 2002) to obtain a p35S::BvSUT4-GFP fusion. Transient transformation of *A. thaliana*  
626 mesophyll protoplasts was performed as described (Abel and Theologis, 1994). Isolation and  
627 transient transformation of *B. vulgaris* mesophyll protoplasts were performed as described (Nieberl  
628 et al., 2017).

## 629 **Data availability**

630 Transcriptome sequencing data has been deposited in the GenBank Sequence Read Archive under  
631 BioProject PRJNA602804.

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636 of *Beta vulgaris* seed material and management of sugar beet growth.

## 637 **Author contributions**

638 H.E.N., F.L., W.K., K.H., U.S., B.P., designed the research;  
639 C.M.R., C.M., I.K., W.Z., F.R., P.N., B.P performed research;  
640 O.C., J.M.C.G, T.M. contributed new analytic/computational/etc. tools;  
641 C.M.R., C.M., B.P., analyzed data;  
642 B.P., C.M., and H.E.N. wrote the paper.

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820

## 821 **Figure legends**

822 **Figure 1.** Biomass and sugar accumulation response to cold temperatures in shoots and taproots of 6-week old sugar  
823 beet plants from three different genotypes (GT1 = grey square; GT2 = blue circle; GT3 = brown triangle). Plants were  
824 grown for six weeks at 20°C, then transferred to 12°C for one week and then to 4°C (start of recording of biomass and  
825 sugar accumulation) for 19 days. For each data point, whole organs (shoots or taproots) were harvested at midday.  
826 Data points show means from n=6 to 10 plants ± SD. **(A, B)** Fresh weight (FW), dry weight (DW) and water content  
827 of shoots and roots. **(C, D)** Sugar and starch accumulation during the course of the chilling (4°C) period in shoots and  
828 taproots, respectively. Significant changes to the control condition (first data point) were calculated using double sided  
829 Student's *t*-test (\* =  $p < 0.05$ ).

830  
831 **Figure 2.** Photosynthetic parameters, CO<sub>2</sub> assimilation, and expression data of sugar beet leaves after cold exposure.  
832 Sugar beet plants of three genotypes (GT1 = grey square; GT2 = blue circle; GT3 = brown triangle) were grown for  
833 six weeks at 20°C and then transferred to 12°C for one week and then to 4°C for three weeks. **(A)** PAM measurements  
834 of leaves of the three different genotypes. Quantum yield of photosynthesis [Y(II)], of non-photochemical quenching  
835 [Y(NPQ)], and of non-regulated quenching [Y(NO)]. At each time point four plants per genotype were analyzed. **(B)**  
836 Gas exchange measured for the same plants as used in **(A)**. Intercellular leaf CO<sub>2</sub> concentration (*C<sub>i</sub>*), CO<sub>2</sub> assimilation  
837 rate (*A*), and transpiration rate (*E*) are depicted. For each measurement, four independent plants were used. The very  
838 same plants were used for the measurements at the different time points after transfer to cold conditions. Significant  
839 changes to the control condition (first data point) were calculated using Student's *t*-test (\* =  $p < 0.05$ ). **(C)** Principal  
840 component analysis (PC1 versus PC2) for three genotypes based on expression values of 162 photosynthesis-related  
841 genes extracted from RNA-seq data of source leaves from plants grown at 20°C after exposure to 4°C or to control  
842 conditions (20°C) for 14 days, respectively. **(D)** Percentage of RNA-Seq reads annotated as genes coding for  
843 photosynthesis (PS) related proteins. Pie charts represent the averaged means from three different genotypes at 20°C  
844 (control) and after 14 days at 4°C. **(D)** Expression of *RubisCO Activase* (*Bv2\_025300\_tzou.t1*), *RubisCO small subunit*  
845 (*Bv2026840\_jyca.t1*), *Chlorophyll A/B binding protein A* (*Bv\_002570\_dmif.t1*), *Plastocyanin* (*Bv\_004160\_hgjn.t1*),  
846 *Glutathione reductase1* (*Bv3\_069540\_erom.t1*), *Glutathione reductase2* (*Bv5\_120360\_jpwm.t1*), *Superoxide*  
847 *dismutase1* (*Bv5\_102420\_sxsu.t1*), *Ascorbate peroxidase1* (*Bv1\_007470\_ymzt.t1*). Data represent the mean  
848 normalized cpm values of three independent RNA-seq analyses per genotype and temperature condition ± SD.  
849 Asterisks represent *p*-values < 0.05 according to double sided *t*-test in comparison to the values at control condition  
850 (20°C).

851  
852 **Figure 3.** Changes in major carbohydrate metabolism and energy state in response to cold. **(A)** Respiration (CO<sub>2</sub>  
853 production) of different taproot regions from GT1 under control conditions (20°C, yellow bars) or after one week  
854 transfer to 4°C (blue bars). **(B)** Respiration (CO<sub>2</sub> production) from leaf tissue of three genotypes (GT1, GT2, GT3)  
855 under control conditions (20°C, yellow bars) or after 1-week transfer to 4°C (blue bars). **(C)** Principal component (PC)  
856 analysis (PC1 versus PC2) for three genotypes based on expression values of 112 genes with GO annotation “major  
857 CHO metabolism” (loadings) extracted from RNA-seq data of source leaves from plants grown at 20°C and transferred

858 for 1 week at 12°C followed by 14 days at 4°C or control conditions (20°C). **(D)** Heatmap analysis of grouped  
859 expression values extracted from RNA-seq data. Unit variance scaling was applied to rows. Rows are clustered using  
860 Manhattan distance and average linkage. **(E)** Expression values for two Sucrose Phosphate Synthase genes (*BvSPSA1*  
861 and *BvSPSA2*) extracted from RNA-seq data of shoots and roots and expression values for two Sucrose Synthase genes  
862 (*BvSUS1* and *BvSUS2*) extracted from RNA-seq data of shoots and roots from GT1, GT2, GT3. Data represent the  
863 mean normalized cpm values of three independent RNA-seq analyses per genotype and temperature condition  $\pm$  SD.  
864 **(F)** ATP, ATP/ADP ratio, energy charge,  $EC = [ATP] + 0.5 [ADP]/[ATP] + [ADP] + [AMP]$ . **(E/F)** Data are means  
865  $\pm$  SD. Asterisks represent *p*-values < 0.05 according to double sided *t*-test in comparison to the values at control  
866 condition (20°C).

867  
868 **Figure 4.** Distribution of <sup>14</sup>C-sucrose and esculin in leaves. **(A-D)** Autoradiography of <sup>14</sup>C-sucrose in leaves. **(A)**  
869 Schematic depiction of experiment. Taproots were inoculated with <sup>14</sup>C-sucrose solution and harvested and dried leaves  
870 were autoradiographed one week later. **(B)** Source leaf from a representative plant grown for one week under at 4°C.  
871 Blackening of veins indicates radioactivity incorporated and distributed into leaf tissue after injection of radiolabeled  
872 sucrose into taproots. Abbreviations: p = petiole; mv = middle vein; 1° = first order lateral vein; 2° = second order  
873 lateral vein. **(C)** Source leaf from representative control plant grown at 20°C. **(D)** radioactivity in cpm (counts per  
874 minute) measured in isolated petioles from plants grown under 4 or 20°C. Center lines show the medians; box limits  
875 indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th  
876 percentiles, outliers are represented by dots; crosses represent sample means; n = 16 sample points. **(E-K)** Esculin  
877 loadings. Yellow fluorescence indicates lignified xylem vessels, blue fluorescence indicates esculin trafficking. **(E)**  
878 Schematic depiction of experiment. Esculin was loaded onto the scratched surface of a source leaf of plants grown at  
879 20°C. Loaded plants were transferred to 4°C or kept at 20°C. Petioles of neighbored, not loaded source leaves were  
880 analyzed for esculin fluorescence in plants from 4°C or 20°C. **(F-I)** Cross sections through petiole of a source leaf not  
881 loaded with esculin from plants loaded at 20°C. **(F, G)** Petioles from 20°C **(F)** Bright field image. **(G)** UV fluorescence  
882 image. **(H, I):** Petioles from 4°C. **(H)** Bright field image. **(I)** UV fluorescence image. **(J, K)** Longitudinal sections of  
883 a petiole from 4°C. **(J)** Bright field image. **(K)** UV fluorescence image. Abbreviations: xy: xylem, ph: phloem. Bars are  
884 50µm in G and H and 100µm in E, F, I, and J.

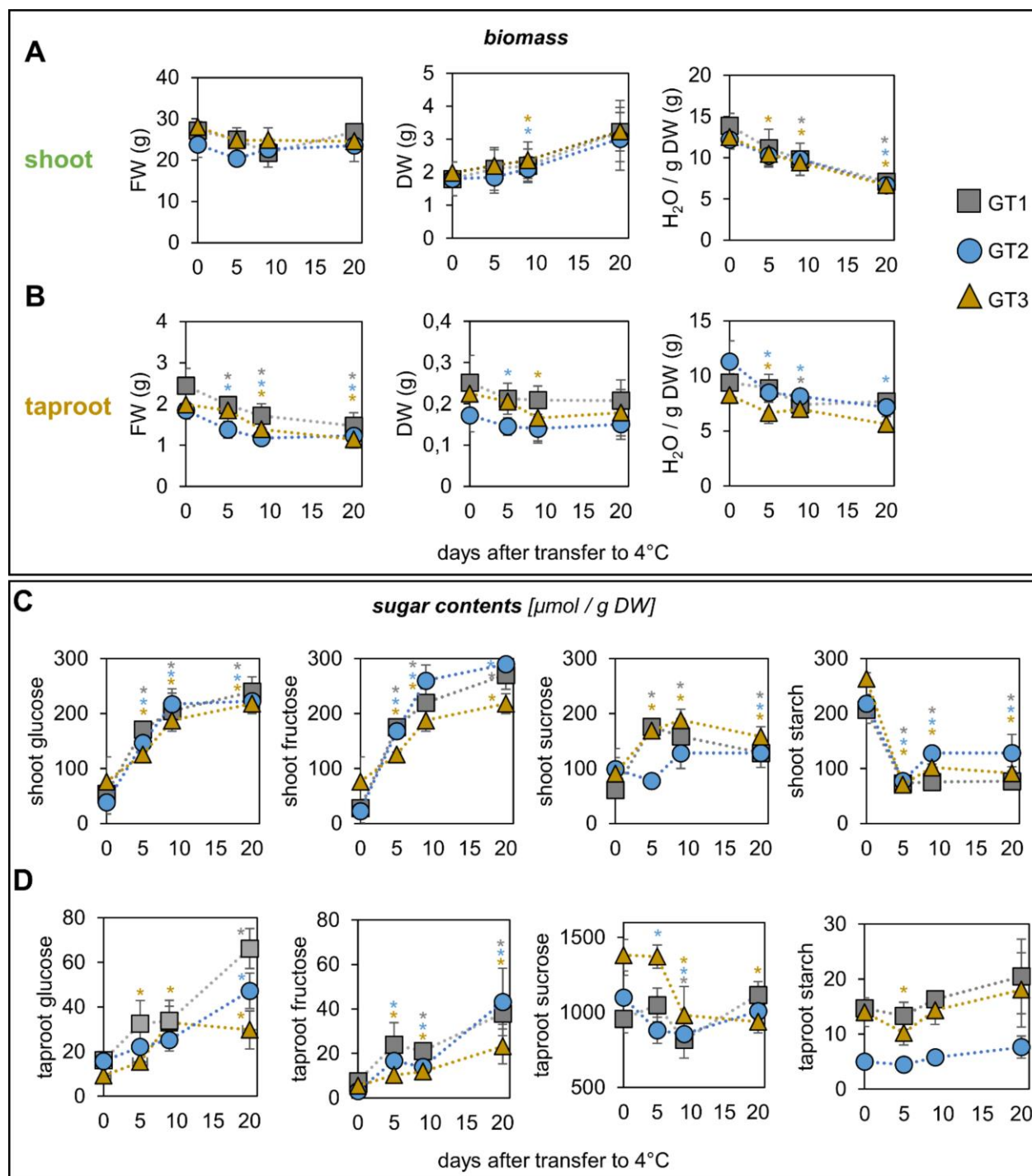
885  
886 **Figure 5.** Cold-dependent accumulation of *BvTST2;1* and *BvSUT4* in three different sugar beet genotypes. **(A)**  
887 Illustration on cold-induced processes. Upper image: Cold-dependent sugar relocations from taproots to shoots. Middle  
888 image: schematic of taproot vacuolar transport processes and factors. Vacuolar ATPase (V-H<sup>+</sup>-ATPase) establishes a  
889 proton motif force (pmf) across the vacuolar membrane; TST2;1 acts as proton/sucrose antiporter using pmf for sucrose  
890 import into vacuoles. SUT4 acts as proton/sucrose symporter using pmf for vacuolar sucrose export. Bottom image:  
891 reciprocal cold-induced regulation of *BvTST2;1* and *BvSUT4* mRNA levels in taproots **(B)** Transcript abundance of  
892 *BvTST2;1* (*Bv5\_115690\_zuju*) mRNA based on RNA-seq reads. Values represent means from n=3 biological  
893 replicates per genotype  $\pm$  SE. **(C)** Transcript abundance of *BvSUT4* (*Bv5\_124860\_zpft.t1*) mRNA based on RNA-seq  
894 reads. Values represent means from n=3 (mRNA) biological replicates  $\pm$  SE. Asterisks indicate significant differences  
895 between the 20°C and 4°C treatments according to t-test (\* = *p* < 0.05). **(D)** Subcellular localisation of *BvSUT4*-GFP

896 in *Arabidopsis* or *Beta vulgaris* leaf mesophyll protoplasts. Single optical sections in all pictures. The green colour  
897 shows the GFP-signal; the chlorophyll auto fluorescence is shown in red. Bars = 5  $\mu$ m. Arrowheads point towards the  
898 vacuolar membrane (tonoplast).

899  
900 **Figure 6.** Expression of floral regulator genes. Transcript abundances of *BvBBX19* (Bv9\_216430\_rmw.t1), *BvBTC1*  
901 (Bv2\_045920\_gycn.t1), *BvFT1* (Bv9\_214250\_miuf.t1), and *BvFT2* (Bv4\_074700\_eewx.t1) based on RNA-seq reads  
902 in shoots and taproots of three different genotypes. Values represent means from n=3 biological replicates  $\pm$  SE.  
903 Asterisks indicate *p*-values < 0.05 according to double sided *t*-test.

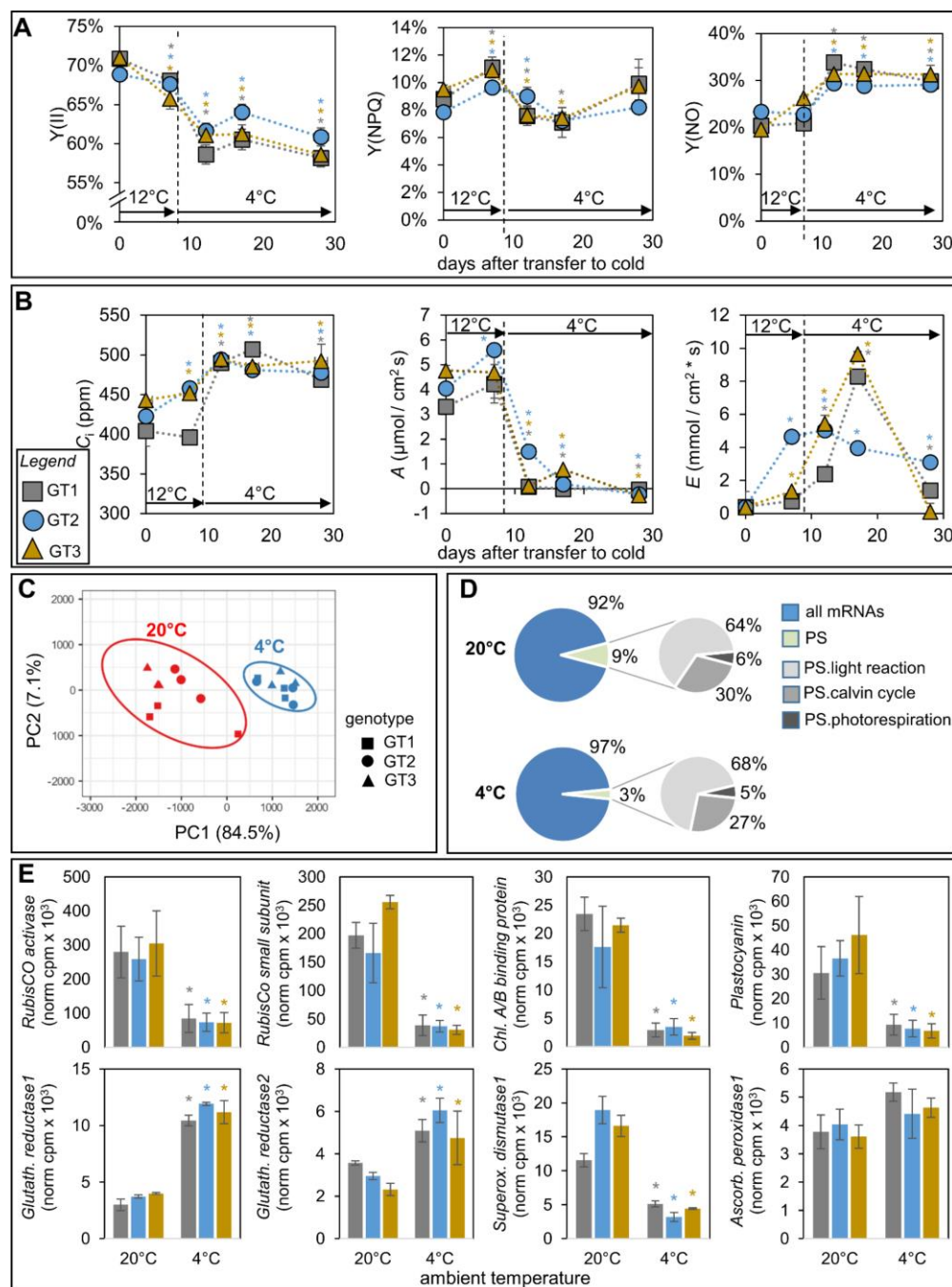
904 **Figure 7.** Schematic illustration of cold-induced sink to source transition. Leaf- and taproot-tissue of sugar beet are  
905 reprogrammed and source and sink identities shifted upon cold. Shoots adopt sink identity during cold. Biomass and  
906 sugar concentration in the shoot increase (**A**) despite reduced photosynthetic activity and inactivation of carbon  
907 assimilation (**B**). Concomitantly, shoot respiration increases (**C**) and cellular starch pools decrease (**D**). Contrastingly,  
908 taproots show a decrease of sucrose levels (**E**) but lower respiration rate (**F**) as well as increased sucrose biosynthesis  
909 (**G**). Taproot sugar is remobilized in the cold due to opposite regulation of taproot-specific vacuolar sucrose importer  
910 (*BvTST2;1*) and exporter (*BvSUT4*) activity (**H**). Taken together, this results in a reversal of the phloem translocation  
911 stream (**I**) triggered by a reprogramming of source and sink identities, which might correlate with inflorescence  
912 initiation.

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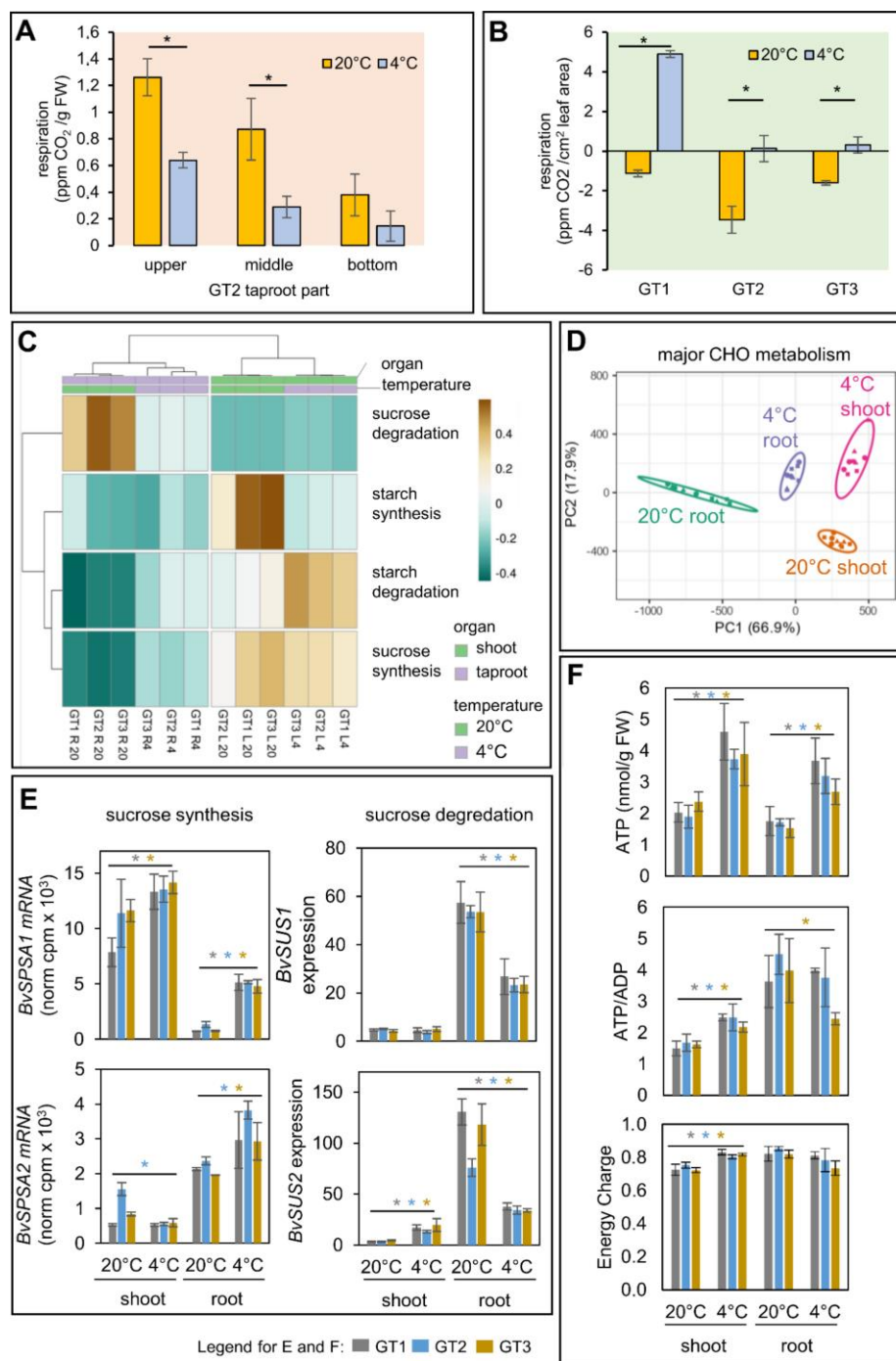
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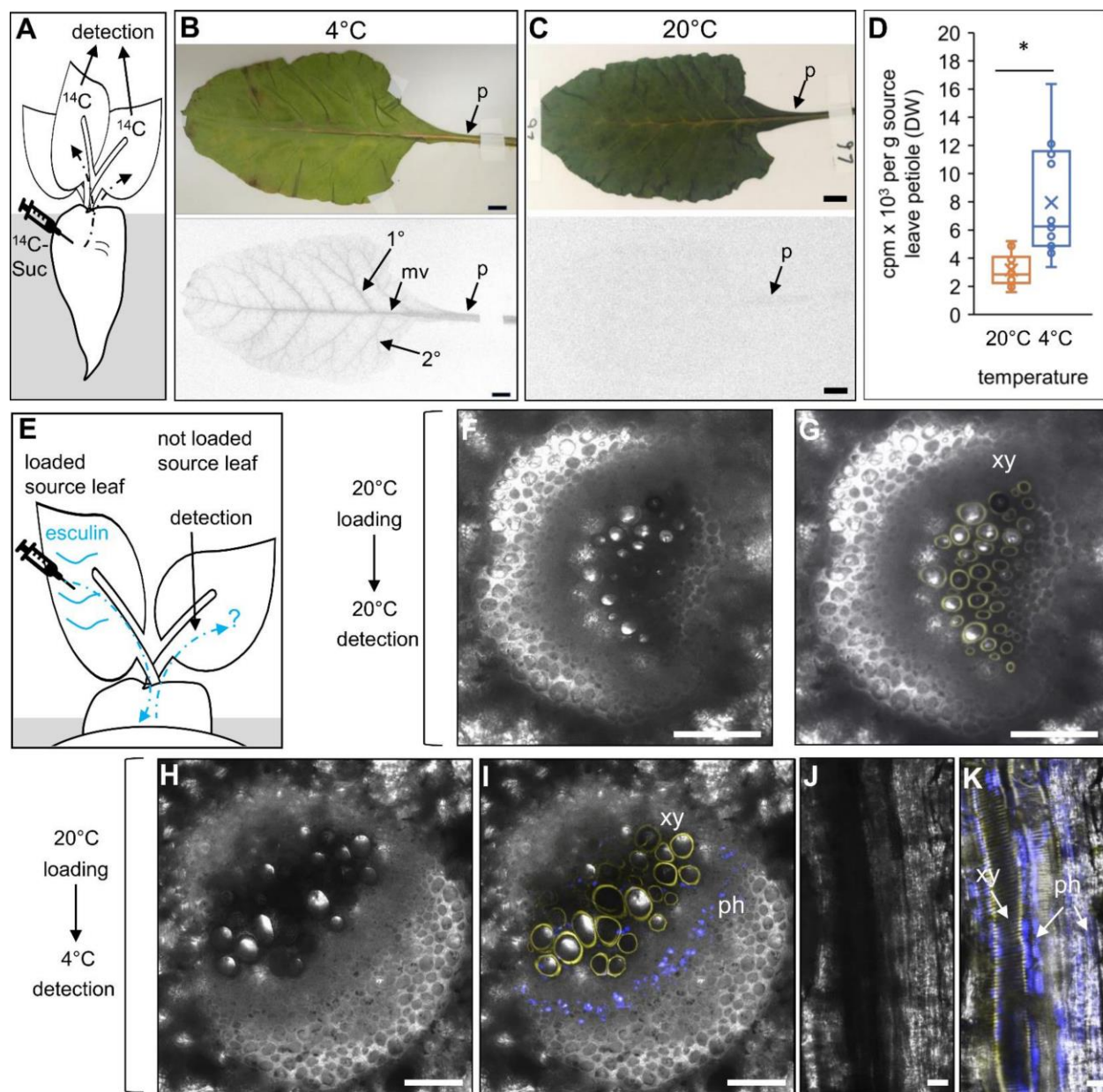
**Figure 2.** Photosynthetic parameters, CO<sub>2</sub> assimilation and expression data of sugar beet leaves after cold exposure.

Sugar beet plants of three genotypes (GT1 = grey square; GT2 = blue circle; GT3 = brown triangle) were grown for six weeks at 20°C and then transferred to 12°C for one week and then to 4°C for three weeks. **(A)** PAM measurements of leaves of the three different genotypes. Quantum yield of photosynthesis [Y(II)], of non-photochemical quenching [Y(NPQ)], and of non-regulated quenching [Y(NO)]. At each time point four plants per genotype were analyzed. **(B)** Gas exchange measured for the same plants as used in **(A)**. Intercellular leaf CO<sub>2</sub> concentration (C<sub>i</sub>), CO<sub>2</sub> assimilation rate (A), and transpiration rate (E) are depicted. For each measurement, four independent plants were used. The very same plants were used for the measurements at the different time points after transfer to cold conditions. Significant changes to the control condition (first data point) were calculated using Student's *t*-test (\* = *p* < 0.05). **(C)** Principal component analysis (PC1 versus PC2) for three genotypes based on expression values of 162 photosynthesis-related genes extracted from RNA-seq data of source leaves from plants grown at 20°C after exposure to 4°C or to control conditions (20°C) for 14 days, respectively. **(D)** Percentage of RNA-Seq reads annotated as genes coding for photosynthesis (PS) related proteins. Pie charts represent the averaged means from three different genotypes at 20°C (control) and after 14 days at 4°C. **(E)** Expression of *RubisCO Activase* (*Bv2\_025300\_izou.t1*), *RubisCO small subunit* (*Bv2026840\_jycs.t1*), *Chlorophyll A/B binding protein A* (*Bv\_002570\_dmif.t1*), *Plastocyanin* (*Bv\_004160\_hgin.t1*), *Glutathione reductase1* (*Bv3\_069540\_erom.t1*), *Glutathione reductase2* (*Bv5\_120360\_jpwm.t1*), *Superoxide dismutase1* (*Bv5\_102420\_sxsu.t1*), *Ascorbate peroxidase1* (*Bv1\_007470\_ymzt.t1*). Data represent the mean normalized cpm values of three independent RNA-seq analyses per genotype and temperature condition ± SD. Asterisks represent *p*-values < 0.05 according to double sided *t*-test in comparison to the values at control condition (20°C).





**Figure 3.** Changes in major carbohydrate metabolism and energy state in response to cold. **(A)** Respiration (CO<sub>2</sub> production) of different taproot regions from GT1 under control conditions (20°C, yellow bars) or after one week transfer to 4°C (blue bars). **(B)** Respiration (CO<sub>2</sub> production) from leaf tissue of three genotypes (GT1, GT2, GT3) under control conditions (20°C, yellow bars) or after 1-week transfer to 4°C (blue bars). **(C)** Principal component (PC) analysis (PC1 versus PC2) for three genotypes based on expression values of 112 genes with GO annotation “major CHO metabolism” (loadings) extracted from RNA-seq data of source leaves from plants grown at 20°C and transferred for 1 week at 12°C followed by 14 days at 4°C or control conditions (20°C). **(D)** Heatmap analysis of grouped expression values extracted from RNA-seq data. Unit variance scaling was applied to rows. Rows are using Manhattan distance and average linkage. **(E)** Expression values for two Sucrose Phosphate Synthase genes (*BvSPSA1* and *BvSPSA2*) and for two Sucrose Synthase genes (*BvSUS1* and *BvSUS2*) extracted from RNA-seq data of shoots and roots from GT1, GT2, GT3. Data represent the mean normalized cpm values of three independent RNA-seq analyses per genotype and temperature condition ± SD. **(F)** ATP, ATP/ADP ratio, energy charge, EC = [ATP] + 0.5 [ADP]/[ATP] + [ADP] + [AMP]. **(E,F)** Data are means ± SD. Asterisks represent *p*-values < 0.05 according to double sided *t*-test in comparison to the values at control condition (20°C).

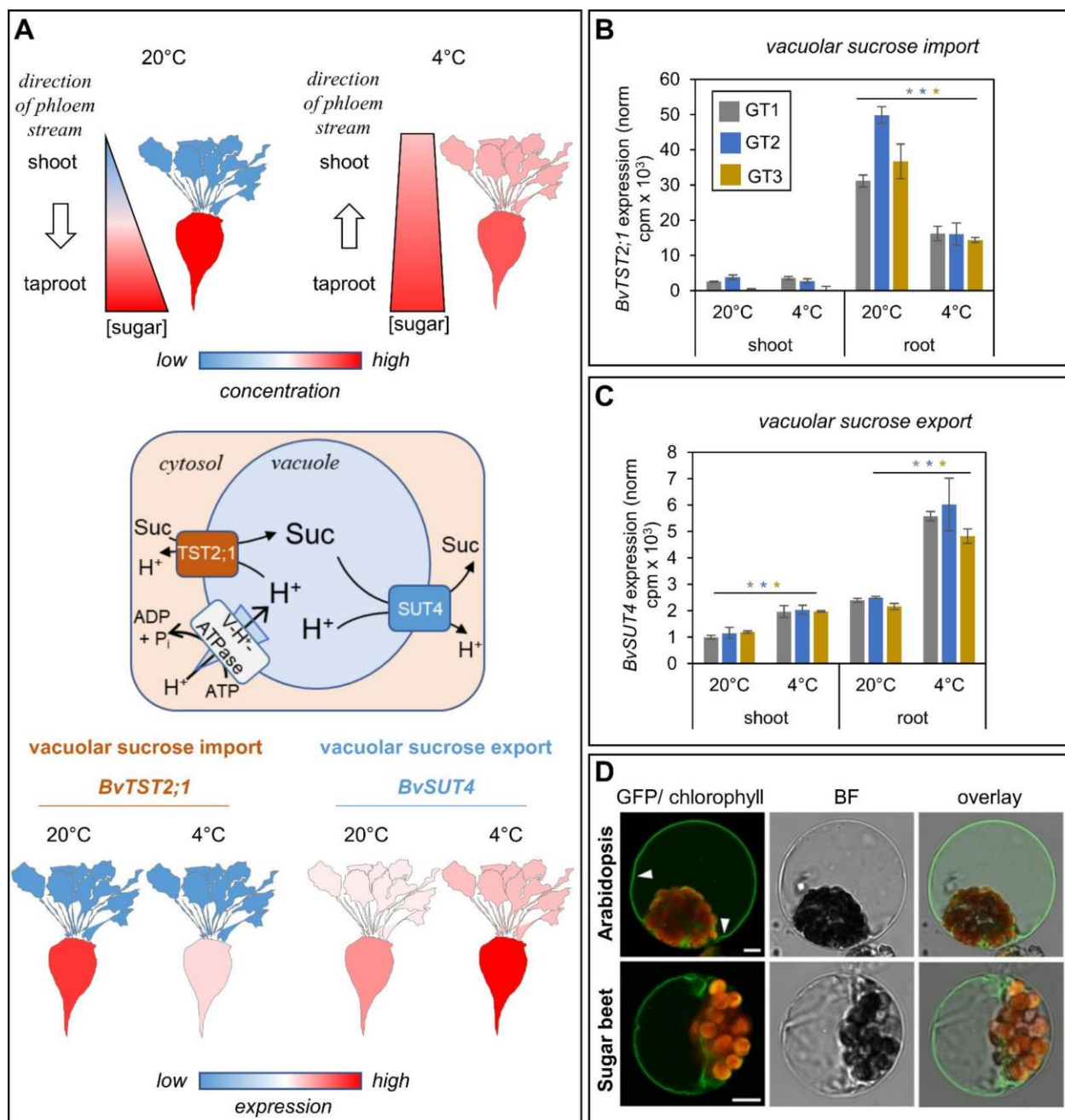


**Figure 4.** Distribution of <sup>14</sup>C-sucrose and esculin in leaves.

(A-D) Autoradiography of <sup>14</sup>C-sucrose in leaves. (A) Schematic depiction of experiment. Taproots were inoculated with <sup>14</sup>C-sucrose solution and harvested and dried leaves were autoradiographed one week later. (B) Source leaf from a representative plant grown for one week under at 4°C. Blackening of veins indicates radioactivity incorporated and distributed into leaf tissue after injection of radiolabeled sucrose into taproots. Abbreviations: p = petiole; mv = middle vein; 1° = first order lateral vein; 2° = second order lateral vein. (C) Source leaf from representative control plant grown at 20°C. (D) Radioactivity in cpm (counts per minute) measured in isolated petioles from plants grown under 4 or 20°C. Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots; crosses represent sample means; n = 16 sample points. (E-K) Esculin loadings. Yellow fluorescence indicates lignified xylem vessels, blue fluorescence indicates esculin trafficking. (E) Schematic depiction of experiment. Esculin was loaded onto the scratched surface of a source leaf of plants grown at 20°C. Loaded plants were transferred to 4°C or kept at 20°C. Petioles of neighboring, not loaded source leaves were analyzed for esculin fluorescence in plants from 4°C or 20°C. (F-K) Sections through a petiole of a source leaf not loaded with esculin. (F,G) Cross sections of petioles from 20°C (F) Bright field image. (G) UV fluorescence image. (H, I) Petioles from 4°C. (H) Bright field image. (I) UV fluorescence image. (J, K) Longitudinal sections of a petiole from 4°C. (J) Bright field image. (K) UV fluorescence image. Abbreviations: xy: xylem, ph: phloem. Bars are 100µm in F-I and 100µm in J and K.

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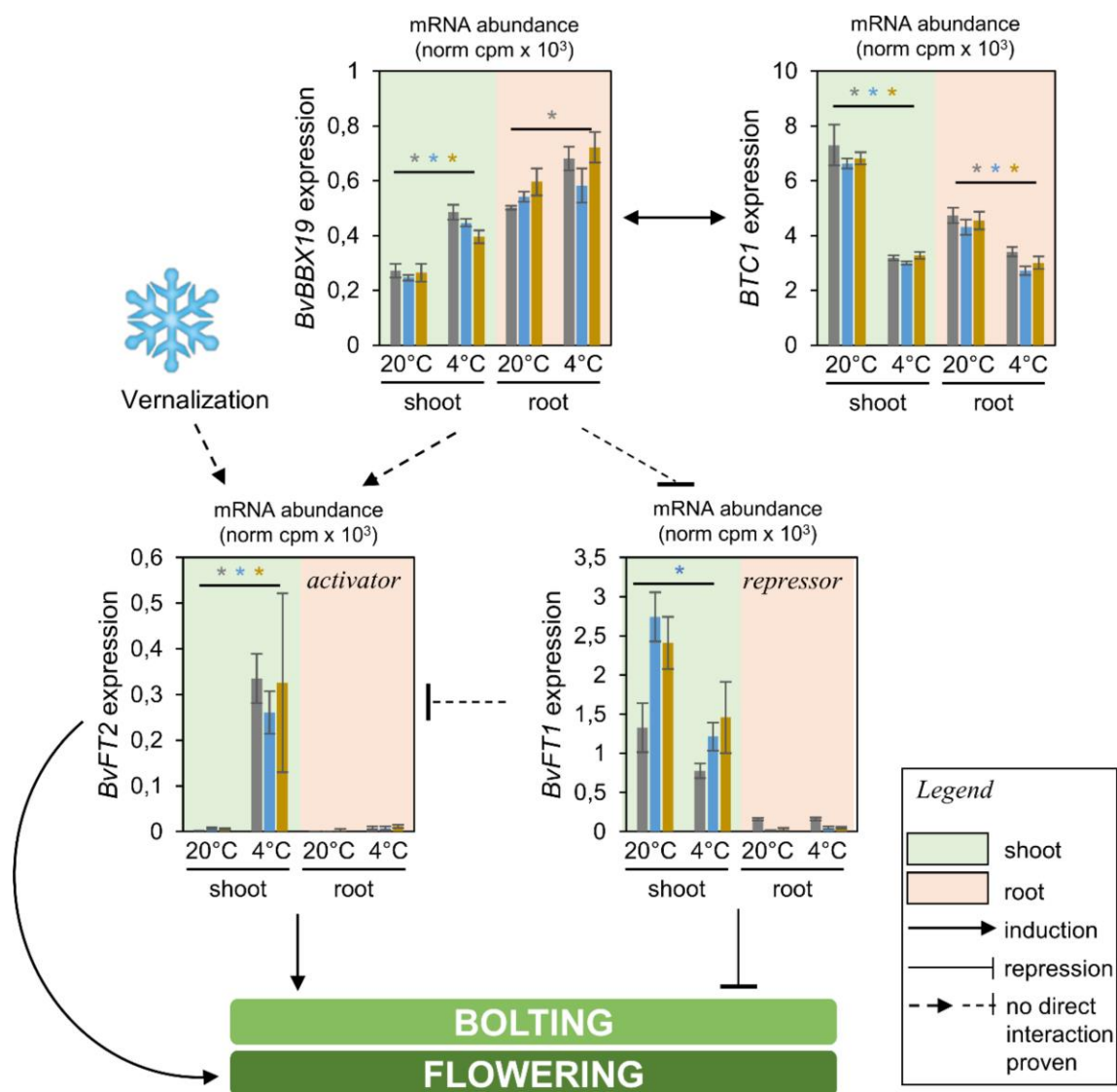


**Figure 5.** Cold-dependent accumulation of *BvTST2;1* and *BvSUT4* in three different sugar beet genotypes.

(A) Illustration on cold-induced processes. Upper image: Cold-dependent sugar relocations from taproots to shoots. Middle image: schematic of taproot vacuolar transport processes and factors. Vacuolar ATPase (V-H<sup>+</sup>-ATPase) establishes a proton motif force (pmf) across the vacuolar membrane; TST2;1 acts as proton/sucrose antiporter using pmf for sucrose import into vacuoles. SUT4 acts as proton/sucrose symporter using pmf for vacuolar sucrose export. Bottom image: reciprocal cold-induced regulation of *BvTST2;1* and *BvSUT4* mRNA levels in taproots. (B) Transcript abundance of *BvTST2;1* (*Bv5\_115690\_zuju*) mRNA based on RNA-seq reads. Values represent means from n=3 biological replicates per genotype ± SE. (C) Transcript abundance of *BvSUT4* (*Bv5\_124860\_zpft.t1*) mRNA based on RNA-seq reads. Values represent means from n=3 (mRNA) biological replicates ± SE. Asterisks indicate significant differences between the 20°C and 4°C treatments according to t-test (\* = *p* < 0.05). (D) Subcellular localisation of *BvSUT4*-GFP in Arabidopsis or *Beta vulgaris* leaf mesophyll protoplasts. Single optical sections in all pictures. The green colour shows the GFP-signal, the chlorophyll auto fluorescence is shown in red. Bars = 5 μm. Arrowheads point towards the vacuolar membrane (tonoplast).

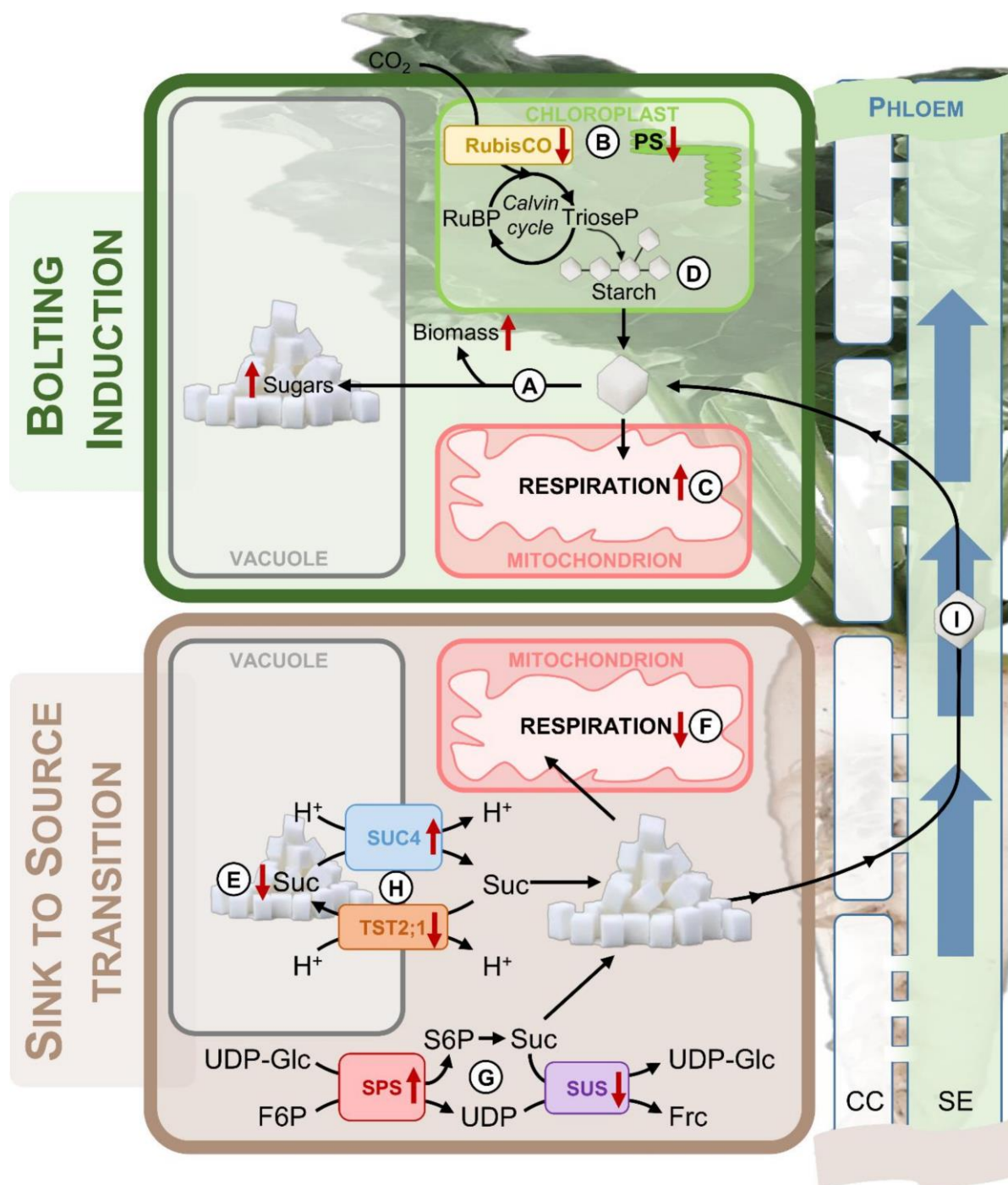
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**Figure 7.** Schematic illustration of cold-induced sink to source transition.

Leaf- and taproot-tissue of sugar beet are reprogrammed and source and sink identities shifted upon cold. Shoots adopt sink identity during cold. Biomass and sugar concentration in the shoot increase (A) despite reduced photosynthetic activity and inactivation of carbon assimilation (B). Concomitantly, shoot respiration increases (C) and cellular starch pools decrease (D). Contrastingly, taproots show a decrease of sucrose levels (E) but lower respiration rate (F) as well as increased sucrose biosynthesis (G). Taproot sugar is remobilized in the cold due to opposite regulation of taproot-specific vacuolar sucrose importer (BvTST2;1) and exporter (BvSUT4) activity (H). Taken together, this results in a reversal of the phloem translocation stream (I) triggered by a reprogramming of source and sink identities, which might correlate with inflorescence initiation.