THE ROLE OF ORGAN - AND DAYLENGTH - SPECIFIC GENE EXPRESSION IN BULB DEVELOPMENT AND RESOURCE MANAGEMENT IN ONION (ALLIUM CEPA L.)

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Abstract
Onion development is a complex process with each developmental event depending on the adequate supply of resources. Also, these resource components themselves are crucial nutrients in onion when consumed as food. For higher yield and better quality in onion production, it is essential to not only have a high level of photosynthesis but also effective transportation of photosynthetic products. A comprehensive set of spatial and developmental time-course experiments detailed the gene expression in onion leaf and bulb tissues during the development of a long-day onion cultivar under long-day and short-day conditions. Candidates included well characterised functional clock genes in Arabidopsis flowering pathway, as well as some novel sequences differentially expressed in bulb tissues. Both AcFT1 and AcFT4 showed significant increasing expression level during plant development, with AcFT1 being expressed in long-days during bulb formation and AcFT4 in short-days where bulbs are not formed. The data further support the role of AcFT1 as a promoter and AcFT4 as inhibitor in onion bulb formation. A number of genes associated with onion carbohydrate metabolism, sulphur metabolism and bulb development were identified as having different tissue-specific expression and provide targets for future studies.

Keywords. Onion; bulbing; daylength; FT genes; sugar sensing; resource allocation

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1 INTRODUCTION

The common onion varieties are now cultivated over a worldwide geographic range from temperate to tropical regions. In terms of global weight produced, onion presents as second in importance among vegetables, only exceeded by tomatoes (FAO statistics, 2018). Besides its unique flavour, onion has a unique combination of diverse and highly valuable families of phytochemicals: organosulfur, fructans and flavonoids. These families of compounds, working independently or in combination, contribute to several salutary effects on human health as well as, in some cases, helping to provide protection against pests and disease.

Examples of organosulphur compounds include defensins (DFS), which are small peptides that are rich in disulfide-linked cysteines and are widely distributed throughout animals and plants. Plant defensins were firstly isolated from wheat and barley by Mendez et al., 1990, before being studied mainly with regard to their effects in enhancing the ability of plants to counteract pathogens and insects. A second beneficial organosulphur compound is lachrymatory factor (propanthial S-oxide). The enzyme lachrymatory factor synthase (LFS) causes the characteristic pungency of onion when chopped up, giving the savour and the ability to defend against wild animals and microbes.

Plant defense compounds may also be beneficial to humans when they include flavonoids, and health-related polyphenolic natural products (Koes et al., 1994, Gurjar et al., 2012). Onion is the richest source of dietary flavonoids contributing to a large extent to the overall intake. There are two types of flavonoid
subgroups found in onion: the anthocyanins in red and purple varieties, and the flavonols such as quercetin in yellow and brown varieties.

Fructans are long chain polymers of fructose that contribute to human health through multiple mechanisms, despite their resistance to hydrolysis by human digestive enzymes. They are the principal type of carbohydrate storage in onion, making up 80% of the dry matter (Brewster, 1994). In a study of 60 common vegetables, onions are reported to have the highest quantity of fructans (Roberfroid, 2004). Studies show fructans can help maintain gastrointestinal health by sustaining beneficial bacteria (Franco-Robles and López, 2015). Established beneficial properties of fructans also include antifungal, antibacterial, antitumor, anti-inflammatory, antithrombotic and, hypocholesterolaemia (Lanzotti, 2006; Kumari and Augusti, 2007). They may also play a part in treating diabetes as research shows that fructans in onions can lower insulin levels and improve tolerance for glucose (Dey et al., 2002; Liu, 2007).

The target of onion production is a high yield of high-quality product, which is the result of many processes of plant growth and development (Brewster, 2008). Each leaf is composed of a photosynthetic blade and a non-photosynthetic storage leaf base or sheath. The edible bulbs are formed from modified plant leaves (scale leaves) which do not form blades but swell and develop into the storage organ. The change from photosynthetic leaf production to scale and bulb formation is coordinated with source-to-sink carbohydrate reallocation throughout the plant. In onion, the source for bulb is the fructans produced from
sucrose and fructose in the leaf blade before being accumulated in the basal scales (sink) during bulb formation (Pak et al. 1995).

Our aim in this work was to gain an improved understanding of how the different pathways involved in bulb development and composition were co-ordinated during the transition to bulbing by looking at expression across a range of genes with different functions. Twelve candidate genes, including genes involved in leaf to bulb signalling, source and sink activity and phytochemical content were selected and their expression patterns were evaluated under inductive and non-inductive daylengths. AcCOL2 (Rashid and Thomas, 2020) and AcFTs (Lee et al., 2013) were selected for their proposed involvement in the photoperiodic onion bulbing process, based on the well-established parallels with Arabidopsis thaliana flowering pathway.

Two invertase genes (INV, or FFS) were selected, for their putative function in catalysing sucrose hydrolysis to produce hexoses (fructose and glucose) available for cellular activity (Benkeblia et al., 2004). Additionally, three distinct sucrose 1-fructosyltransferase (SST1) genes, coding for the key enzyme that catalyses fructan synthesis from sucrose were selected (see Figure S.1). For organosulfur metabolism, two genes, Lachrymatory factor synthase (LFS) and Defensin (DFS) were included. By exploring the characteristics of these genes, the aim of this work was to gain a better understanding of inter-organ co-ordination of development and resource management during the onion bulbing process.
2 MATERIALS AND METHODS

2.1 Plant material

Seeds of onion (Allium cepa L.) variety “Marco” F1 were obtained from Marshall & Co Ltd, (Cambridgeshire, UK). Marco has a critical daylength requirement of 15-16 h (information provided by seed company) and is thus a LD variety. Marco plants were grown from seed planted Dec 15th 2017 in School of Life Sciences Phytobiology Facility (PBF) at the University of Warwick. At 37 days from sowing (DFS), they were potted up into 7 cm pots and grown on in the PBF with a controlled temperature of 22/18°C day/night and supplementary lighting to give a 16 h daylength. At 100 DFS, when basal swelling was first observed, plants were separated into two groups and transferred to the different daylength treatments. For one group, LD was provided in a compartment with natural daylight (varied from 12h 42min to 15h 24min) plus 16h of supplementary light extension (400W SONT lamps) from 03:00 to 19:00 GMT. For the other group, SD was provided in a compartment fitted with photoperiod blackout blinds to give an 8h daylength from 8:00 to 16:00 GMT, during which they received supplementary light from 400W SONT lamps. Plants in both groups were kept in these condition for 42 days for sampling until the experiment finished.

Sampling was conducted two days after the transfer and at weekly intervals seven times from 102 to 144 DFS in total (Table 1). For each sampling time, five plants were taken from both LD and SD compartments 10 h after the start of the light period (Zeitgeber (ZT)10). Plants for harvesting were selected with a random number generator (Haahr, 2006). Measurements of bulb and neck diameter were conducted using slide callipers and visible leaf number was counted. Bulb initiation was quantified using ‘bulbing ratio’ (maximum bulb diameter/minimum sheath diameter), which is an indication of onion bulbing when the value greater than two (Clark and Heath, 1962).

<table>
<thead>
<tr>
<th>Days from sowing</th>
<th>102</th>
<th>109</th>
<th>116</th>
<th>123</th>
<th>130</th>
<th>137</th>
<th>144</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples for bulbing ratio</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Molecular samples</td>
<td>LB1</td>
<td>LL1</td>
<td>LB2</td>
<td>LL2</td>
<td>LB3</td>
<td>LL3</td>
<td>LB4</td>
</tr>
<tr>
<td></td>
<td>SB1</td>
<td>SL1</td>
<td>SB2</td>
<td>SL2</td>
<td>SB3</td>
<td>SL3</td>
<td>SB4</td>
</tr>
</tbody>
</table>
Table 1 Sampling timepoints and sample names. LB, LL, SB, SL represent long-day bulb, long-day leaf, short-day bulb and short-day leaf, respectively.

2.2 Molecular analysis
At harvest, the middle 1 cm section of the second youngest leaf and a cross section of basal tissue were excised using a scalpel and immediately frozen in liquid nitrogen. Total RNA was extracted from approximately 100 mg of frozen leaf and base tissue from specific daylengths conditions using the Z6 buffer method, following the manufacturer’s (Roche manufacturing Ltd., Republic of Ireland) guidelines. After the isolation, samples were DNase treated using TURBO DNA-free™ (Ambion Inc, Cat. No. AM1907) to eliminate the genomic DNA contamination. The quality and quantity of total RNA were measured with the Thermo Scientific NanoDrop™ 1000 Spectrophotometer (NanoDrop Technologies, Inc., USA). First-strand cDNA was synthesised using 2μg total RNA with ThermoScript™ Reverse transcription polymerase chain reaction System (Invitrogen by Life Technologies, Cat. No. 11146-016) following the manufacturer's guidelines.

2.3 RT-qPCR analysis
The expression of reference and candidate genes was carried out by Real-time PCR quantification using the CFX384 Touch™ Real-time PCR machine from BioRad (Bio-Rad Laboratories Ltd., UK) with the cDNAs obtained. Each sample was run in triplicate and the average CT value calculated. The optimisation and selection of dilution series for making standard curves were conducted (acceptable PCR efficiency between 90-110%, R^2 higher than 0.985 with a unique peak in melt curve). The top three references genes (AcTUA, PP2A1 and UBL) out
of seven tested were chosen by Biogazelle qBase+ software based on the geNorm (Vandesompele et al., 2002) and qBase technology (Hellemans et al., 2007). Normalisation was achieved by dividing the expression of the gene of interest against the expression of the mean of three reference genes at that same sampling-point.

2.4 Gene sequences and primers
The gene sequences were obtained in one of two ways. The genes from Arabidopsis photoperiodic flowering homologs including COL2 and FTs were taken from published papers (Rashid and Thomas, 2019). Others were selected from the onion transcriptome RNA-seq database (Rashid and Thomas, 2019) and putative functions confirmed by BLASTing against the NCBI database. The sequences and q-qRT-PCR primers used are included in the supplementary material. Candidates included invertases (beta-fructofuranosidase, FFS) FFS-1 and FFS-2; sucrose 1-fructosyltransferase (SST1) SST1-B1 (putative bulb specific sequence), SST1-B2 and SST1-L (putative leaf specific sequence); defensins (DFS); and lachrymatory factor synthase (LFS).

2.5 Bioinformatic analysis of the relation between tissue specificity and daylength sensitivity
To interpret biological roles of genes changing expression in response to day length, transcripts were first annotated using TRAPID against a reference of Lilopsidia genomes. The resulting GOterm associations were then tested for enrichment in day-length differential expression groups of genes using hypergeometric test in the goseq package of the R Bioconductor library. BLAST searching of the transcripts against the
NCBI non-redundant database of proteins was also done to find potential functions in more distant homologs.

2.5 Statistical analyses
Means, standard deviations and standard errors were calculated in Microsoft Excel and the significance of the differences was assessed by using factorial analysis of variance (ANOVA) with repeated measures. ANOVA was carried out using statistical software package SPSS.

3 RESULTS
3.1 Bulbing ratio and leaf number:
Measurements of bulbing ratio and leaf number were made as the differences in bulbing responses. Figure 1 shows the bulbing ratio and visible leaf number during plant development in Marco grown in SD and LD. There were slight differences at the starting point for both figures due to the randomization when separating the plants. Plants in which bulbing was just beginning to take place, as judged visually, were preferentially placed in SD.

Along with no obvious change in bulbing ratio, an increase in visible leaf number can be seen after 4 harvests in SD plants (Figure 2), indicating an interrupted bulbing process followed by a restart of leaf production. In LD plants, a clear increase in the bulbing ratio can be seen, coupled with the cessation of leaf production. The apparent reduction in leaf number is because the oldest leaves had senesced and died back. The bulbing ratio for LD plants was less than that of
SD plants at the beginning of the sampling timepoints, but progressively increased after around 127 days from sowing. The reverse was true for leaf number and crossover of the values for visible leaf number between SD and LD occurring at approximately the same time as the bulbing ratios.

Figure 1: Bulbing ratio and visible leaf number after plants transfer. The red arrow indicates the transferring point (2 days before the first sampling date). A indicates SD plants’ bulbing ratio and leaf number; B indicates LD plants’ bulbing ratio and leaf number. Sampling was then conducted at weekly intervals. Error bars represent the SEM (n=5).
3.2 Relative expression of photoperiod genes

Marco plants were grown in LD or SD as described in Table 2 and the expression of target genes was measured by RT-qPCR. *AcCOL2* expression was highly leaf specific and expressed in both daylengths, although at a higher level in the SD treatment (Table S.1). No obvious trend was observed during onion leaf development in either daylength (Figure 2). *AcFT1* was expressed only in LD leaf with increasing transcripts during plant development (Figure 2). The expression of the gene is highly selective between tissues and very sensitive to daylength (Table S.2). The gene showed almost no transcripts in bulb material nor under SD, although foliage leaves continued to be produced. In addition, if the SD expression data is plotted on an expanded scale (Figure S.1), some expression of *AcFT1* can be observed in the newly transferred material, that was just beginning to bulb, but this was sharply reduced after the plants were transferred from LD to SD. *AcFT4* expression was also significantly selective in plant sites and daylengths (Table S.3). It was only expressed in the SD leaf in contrast with *AcFT1*, though also with a rising trend in transcript levels during with *Marco* development (Figure 2). There was no expression of *AcFT4* detected in the basal tissue (leaf bases and scale leaves), and very limited expression in the leaf grown in LD.
Figure 2 Relative expression of AcCOL2, ACFT1 and ACFT4 in Marco leaf and bulb tissue under LD and SD. Error bars represent the SEM. Reference genes were ActUA, PP2A1 and UBL.
AcFT5 mRNA was highly expressed in LD leaf material but very low expression in SD leaf (Figure 3). There was barely any detectable expression in bulb tissues. ANOVA further confirmed AcFT5 expression was significantly affected by daylengths (Table S.4). AcFT6, by contrast to AcFT5, was expressed mostly in the SD leaf material with an increasing pattern during the onion development (Figure 3). Low level of transcripts was presented in basal tissue for both daylengths. The expression of AcFT6 was also significantly affected by daylength and tissue-specific (Table S.5). A reducing amount of mRNA in SD bulb material was observed after the plants being transferred from LD.
3.3 Relative expression of invertases (beta-fructofuranosidase, FFS) genes

The expression of FFS-1 was mostly detected in leaves, but only in the first two harvests (Figure 4) although overall, the difference between leaf and bulb was not statistically significant (Table S.6). FFS-2 expression was detected throughout the plant under both daylengths throughout plant development.
(Figure 4), with significantly more transcripts in bulb tissue (Table S.7). The gene is not sensitive to daylength, and no obvious trend can be seen over time. As FFS-2 was predicted to code for an insoluble invertase, its higher expression in leaf base may be related to thickening of cell walls in sink tissue during onion bulb formation.

Figure 4 Relative expression of FFS-1 and FFS-2 in Marco leaf and bulb tissue under LD and SD. Error bars represent the SEM. Reference genes were ActUA, PP2A1 and UBL.
3.4 Relative expression of sucrose 1-fructosyltransferase (SST1) genes

SST1-B1 was expressed throughout the developmental stages in all tissues under both daylengths (Figure 5). Statistics suggested the gene is not selective between tissues and not sensitive to daylengths treatments (Table S.8). SST1-B2 showed greater abundance in onion bulb materials under both daylengths (Figure 5). ANOVA confirmed the gene is significantly bulb specific (Table S.9). There was no obvious differential expression in response to daylengths. In addition, the gene seemed to have an increasing number of transcripts in all tissues over time, indicating it may have a role in fructan accumulation in both source and sink materials. SST1-L presented a significant leaf-specific pattern with very limited mRNAs detected in the basal tissue (Figure 5). Statistics confirmed the gene is not only tissue selective, but also daylength sensitive with higher expression under SD (Table A.10).
Figure 5. Relative expression of SST1-B1, SST1-B2 and SST1-L1 in Marco leaf and bulb tissue under LD and SD. Error bars represent the SEM. Reference genes were ActUA, PP2A1 and UBL.
3.5 Relative expression of onion organosulphur metabolism genes.

DFS presented a clear bulb-specific pattern visually (Figure 6) and this was confirmed statistically (Table S.11). The transcripts were also detected in leaf material but much lower than in bulb. DFS had no daylength sensitivity nor an accumulation in transcription level (Table S.11). LFS mRNAs were evident throughout basal tissues for both SD and LD presenting a clear increasing trend in both bulb materials over time (Figure 7). It is an indication of the accumulation of its functional protein existing in this part of the plant. The expression was also detected in leaves but much lower than that in bulb. ANOVA showed LFS is tissue specific but not sensitive to daylength (Table S.12).
Analysis of the relation between tissue specificity and daylength sensitivity

It is noteworthy that, when comparing tissue specificity and daylength sensitivity of twelve candidate genes, all daylength sensitive genes showed significant tissue selection, including the sugar metabolism gene SST1-L; whereas the ones with tissue specificity did not necessarily respond to daylength e.g. AcCOL2. We further tested this with the RNA-seq experiment reported in Figure 6.
(Thomas and Rashid 2019). From the total 23280 sequences, only 4.29% of the tissue specific genes were expressed differentially in LD and SD, whilst 68.34% of the daylength sensitive genes were tissue-selective (Table 2). The evidence of organ specific responses to daylength consistent with interorgan signalling between the site of perception in the leaf and response in the bulbing tissues.

<table>
<thead>
<tr>
<th>Tissue selective genes</th>
<th>Leaf: LD-enhanced</th>
<th>Leaf: SD-enhanced</th>
<th>Leaf daylength-insensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulb LD-enhanced</td>
<td>8</td>
<td>3</td>
<td>315</td>
</tr>
<tr>
<td>Bulb SD-enhanced</td>
<td>3</td>
<td>6</td>
<td>334</td>
</tr>
<tr>
<td>Bulb-daylength insensitive</td>
<td>181</td>
<td>148</td>
<td>22282</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Daylength-responsive genes</th>
<th>SD-Bulb</th>
<th>SD-Leaf</th>
<th>SD-tissue selective</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD-Bulb</td>
<td>2375</td>
<td>17</td>
<td>1666</td>
</tr>
<tr>
<td>LD-Leaf</td>
<td>29</td>
<td>5002</td>
<td>2136</td>
</tr>
<tr>
<td>LD- tissue selective</td>
<td>2422</td>
<td>2262</td>
<td>7371</td>
</tr>
</tbody>
</table>

Table 2 Comparison of numbers of the genes with tissue specificity or daylength sensitivity as found by RNA-seq (Rashid and Thomas, 2019). Tissue selective genes are those which show different expression between leaf and bulb. Daylength responsive genes are those that show differential expression in LD and SD.
4 DISCUSSION

4.1 Daylength sensitivity of photoperiod genes and their possible roles in bulb formation

For the genes also found in the Arabidopsis photoperiodic flowering pathway, all the targets other than AcCOL2, showed significant daylength sensitivities and leaf-specific pattern, indicating their role in light perception in sugar source organs. AcCOL2 forms part of the daylength perception mechanism in Arabidopsis and, is also expressed in leaves in both LD and SD (Reference here). AcFT1 was expressed only in the LD leaf material, with a notable increasing trend of transcripts during plant development under inductive daylength conditions. In the expanded scale of expression, a quickly down-regulated AcFT1 mRNA level was observed when plant transferred to non-inductive daylength. By contrast, AcFT4 was expressed only in the SD leaf material, also with an increasing mRNA level during plant growth. The gene was clearly upregulated after the plants were transferred from LD to SD. All these traits of AcFT1 and AcFT4 expression in this time-course experiment can further support their functional role as a promoter and inhibitor in onion bulbing process (Lee et al., 2013).

Additionally, the reverse pattern of AcFT1 and AcFT4 may suggest there is a negative correlation between the two. Besides being a circadian regulator, the FT family has been reported to play extended roles in modifying source-sink interactions. A recent study in potato tuberization suggested that the switch to tuber development may be mediated by the interaction between the tuberization specific FT homolog StSP6A and the sucrose efflux transporter StSWEET11 (Abelenda et al., 2019). The FT homolog is therefore likely to promote symplastic
sucrose transport in potato. The link between the sugar and photoperiodic pathways during tuberization extends the role of proteins of FT family from mobile signals to mediators of source-sink partitioning, which could also be the case in onion, although that would need to be tested.

Additionally, an opposite expression pattern between AcFT5 and AcFT6 was also observed in this experiment. AcFT5 was found mostly in LD leaf while AcFT6 was only expressed in SD leaf, both showed an accumulation of transcripts during maturity at the required daylength. The result indicates they are also daylength regulated in Marco and play a role in leaf tissues under inductive or non-inductive daylength although the functions remain unknown.

4.2 Diversity of invertase-related genes and their specific role in respective organs
In higher plants, the catabolism of sucrose is to allow the subsequent utilization of the component hexoses. This catabolism is mediated by two enzyme activities, invertase and sucrose synthase (Ap Rees, 1988). We chose two invertase related sequences the RNA-seq database. FFS-1 is predicted to be a soluble acid invertase, which means the enzyme functions in the vacuole. The experiment showed the gene was highly expressed in leaf during earlier stage and the mRNA reduced with plant maturity. The reason for its leaf-specific pattern may be that there is a requirement for sucrose catabolism to support heterotrophic tissues (e.g. epidermal cells) or to provide respiratory substrates at night in leaves. In addition, a possible sensing role of invertase in leaves has also been suggested
The mRNA abundance of *FFS-1* is sensed and transduced into altered patterns of gene expression, and further alter patterns of the genes modulating sucrose catabolism in sink tissues. *FFS-2* was predicted to translate an insoluble isoenzyme, meaning it is one type of cell wall-bound invertase. The experiment suggested it is a bulb-specific invertase gene, indicating its role in carbohydrate accumulation at basal tissue during onion bulb formation and growth. It has been suggested in various studies that invertase is closely correlated with growth (Arai et al., 1991) and expansion in many sink tissues (Schaffer et al., 1986). No daylength sensitivities were seen in either *FFS*.

The organ-specific expression pattern of invertase seen in this experiment has been found in many other plant species. In poplar, there are six cell wall invertase genes (*PtCWINVs*) differentially expressed in leaf, stem and root tissue (Chen et al., 2015). In carrots and tomatoes, some cell wall or vacuolar invertase genes showed markedly different organ specific expression patterns with plant development (Sturm et al., 1995; Godt and Roitsch, 1997). This experiment also showed tissue specific invertase activity of *FFS*s, revealing that these genes play an important role in their respective organs in terms of providing carbohydrates for growth and development, and provides a basis for understanding the function of invertases in onion.
4.3 Fructans are synthesised and stored in different specialized organs

Fructans are synthesised and stored in different specialized organs. According to the classical model of Edelman et al. (1968), two enzymes (SST1 and FFT1) are involved in the synthesis of the simplest form of fructan, and this experiment only focused on SST1s (Figure 5). The three SST1s had different tissue-specific sites of expression. SST1-B1 was expressed in all tissues throughout development. SST1-B2 was preferentially expressed in bulb material while SST1-L expression was limited to photosynthetic leaves. The source for onion bulb is the fructans produced from sucrose and fructose then accumulated in sinks, namely basal scales, during bulbing (Pak et al. 1995). There are also fructans in leaf tissues, thus fructans may be translocated to the sink tissues directly through the phloem, or be hydrolysed first, then transported and resynthesized (Sharma et al., 2015). Vijn et al. (1997) cloned SST1 from onion, and found if placing onion leaves under continuous illumination, the excessive sucrose content would induce fructan synthesis and SST1 mRNA accumulation. This experiment provided a supportive result showing a statistically significant daylength sensitivity of SST1-L.

Fructans are often stored in different specialized organs, for example in the taproot of chicory (Cichorium intybus), the tubers of dahlia (Dahlia variabilis), and the bulbs of tulip (Tulipa gesneriana) (Ritsema and Smeekens, 2003). In this experiment, both SST1-B1 and SST1-B2 also were abundant in bulb materials as seen in tulip. Van den Ende et al. (2000) isolated the cDNA for SST1 from Taraxacum officinale, and the data showed the mRNA concentrations of SST1...
were very low in leaves but abundant in young roots. Fructans can also play a role in the expansion of petals. Studies showed petals of daylily (Hemerocallis) and Campanula rapunculoides rapidly degraded fructan during flower opening stage (Bieleski, 1993; Vergauwen et al., 2000). In cereals, fructans temporarily accumulate in stems and early in seed development (Van den Ende et al., 2011; Joudi et al., 2012). Fructans therefore have a range of functions in addition to their role as the major storage carbohydrates in leaves and bulbs in onions.

We also looked at the expression of LFS and DFS, genes involved in organosulfur metabolism. These originally showed up as bulb-specific genes in RNA-seq analysis (Rashid PhD thesis). This was confirmed by qRT-PCR, where both were more highly expressed in the leaf bases or scales and were not daylength-sensitive. As bulbs are a major source of stored nutrients, they are vulnerable to predation by a range of pests and diseases. LFS catalyses the generation of lachyratomory factor during tissue damage (Silvaroli et al., 2017), which would deter predators. DFS genes code for cys-rich peptides that show potent activity against a range of microbial pathogens, including fungi and bacteria (Sathoff et al., 2019) and are proposed to be involved in the defence against pests and diseases (Garcia-Mina, 2012). DFS is commonly expressed in a tissue specific manner, including the peripheral cells of potato tubers and radishes, where they are thought to constitute the first line of defence against pathogens (Broekaert et al. 1995). The tissue specificity of these genes suggests that their role is to help protect the basal storage tissues in onion, which might be particularly vulnerable to soil-borne disease.
5 CONCLUSION

The data in this paper help us to understand how the transition to bulbing involves the coordinated tissue and daylength specific expression of a genes with a range of functions. The daylength-specific expression of AcFT1 and AcFT4 genes in Marco confirms results found in other LD onions. In addition, AcFT5 and AcFT6 were found to have daylength-specific expression unlike in previous studies with Renate (Rashid and Thomas, 2019). This may indicate that there are varietal differences in the daylength responses in LD onions. The results also indicate that genes that are daylength-sensitive tend to be tissue specific. This is not surprising as differential development of leaf and basal tissues is the primary response to daylength. Further studies of these daylength-sensitive genes will help to understand the molecular mechanisms underlying bulb formation.

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