Genes & Genomics (2020) 42:13–24
https://doi.org/10.1007/s13258-019-00873-z

Online ISSN 2092-9293
Print ISSN 1976-9571

RESEARCH ARTICLE

Identification of source-sink tissues in the leaf of Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) by carbohydrate content and transcriptomic analysis

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Received: 23 July 2019 / Accepted: 25 September 2019 / Published online: 14 October 2019

Abstract

Background A leaf of Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) is composed of a photosynthetic blade and a non-photosynthetic large midrib; thus each leaf contains both source and sink tissues. This structure suggests that, unlike in other plants, source-sink metabolism is present in a single leaf of Chinese cabbage.

Objective This study was designed to identify the transport route of photosynthetic carbon and to determine whether both source and sink tissues were present in a leaf.

Methods Plant samples were collected diurnally. Their carbohydrate contents were measured, and a genome-wide transcriptome analysis was performed using the Br300K microarray. Expression profiles of selected genes were validated using qRT-PCR analysis.

Results The presence of two contrasting tissues (blade as source and midrib as sink) in a leaf was demonstrated by (1) diurnal distribution patterns of starch and sucrose content; (2) Gene Ontology (GO) enrichment analysis of microarray data; (3) expression profiles of photosynthetic and sucrose biosynthetic genes; and (4) expression patterns of a variety of sugar transporter genes.

Conclusion Source and sink tissues were both present in Chinese cabbage leaves, but the midrib functioned as a sink tissue as well as a site exporting to roots and other sink tissues. Function of most genes discriminating between source and sink tissue appeared to be regulated largely at the post-transcriptional level, not at the transcriptional level.

Keywords Carbohydrate content · Br300K microarray · Large midrib · Photosynthetic genes · Sugar transporters

Abbreviations

ACT2	Actin 2
ADG1	ADP glucose pyrophosphorylase small subunit 1
AKIN10	Arabidopsis SNF1 kinase homolog 10
AKIN11	Arabidopsis SNF1 kinase homolog 11; SnRK1.1
AMY3	Alpha-amylase-like 3
BAM6/BMY5	Beta-amylase 6/beta-amylase 5
CCA1	Circadian clock-associated 1
CHLSYN	Chlorophyll synthase
CINV1	Cytosolic invertase 1
CpFBPase	Chloroplastic fructose-1,6-bisphosphatase
CWINV1	Cell wall invertase 1
CytFBPase	Cytosolic fructose-1,6-bisphosphatase
DBE1	Debranching enzyme 1
EMB2729	Alpha-amylase family protein
ERD6	Early response to dehydration 6
ERD6-Like 7	Major facilitator superfamily protein
GLT1	Glucose transporter 1
HKL1	Hexokinase-like 1
HKL3	Hexokinase-like 3

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13258-019-00873-z) contains supplementary material, which is available to authorized users.

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HXK1	Glucose insensitive 2
HXK2	Hexokinase 2
HXK3	Hexokinase 3
HXK4	Hexokinase 4
INT1	Inositol transporter 1
INT4	Inositol transporter 4
IPMI	Invertase/pectin methylesterase inhibitor family protein
ISA1	Isoamylase 1/alpha-amylase
ISA3	Isoamylase 3
LDA/PU1	Limit dextrinase/Pullulanase 1
LHY	Late elongated hypocotyl
LSH1	Light-dependent short hypocotyl 1
OCT4	Organic cation/carnitine transporter 4
PETE1	Plastocyanin
PHT2	Phosphate transporter 2
PHT6	Phosphate transporter 6
PMT5/PLT5	Polyol/monosaccharide transporter 5
PORB	Protochlorophyllide oxidoreductase B
PORC	Protochlorophyllide oxidoreductase C
PSBA	Photosystem II protein D1
RUBISCO	Ribulose 1,5-bisphosphate carboxylase/oxygenase
RBCS-1A	RuBisCO small subunit 1A
RBCS-2B	RuBisCO small subunit 2B
RCA	RUBISCO ACTIVASE
SBE2.1	Starch branching enzyme 2.1
SBE2.2	Starch branching enzyme 2.2
SNF	Sucrose non-fermenting
SnRK1	SNF-related kinase 1
SPP1	Sucrose-phosphatase 1
SPP3	Sucrose-phosphatase 3
SPS	Sucrose phosphate synthase
SS3	Starch synthase 3
STP1	Sugar transporter 1
STP13	Sugar transporter protein 13
STP4	Sugar transporter 4
STP6	Sugar transporter 6
STP7	Sugar transporter 7
SUC1	Sugar-proton symporter 1
SUC2	Sugar-proton symporter 2
SUC3/SUT2	Sucrose transporter 3
SUC4/SUT4	Sucrose transporter 4
SUC7	Sucrose-proton symporter 7
SUS1	Sucrose synthase 1
SUS3	UDP-glycosyltransferase/sucrose synthase 3
SUS6	UDP-glycosyltransferase/sucrose synthase 6
TMT1	Tonoplast monosaccharide transporter 1
TMT2	Tonoplast monosaccharide transporter 2
TOC1	Timing of CAB expression
TOR	Target of rapamycin
UTR3	UDP-galactose transporter 3
UTR6	UDP-galactose transporter 6
VAC-INV	Vacuolar invertase
VGT1	Vacuolar glucose transporter 1
3008	Pollen Ole e 1 allergen and extensin family protein

Introduction

In plants, a "source" tissue is a producer and exporter of nutrients and assimilates, whereas a "sink" tissue is an importer and consumer (Foyer and Paul 2001). Mature leaves and other green tissues with photosynthetic activity are sources of fixed carbon (C), while heterotrophic organs, such as roots and growing tubers, fruits, and seeds, are sinks (Chang et al. 2017). The regulation of source-sink balance of carbon assimilates is controlled by sugar signaling (Smith and Stitt 2007). A typical plant leaf consists of a petiole and a blade containing veins. The leaf blade is the source of most nutrients, with the veins serving as a translocation route from source to sink.

The transport and partitioning of sugars from source to sink through the phloem are major parameters controlling crop productivity (Ainsworth and Bush 2011). These processes are closely associated with photosynthetic capacity, starch metabolism in chloroplasts, and transient storage of sugars inside vacuoles. Sugar transport towards sinks is mediated either by symplastic flow through plasmodesmata or by the apoplastic pathway involving membrane transporters, such as monosaccharide transporters (MSTs), H^+/sucrose transporters (SUT/SUC), and SUGAR WILL EVENTUALLY BE EXPORTED TRANSPORTERS (SWEETs), which control monosaccharide and sucrose fluxes (Hennion et al. 2019). In Arabidopsis (*Arabidopsis thaliana*), AtSUC2/AtSUT1, AtSWEET11 and AtSWEET12 control phloem loading, while AtSUC1 is responsible for phloem unloading (Durand et al. 2018). Sucrose produced by photosynthetic mesophyll cells may be transported via SWEET11 and SWEET12 directly into the apoplasm, from which it is imported (or loaded) into companion cells or sieve elements by SUC2/SUT1. Alternatively, sucrose may be transported symplastically from cell to cell through plasmodesmata before being transported to the apoplasm by SWEET11 and SWEET12, and loaded into companion cells or sieve elements by SUC2/SUT1 (Stadler and Sauer 1996; Gottwald et al. 2000; Braun et al. 2014; Chen et al. 2012). Sucrose from phloem is unloaded into sink tissues by AtSUC1 (Sivitz et al. 2008; Durand et al. 2018). Differences in activity and expression levels of these transporters between source and sink organs are therefore expected.

A plant's carbon balance shows diurnal fluctuations in photosynthates and respiratory losses, as well as in

partitioning of assimilates between phototrophic and heterotrophic organs (Brauner et al. 2018). Starch or sucrose is the main storage form of photosynthates in various crop species (Goldschmidt and Huber 1992). Sugars and sugar alcohols, which are the main energy source for numerous biosynthetic reactions, play an essential role in the transport of fixed carbon to different sink tissues (Schneider et al. 2008). Diurnal fluctuations in high energy signals, such as sugar phosphate, sucrose, and glucose, regulate plant growth and development, and also coordinate source-sink activity, which is mediated by SUCROSE NON-FERMENTING 1-RELATED KINASE 1 (SnRK1; Crepin and Rolland 2019; Rodrigues et al. 2019). In addition to sugars, hexokinases have been identified as glucose sensors. They affect development throughout the whole plant cycle and repress expression of some photosynthetic genes in response to high internal glucose concentrations; this is especially important in crops that specialize in accumulating sugar and biomass (Aguilera-Alvarado and Sánchez-Nieto 2017; Rodrigues et al. 2019).

Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) is an important leafy vegetable crop in Korea and other parts of eastern Asia. It is characterized by large leaves with a wrinkled surface, a pale-green color, large white midribs, and heads of different shapes (Fig. 1). The large white midrib is greatly expanded and appears to be a sink tissue; sucrose can be directly obtained from the blade without phloem loading and unloading. Our previous study (Mun et al. 2009) examined levels of different nutrients and associated gene expression in blades and midribs from different developmental stages. Diurnal fluctuations and the source-sink relationship have not yet been examined, and may be a phenomenon peculiar to Chinese cabbage leaves. We aimed to reveal the transportation route of photosynthetic carbon and to clarify whether source and sink tissues were present in a leaf. We therefore measured carbohydrate contents at three different time points across a day and determined gene expression profiles at the same times using microarray and qRT-PCR analyses. Our results indicated that the leaf blade was a source tissue, and that phloem loading occurred in both the blade and the midrib; thus the midrib acted as a sink as well as a supporting tissue.

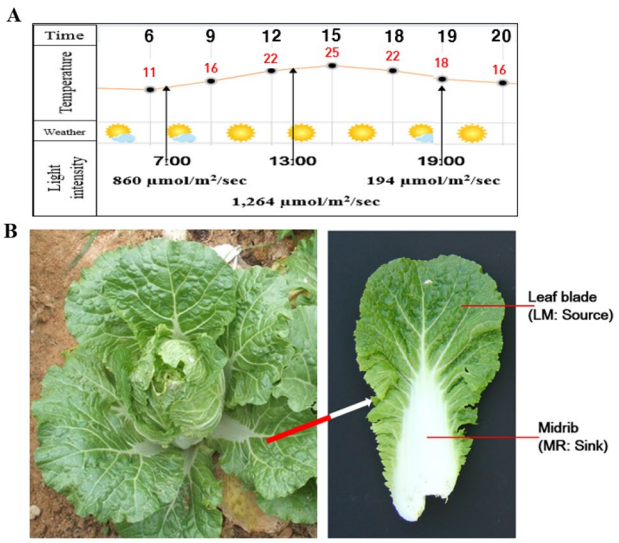

Fig. 1 Growth conditions in the field and collection points of Chinese cabbage samples used in this study. **a** Representative weather conditions including temperature and light intensity on the sampling days. **b** Sampling points on the leaf blade (LM) and midrib (MR)

Materials and methods

Plant materials

Brassica rapa ssp. *pekinensis* cultivar Huessen (Woori Seed Co., Korea) plants were grown in the field at Daejeon city, Korea, from April to June. Samples were collected three times each day at 07:00 (shortly after dawn), 13:00 (middle of the day), and 19:00 (shortly before sunset) at 4 day intervals between May 30 and June 12. Sampling times, weather conditions, light intensity, and sampled tissues are shown in Fig. 1a. Tissue samples were frozen soon after collection in liquid nitrogen and stored at − 70 °C until use.

Soluble sugar and starch content

Megazyme kits (Megazyme, Ireland) were used to measure levels of sucrose, glucose, fructose, and starch in leaf blade and midrib tissues using the method described in Mun et al. (2009). The measurements were repeated three times for each sample; data are expressed as means ± standard errors.

RNA isolation

Total RNA was isolated from samples using an easy-BLUE™ total RNA extraction kit (Invitrogen, USA) and purified using the RNeasy MinElute™ Cleanup Kit (Qiagen, Germany). For biological repeats, RNA extracted from three independent samples was subjected to microarray or qRT-PCR analysis.

Br300K microarray and data analysis

A 300 K microarray chip for *Brassica rapa* (Br300K; v2.0) was designed containing 47,548 unigenes, as described in detail in a previous report (Dong et al. 2013). Cleaned-up samples of total RNA extracted from leaf blades and midribs were used for Cy3-labeled cDNA synthesis. The microarray was scanned with a Genepix 4000 B (Axon, USA),

preset with a 5 µm resolution for a Cy3 signal. Signals were digitized and analyzed by NimbleScan (Nimblegen, USA). Only genes with either an adjusted *p* value or a false discovery < 0.05 were collected and analyzed. To assess the reproducibility of the microarray analysis, the experiment was repeated twice using independently prepared total RNA samples. Data were processed with cubic spline normalization, using quantiles to adjust the signal variation between chips (Workman et al. 2002). Call files were produced from a probe-level summarization in a Robust Multi-Chip Analysis that used a median polish algorithm implemented in NimbleScan (Workman et al. 2002; Irizarry et al. 2003). Gene annotation initially used genes from *Arabidopsis thaliana* TAIR7 (http://www.arabidopsis.org/). As above, only genes for which either the adjusted p value or the false discovery was < 0.05 were assembled. Final data were expressed as probe intensity (PI) values. Gene Ontology (GO) enrichment analysis was carried out using the agriGO tool (http://bioinfo.cau.edu.cn/agriGO/index.php).

RT-PCR analysis

Each 5 µg sample of total RNA was combined with random hexamer primers in a SuperScript first-strand cDNA synthesis system, according to the manufacturer's instructions (Invitrogen, USA). Complementary DNA was diluted tenfold, and 1 µl of diluted cDNA was used in a 20 µl PCR mixture. All RT-PCR primers, including the *BrACT2* primers used as a control, are listed in Table S1. PCR was performed using the following program: 5 min denaturation at 94 °C, followed by 25 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s. PCR products were analyzed following electrophoresis through a 1% agarose gel.

Results

Carbohydrate content

Relative carbohydrate content was measured in samples of leaf blade and midrib tissues collected at 07:00, 13:00, and 19:00 (Fig. 2). Relative levels of starch and sucrose were high in the leaf blade except sucrose level at 19:00 (end of the day). Absolute starch content (%) was higher than that of sucrose, indicating that Chinese cabbage was a starch accumulator. The sucrose content in the midrib gradually increased across a day but remained almost constant in the leaf blade, indicating that the midrib was a photosynthate storage organ or sink tissue. The absolute content of glucose and fructose in the leaf blade was similar, but the absolute glucose content in the midrib was higher than that of fructose.

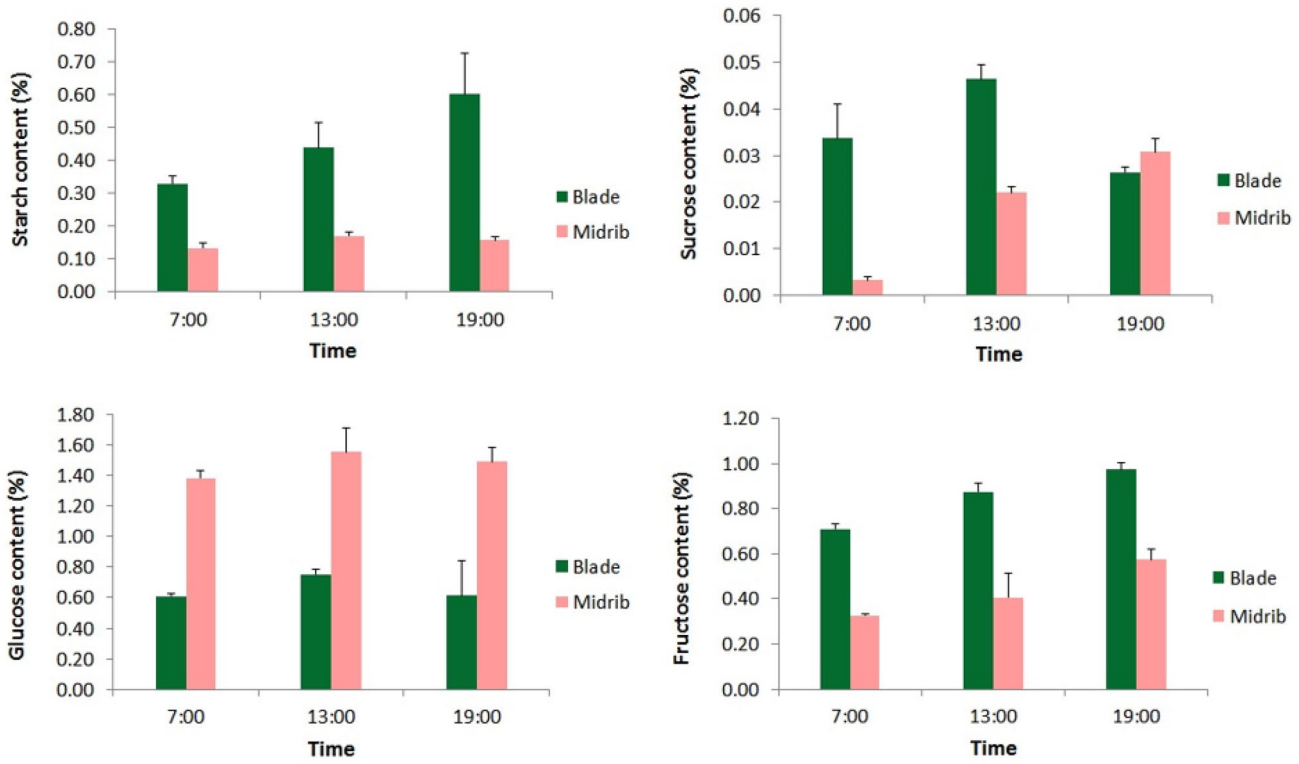

Fig. 2 Carbohydrate content in blade and midrib at indicated times across a day expressed as % (w/w)

Diurnal fluctuations in starch levels in the leaf blade and sucrose levels in the midrib were observed; both the starch level in the photosynthetic blade tissue and the sucrose level in the midrib were highest at the end of the day. The starch level in the midrib and the sucrose level in leaf blade tissue were relatively constant, however, across the day. The temporary storage forms of photosynthate thus appeared to be starch in the leaf blade and sucrose in the midrib.

Gene Ontology (GO) enrichment analysis

We analyzed the transcriptome in the leaf blade and midrib at three different time points over a day using the Version 2 Br300K microarray, which includes 47,548 *B. rapa* genes as probes. About 18% (8542) of the *B. rapa* genes had no Arabidopsis counterpart (no_hits_found at TAIR7_cds ID; Table S2). Of the 47,548 genes on the array, 39,879 (84%) genes showed a probe intensity (PI) value over 500 in at least one of the samples tested. We determined that transcript levels of genes with a cutoff PI value of 500 could be easily examined using standard RT-PCR.

To identify which categories of genes were expressed in a tissue-specific manner, leaf blade- (Table S3) and midrib- (Table S4) specific genes were analyzed. Specific genes were defined as genes with PI values below 500 in all three samples of one tissue, but over three times higher in the three samples of the other tissue; using this definition, 153 genes were selected as leaf blade-specific and 120 genes as midrib-specific. The GO terms "response to stress", "photosynthetic membrane", and "thylakoid membrane" were highlighted in the GO enrichment analysis of genes specifically expressed in the leaf blade; this was almost certainly a result of the blade region's functions (Fig. 3b). The terms "symplast", "external encapsulating structure", "cell–cell junction", "cell wall", and "plasmodesma" were over-represented among genes specifically expressed in the midrib, which reflected its transport and support functions (Fig. 3c). These results suggest that the leaf blade was a source tissue but the midrib was a translocation route and/or sink tissue.

Several categories of genes were selected using these microarray data and previously known information. RT-PCR was performed to determine the transcript levels and identify the biological roles of these genes; in most cases, this revealed a similar pattern of gene expression to that observed in the microarray experiment. Photosynthesis-related genes, transporter genes, *SWEET* genes, and sugar sensing and energy homeostasis-related genes were analyzed using RT-PCR. Expression of circadian clock-related genes was examined as a reference experiment. The circadian clock-associated genes *CIRCADIAN CLOCK-ASSOCIATED 1* (*BrCCA1*), *LATE EXTENDED HYPOCOTYL* (*BrLHY*) (two morning loop components), and *TIMING OF CAB 1*

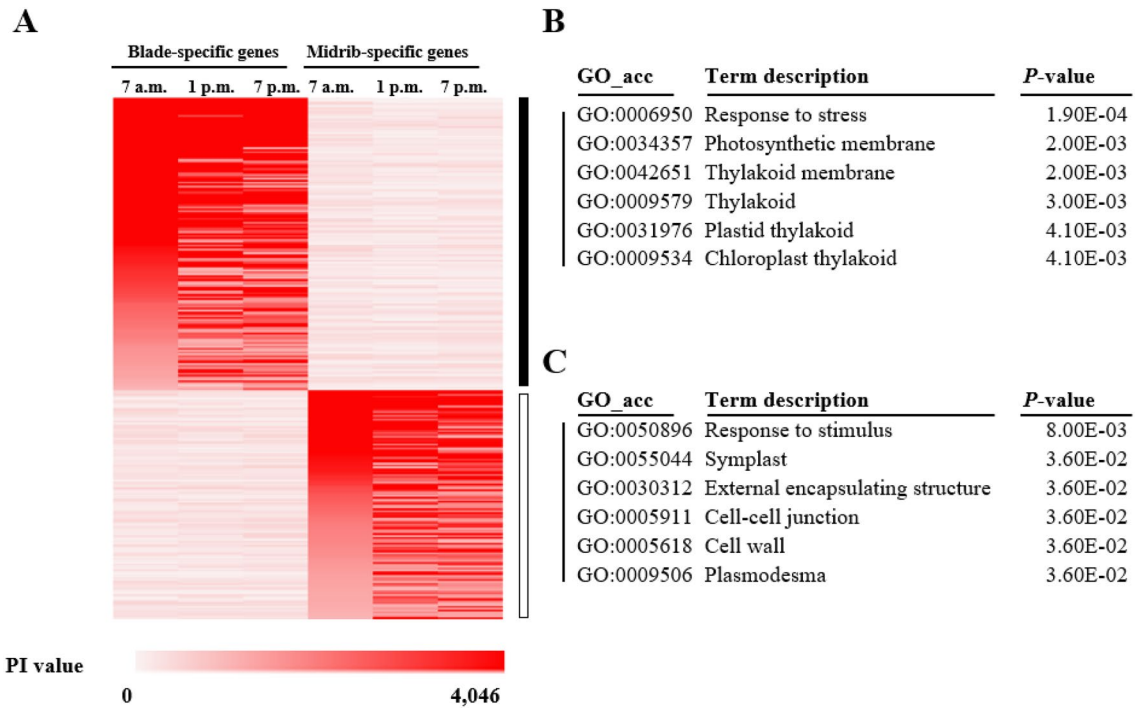

Fig. 3 Analysis of genes expressed specifically in the leaf blade and midrib of Chinese cabbage. **a** Heatmap of genes specifically expressed in blade and midrib. **b** Gene Ontology (GO) enrichment analysis of genes specifically expressed in leaf blade. **c** GO enrichment analysis of genes specifically expressed in midrib. GO enrichment analysis was carried out using agriGO tool (http://bioinfo.cau.edu.cn/agriGO/index.php)

(BrTOC1; an evening loop component) showed the expected pattern of expression (Ito et al. 2009; Fig. S1), indicating that the sampling times were appropriate for examining diurnal changes in gene expression.

Photosynthesis-related gene expression

The category "photosynthesis-related genes" included genes associated with chlorophyll biosynthesis, the light reaction, and CO_2 fixation (Fig. 4), starch biosynthesis and degradation (Fig. 5), and sucrose biosynthesis and degradation (Fig. 6). Chinese cabbage *PLASTOCYANIN* (*BrPETE1*) was expressed only in the leaf blade, as expected. Of the several Rubisco small subunit genes examined, only *RBCS-1A* was specifically expressed in the leaf blade, consistent with a previous report describing the roles of *RBCS-1A* and *RBCS-3B* in Rubisco accumulation (Izumi et al. 2012). All the other tested genes involved in chlorophyll biosynthesis, the light reaction, and CO_2 fixation were expressed in both leaf blade (source) and midrib (sink) tissues (Fig. 4), suggesting that regulation occurred at the post-transcriptional level. Expression

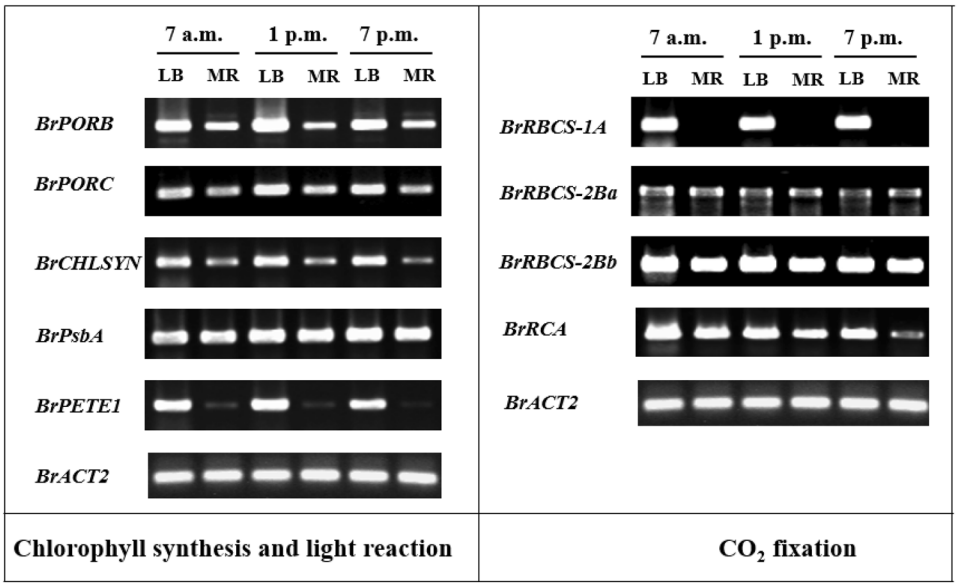

Fig. 4 Transcript levels of genes involved in the light reaction (left) and CO_2 fixation (right). Expression of *B. rapa ACTIN 2* (*BrACT2*) was used as a control

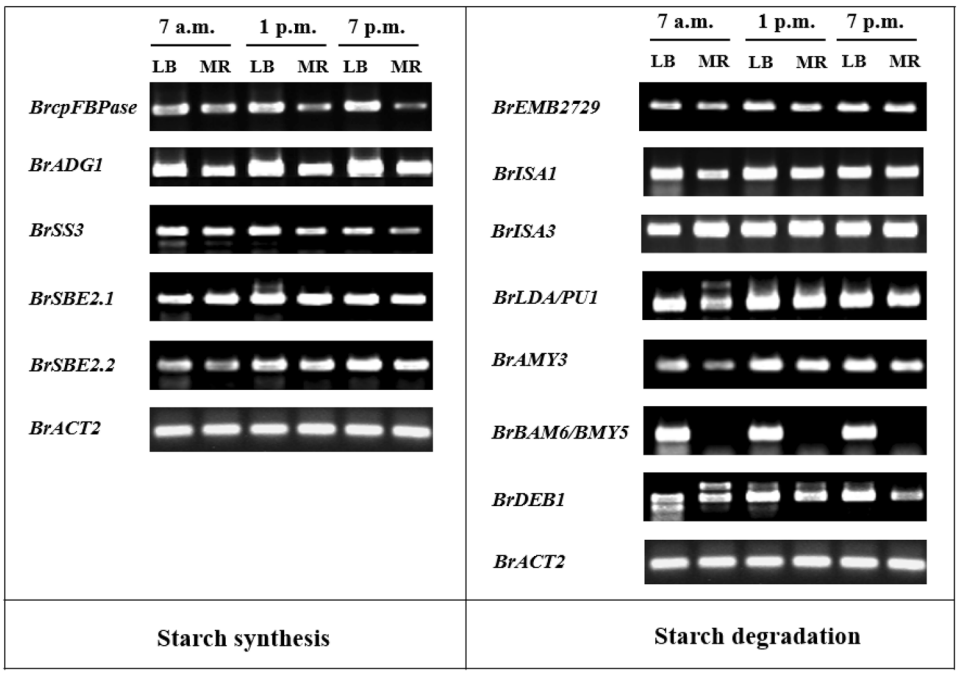

Fig. 5 Transcript levels of genes involved in starch synthesis (left) and degradation (right). Expression of *B. rapa ACTIN 2* (*BrACT2*) was used as a control

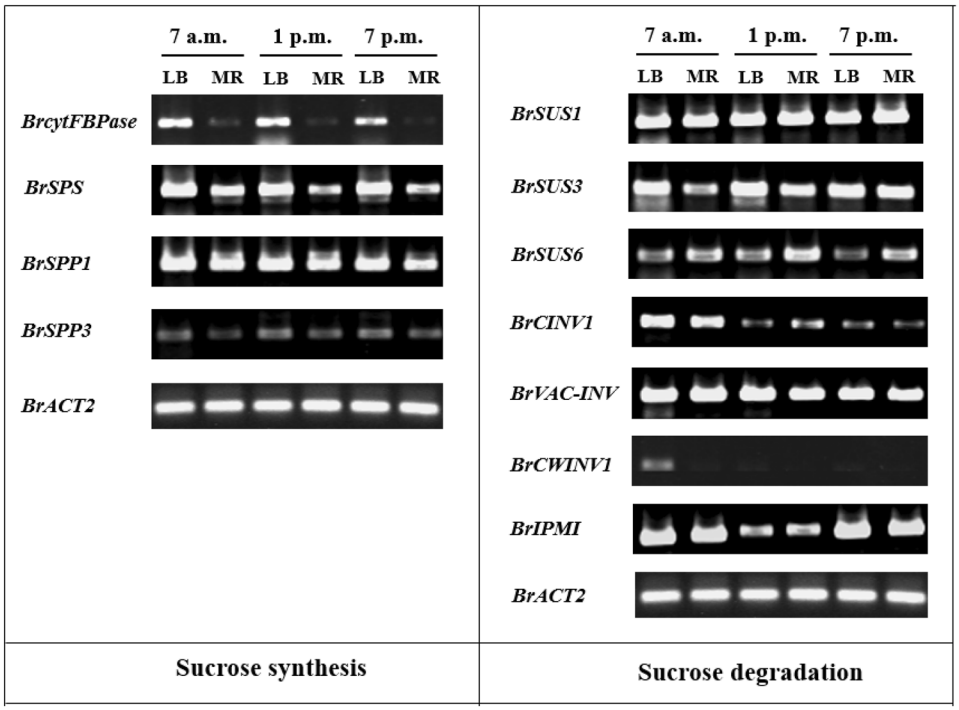

Fig. 6 Transcript levels of genes involved in sucrose synthesis (left) and degradation (right). Expression of *B. rapa ACTIN 2* (*BrACT2*) was used as a control

levels of the starch degradation-related genes *ISOAMYLASE 1* (*BrISA1*) and *ALPHA-AMYLASE-LIKE 3* (*BrAMY3*) increased slightly in the leaf blade. Although the midrib contained low levels of starch, most genes associated with starch synthesis and degradation were expressed at similar levels in the midrib and leaf blade (Fig. 5), with the exception of *BrBAM6/BMY5*, whose expression in the leaf blade implied high starch content in that tissue (Monroe et al. 2014; Monroe and Storm 2018).

Genes related to sucrose biosynthesis were also expressed in the midrib (Fig. 6, left-hand panel), implying that the function of such genes depended on post-transcriptional regulation. *CYTOSOLIC FRUCTOSE-1,6-BISPHOSPHATASE* (*BrcytFBPase*), which encodes the protein responsible for catalyzing the first irreversible step of sucrose biosynthesis (Stitt 1990; Daie 1993), was specifically expressed in the leaf blade, a photosynthetic source. This implied that conversion of photosynthetic carbon (C3 carbon) to sucrose was restricted to photosynthetic blade tissue and sucrose was then moved to the midrib. Sucrose phosphate synthase post-translationally regulates this activity (Jones and Ort 1997) and was expressed at higher levels in source than in sink tissues. Expression levels of sucrose degradation-related genes were similar in both leaf blade and midrib tissue (Fig. 6, right-hand panel). The gene expression profiles and carbohydrate content in these tissues suggested that sucrose synthesis occurred in a source tissue (blade), and was subsequently transported to the midrib either via symplastic or apoplastic flow.

Transporter gene expression

As veins provide a translocation route from sources to sinks, expression of various transporter genes is likely to provide information on the role of midrib in Chinese cabbage leaves. *PHOSPHATE TRANSPORTER 2* (*BrPHT2* [*PHT1;4*]), whose homolog is expressed in Arabidopsis roots (Karthikeyan et al. 2009), was expressed in both tissues of Chinese cabbage leaves, but transcript levels were higher in the leaf blade than in the midrib (Fig. 7, left-hand panel). Of the sucrose transporter genes examined, only *BrSUC2*, which plays a major role in phloem loading in Arabidopsis (Srivastava et al. 2008), was highly expressed in both tissues. Its expression was particularly high in the leaf blade tissue, which contains large numbers of small veins. This observation implied that phloem loading occurred in both the blade and midrib. Most of the glucose transporter genes tested were expressed in both tissues, although *SUGAR TRANSPORTER 6 (BrSTP6)*, *BrSTP7*, and *BrSTP13* were expressed specifically in source blade tissue (Fig. 7, right-hand panel).

The expression profiles of most genes encoding monosaccharide transporters and other transporters were similar in the blade and midrib. Several genes, including one encoding a fatty acid transporter, *ORGANIC CATION/CARNITINE TRANSPORTER 4* (*OCT4*), showed tissue-specific expression (Fig. 8, left-hand panel): *POLYOL/MONOSACCHARIDE TRANSPORTER 5* (*BrPMT5/PLT5*) and *BrOCT4* were preferentially expressed in the leaf blade, whereas *UDP-GALACTOSE TRANSPORTER 6* (*BrUTR6*), *INOSITOL TRANSPORTER 1* (*BrINT1*), and *EARLY RESPONSE TO*

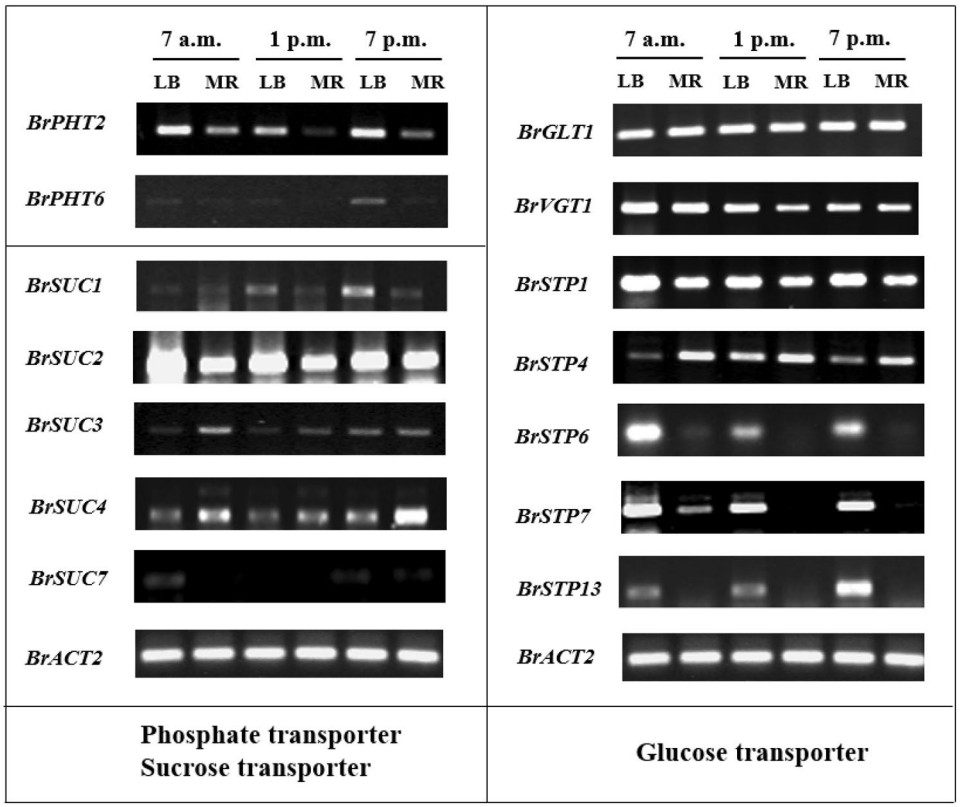

Fig. 7 Transcript levels of genes involved in phosphate and sucrose transport (left) and glucose transport (right). Expression of *B. rapa ACTIN 2* (*BrACT2*) was used as a control

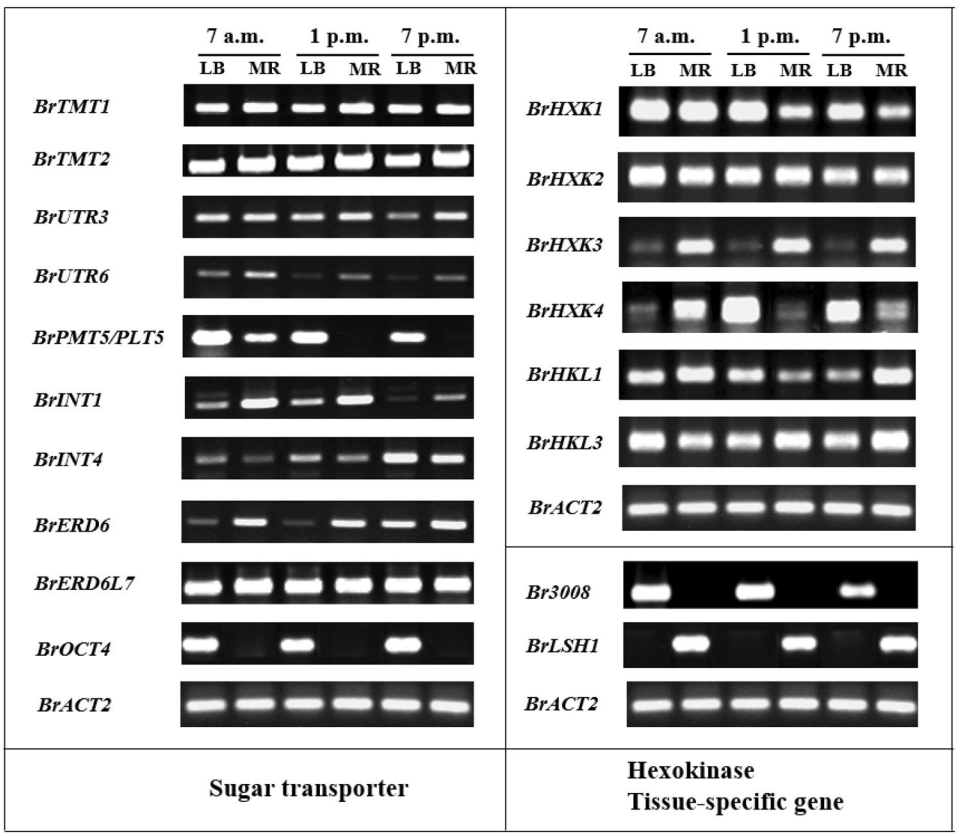

Fig. 8 Transcript levels of genes involved in sugar transport (left) and hexose transport and tissue-specific expression (right). Expression of *B. rapa ACTIN 2* (*BrACT2*) was used as a control

DEHYDRATION 6 (*BrERD6*) transcripts were expressed at higher levels in the midrib.

SWEET gene expression

SUGAR WILL EVENTUALLY BE EXPORTED TRANSPORTER (SWEET) proteins are a recently identified family of sugar transporters. The Arabidopsis genome contains 17 SWEET homologs, AtSWEET1–17 (Chen et al. 2010). SWEETs play key roles in a variety of plant biological processes, including phloem loading for long-distance sucrose translocation (Chen et al. 2012). Expression profiling of *BrSWEET* genes in the leaf blade and midrib is therefore central to a full understanding of the source-sink relationship in a leaf. The Br300K microarray contained 14 *SWEET* genes (20 alleles) (Table S2). Of these *SWEET* genes, eight (13 alleles) were highly expressed in either or both tissues, but six genes (seven alleles) showed relatively low levels of expression (Figs. S2 and S3). *BrSWEET11* s and *BrSWEET12* s, which may be responsible for phloem loading of sucrose, were more highly expressed in the leaf blade than in the midrib. Although expression of most genes was high in the leaf blade or similar in both tissues, *BrSWEET1a* and *BrSWEET1b* were highly expressed in blade tissue and three genes (*BrSWEET14a*, *BrSWEET15b* and *BrSWEET17a*) were more highly or solely expressed in the midrib. These three genes are expected to play a novel role in the midrib of Chinese cabbage.

Expression of genes involved in sugar sensing and energy homeostasis

Sugar sensing and energy homeostasis are essential for plant growth and development, as well for regulating the source-sink balance. Hexokinases (HXKs) are sensors involved in detecting sugar levels (Price et al. 2004) and two components, SnRK1 and TARGET OF RAPAMYCIN (TOR), are responsible for energy homeostasis (Crepin and Rolland 2019; Rodrigues et al. 2019). Genes involved in energy homeostasis did not show tissue-specific expression (Fig. S4); although expression of *B. rapa ARABIDOPSIS SNF1 KINASE HOMOLOG 11* (*BrAKIN11* [*BrSnRK1.2*]) and *BrTOR* was slightly elevated in midrib tissues, levels of transcript of all three genes were very high.

Of the genes involved in sugar sensors, *BrHXK3* was preferentially expressed in the midrib, while *BrHXK4* was expressed more highly in the leaf blade (Fig. 8, right-hand panel); expression levels of the other hexokinase genes tested were similar in both tissues. Figure 8 also shows the expression profiles of two tissue-specific genes, *LIGHT-DEPENDENT SHORT HYPOCOTYL 1* (*BrLSH1*; an organ boundary gene), expressed in the midrib, and *Br3008* (an unknown gene), expressed in blade tissue.

Discussion

Carbohydrate content and GO analysis

Plants can be divided into three groups depending upon their accumulation of photosynthetic product: starch storers (or accumulators), including soybean, cotton, cucumber, and Arabidopsis; sucrose storers such as spinach; and intermediate plants, which include tomato, beans, and pea (Goldschmidt and Huber 1992; Smith and Stitt 2007). In starch storers, starch content gradually increases over the day, reaches its maximum before dark, and is converted into sucrose at night (Smith and Stitt 2007). The diurnal fluctuations in starch content in the leaf blade and of sucrose in the midrib (Fig. 2) suggested that Chinese cabbage was a starch accumulator with the midrib acting as a sucrose storage organ and/or intermediate transportation site prior to transport to roots. This new finding implied that the midrib was a sucrose-importing sink as well as an exporting source tissue. Fructose, which tastes sweeter than sucrose and glucose, is usually present in high levels in fruits (Muir et al. 2009). Fructose levels were higher in the leaf blade than in the midrib (Fig. 2), implying that the leaf blade was a source of sweetness in Chinese cabbage.

Consistent with the changes in starch content, transcript levels of genes encoding enzymes involved in starch metabolism, including *STARCH BRANCHING ENZYME 3* (*SBE3*), *ISA3*, and *AMY3*, change diurnally; their levels gradually increase during the day and then gradually decrease at night (Smith and Stitt 2007). The Arabidopsis genome contains nine β-amylase (*BAM*) genes; of these, *AtBAM6* is responsible for starch accumulation in old leaves (Monroe and Storm 2018). We found that *BrBAM6* was specifically expressed in source tissue (leaf), while expression of other starch metabolism genes was slightly higher in the leaf blade and also increased during the day (Fig. 5), supporting the classification of *B. rapa* as a starch-storing plant.

As expected, GO categories enriched in the leaf blade included those associated with chloroplast function and photosynthesis; by contrast, categories enriched in the midrib included those functioning in cell support, including "cell wall" and "external encapsulating structure", or assimilate transport, such as "symplast", "cell–cell junction", and "plasmodesma" (Fig. 3). Preferential expression of *BrINT1* and *BrERD6* in the midrib (Fig. 8) might indicate active cell wall biosynthesis in this tissue, as described by Schneider et al. (2008) and Rautengarten et al. (2014). Chinese cabbage leaves lack a petiole (Fig. 1b), and thus one of the functions of the large midrib may be to act as a petiole substitute by supporting the leaf blade and transporting materials from the leaf (Tsukaya

et al. 2002; Kozuka et al. 2005, 2010). Expression profiles of transcripts of sugar transporter genes (Figs. 7 and 8) suggested that the midrib also functioned in phloem loading and unloading.

Genes related to phloem loading and unloading

AtSUC2/AtSUT1, AtSWEET11, and AtSWEET12 function together in phloem loading, while AtSUC1 is responsible for phloem unloading (Durand et al. 2018). Although they have slightly different effects on phloem loading, both AtSWEET11 and AtSWEET12 are involved in the efflux of sugar from parenchyma cells to the apoplasm, from where it is loaded into sieve elements and companion cells by AtSUC2/SUT1 (Stadler and Sauer 1996; Gottwald et al. 2000; Chen et al. 2012; Braun et al. 2014). Expression of all of the BrSWEET11 and BrSWEET12 alleles tested was higher in the leaf blade than in the midrib, although expression levels in the midrib were not especially low (Fig. S2), implying possible regulation by post-transcriptional mechanisms. Preferential or specific expression of *BrSWEET14a*, *BrSWEET15b*, and *BrSWEET17a* in the midrib (Fig. S2) indicated that these genes were involved in phloem loading in the tissue as sucrose levels fluctuated diurnally (Fig. 2) and AtSWEET16 and 17 are vacuolar hexose transporters (Eom et al. 2015). Expression of *BrSWEET17* resembled that of its Arabidopsis homolog *SWEET17*, which is expressed at relatively low levels in mature leaves (Chardon et al. 2013), but at high levels in the root cortex (Guo et al. 2014). AtSWEET1 is a glucose uniporter localized to the plasma membrane and highly expressed in flowers (Chen et al. 2010); however, *BrSWEET1a/b* was highly and specifically expressed in blade tissue (Fig. S2), implying it may have other functions in Chinese cabbage.

Phloem unloading into sink tissues requires AtSUC1 function in Arabidopsis (Sivitz et al. 2008; Durand et al. 2018), but *BrSUC1* was neither preferentially nor specifically expressed in the midrib. This implied that the midrib was a sink tissue that did not require phloem unloading to obtain sugar from the leaf blade but could obtain it either symplastically (via plasmodesmata) or apoplastically. Despite this observation, expression levels of *BrSUC2* supported the idea that phloem loading might occur in the midrib (Fig. 7).

Genes specifically or preferentially expressed in sinks

Sugar levels regulate source-sink metabolic activities such as photosynthesis, growth, cell cycle, and cell wall synthesis via SnRK1 and TOR. Lower sucrose levels activate SnRK1, which represses TOR and phosphorylates many proteins, whereas higher sucrose levels inactivate SnRK1 and increase levels of hexokinases and TOR, promoting growth (Kim 2019; Rodrigues et al. 2019). Transcript levels of *BrSnRK1.1*, *BrSnRK1.2*, and *BrTOR* did not show obvious differences between tissues, being high in both the leaf blade and midrib (Fig. S4), indicating that either regulation of energy homeostasis was similar in both tissues or regulation occurred at post-transcriptional levels.

In Arabidopsis, most monosaccharide transporters belonging to the *SUGAR TRANSPORT PROTEIN* (*STP*) gene family contribute to the uptake of sugars into sink cells and thus are mainly expressed in sink tissues (Büttner 2010; Poschet et al. 2010). Most *BrSTP*s were expressed in source tissues (Fig. 7), and only expression of *BrSTP4* was slightly high in the midrib. Unlike other STPs, STP7 does not transport hexose sugars but is specific for the pentose sugars l-arabinose and d-xylose. *STP7* expression is high in tissues with a high cell wall turnover, indicating that it contributes to the uptake and recycling of cell wall sugars (Rottmann et al. 2018). *BrSTP7* was preferentially expressed in the leaf blade, indicating that Chinese cabbage has a higher level of cell wall turnover in this tissue than in the midrib. These results suggest that STPs have a variety of functions and/or different roles in different plant species.

In contrast to *SUT1*, *SUT4* (*SUC4*) is localized to the tonoplast (Endler et al. 2006) and expressed predominantly in the minor veins of source leaves (Weise et al. 2000), which require a high-capacity sucrose transporter for phloem loading. *BrSUT4* expression was slightly higher in the midrib than in the leaf blade, indicating that the midrib was involved in sucrose transport. POLYOL/MONOSACCHARIDE TRANSPORTER 5 (PMT5) is a H^+ symporter localized in the plasma membrane that is responsible for sugar uptake into non-photosynthetic sink tissues, such as the root elongation zone, inflorescence stems, floral structures, and the abscission zone (Reinders et al. 2005). *BrPMT5/PLT5* was expressed preferentially or specifically in the leaf blade.

BrINT1, *BrERD6*, *BrUTR6*, *BrHXK3*, and *BrLSH1* were identified as showing preferential or specific expression in the midrib of Chinese cabbage leaves (Fig. 8). *INT1* expression is root-specific in Arabidopsis; it encodes the tonoplast-localized *myo*-inositol transporter, which regulates inositol content and is involved in various metabolic processes including cell wall synthesis (Schneider et al. 2008). Expression of *EARLY RESPONSE TO DEHYDRATION 6* (*ERD6*), which encodes a putative sucrose transporter, is induced by dehydration and cold. *UDP-GALACTOSE TRANSPORTER 6* (*UTR6*) encodes a protein involved in cell wall biosynthesis (Rautengarten et al. 2014). All three genes were specifically or preferentially expressed in the midrib of Chinese cabbage leaves. *AtHXK3* is mostly expressed in the stroma of the plastid, a sink organ (Karve et al. 2008). It is a putative plastid sugar signal sensor (Zhang et al. 2010; Aguilera-Alvarado and Sánchez-Nieto 2017) and phosphorylates

glucose imported into the plastid for biosynthetic processes (Karve et al. 2008). *BrHXK3* was preferentially expressed in the midrib (Fig. 8). Expression of *BrLSH*s and *BoLSH*s is specific to the midrib and veins (Dong et al. 2014), but their functions have not yet been characterized. All these data suggested that the midrib was a sink tissue.

Taken together, the carbohydrate content and gene expression profiles support the idea that a Chinese cabbage leaf is composed of both source (leaf blade) and sink (midrib) tissues. Unlike the situation in roots, the midrib did not depend on phloem unloading for the uptake of sugars but instead functioned in phloem loading of other sink tissues.

Acknowledgements This work was supported by a grant from the Research Fund of Chungnam National University (CNU), Daejeon, Korea, to Yoonkang Hur (2018-1201-01).

Compliance with ethical standards

Conflict of interest The authors have declared that they have no conflicts of interest.

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