Growth and Gene Expression Related to Bulb Development and Day-length Responses in Onion Cultivars During Overwinter Cultivation

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Onion (Allium cepa L.) is one of the most important vegetable crops in the world, and its cultivation is roughly divided into two types: autumn- (overwinter) and spring-sowing. In this study, we compared the changes in plant growth and bulb development of four short-day and intermediate-day onion cultivars under two years of varying environmental conditions to understand autumn-sowing cultivation and growth characteristics. A comparison of the growth parameters of the four cultivars throughout the growth period revealed that the increase in total leaf number and area, and plant height were almost completely inhibited in winter. In spring, these growth parameters increased rapidly in the early maturing cultivars and reached a plateau depending on the cultivar’s maturity type, as previously shown in spring-sowing cultivation. It is known that AcFT1 and AcFT4 play a key role in the bulb development of cultivated onions. Therefore, we conducted expression analysis of these genes for the four cultivars grown in the field and confirmed that AcFT1 was expressed following the maturity, irrespective of cultivation methods. We also analyzed AcGI expression in leaf blades, and a certain relationship between changes in bulb development and AcGI expression was observed. Correlation analysis of AcFT1 expression and total leaf number and area was conducted, and strong positive correlations were observed. In conclusion, our study demonstrated genetically that leaf number and area are important for inducing onion bulb development.

Key Words: AcFTs, AcGI, Allium cepa L., autumn-sowing cultivation, growth properties.

Introduction

The Allium genus includes approximately 500 species and is important for its economic value and great diversity in various morphological characteristics, particularly in the form of bulbs and rhizomes (Ricrochet et al., 2005; Sengupta et al., 2004). Onion (Allium cepa L.) is one of the most widely produced and consumed vegetable crops worldwide. According to data from the Food and Agriculture Organization of the United Nations (<http://faostat.fao.org/>, Accessed: September 28, 2021), the total global onion production in 2019 was approximately 99.9 million tons, and the main production areas were China, India, the US, Egypt, Turkey, and Pakistan. Bulb size determines the yield, and bulb development is controlled by photoperiod and temperature (Ikeda et al., 2019). To obtain sufficient yield, the sowing dates of onion cultivars are limited by the climate and a short period in spring or autumn (Brewster, 2008b). In overwinter cultivation, early sowing induces many bolters, and late sowing produces small seedlings that cannot survive under cold winter temperatures (Brewster, 2008b). In addition, previous studies on spring-sowing cultivation in the Northern Hemisphere demonstrated that the sowing and transplanting dates affected both bulb diameter and fresh weight, and the bulb size decreased gradually as the sowing and planting dates were delayed (Caruso et al., 2014; Ikeda et al., 2019).

Onion bulb development is controlled by day-length and temperature (Khokhar, 2017); FLOWERING LOCUS T (FT)-like genes related to day-length responses may play a key role in bulb development (Lee et al., 2013; Lyngkhoi et al., 2019; Manoharan et al., 2016; Rashid et al., 2019). The expression of AcFT1, which encodes an FT-like protein for bulb development, is upregulated under long-day lengths and
downregulated under short-day lengths (Lee et al., 2013; Rashid et al., 2019). Another AcFT1, AcFT4, which regulates the initiation of leaf blades and suppression of bulb development, is upregulated under short-day lengths and downregulated under long-day lengths (Lee et al., 2013; Rashid et al., 2019). Expression analysis of AcFT1 and AcFT4 for three cultivars grown in the field with spring-sowing cultivation was conducted in Japan, and the expression of these genes was in accordance with the maturity of the cultivars (Ikeda et al., 2020).

Onion cultivation is roughly divided into two types, autumn- and spring-sowing. Although previous studies have shown that AcFT1 and AcFT4 act in the bulb development of cultivated onions (Ikeda et al., 2020), their expression patterns in autumn-sowing (overwinter) cultivation are unknown. In addition, the relationship between these genes and plant growth is also unclear. In many plant species, such as Arabidopsis thaliana, tomato (Solanum lycopersicum), potato (Solanum tuberosum), and rice (Oryza sativa), day length is sensed by the leaves, and mobile FT protein is transported to the shoot apex or to the tips of the underground stem to induce a flowering or tuberization transition (Navarro et al., 2011). AcFT1 and AcFT4 may be specifically expressed in leaf blades; thus, plant parts and growth, especially the number and area of leaf blades, are hypothesized to be important in understanding onion bulb development.

Therefore, in this study, we investigated various aspects of plant growth, including bulb development, and the expression of AcFT1 and AcFT4 during plant growth throughout the growing period of overwintering cultivation in Japan. We used four onion cultivars with different maturity and suitability characteristics for the experimental field. In Ikeda and Tsukazaki (2021), GIGANTEA (AcGI) was identified as a candidate gene for bulb development using transcriptome (RNA-seq) analysis. Thus, it is possible that onion GI regulates AcFTs, which are located upstream of this gene. GI regulates circadian rhythms and flowering, and acts earlier than FTs in A. thaliana (Mizoguchi et al., 2005). Therefore, in addition to the expression analysis of AcFT1 and AcFT4, we investigated AcGI expression to obtain further insights into the molecular function of the day-length response of onion.

Materials and Methods

Plant materials

Experiments were conducted from 2018 to 2019 and from 2019 to 2020 at the University farm of Utsunomiya University in Tochigi, Moka, Japan (36.49° N, 139.98° E). In total, four commercially grown onion cultivars, ‘Sonic’, ‘Turbo’ (Takii & Co., Ltd., Kyoto, Japan), ‘Kei’ (Tohoku Seed Co., Ltd., Tochigi, Japan), ‘Momiji No. 3’ (Shippo Co., Kagawa, Japan), were used in this study. ‘Sonic’ and ‘Turbo’ are short-day cultivars, while ‘Kei’ and ‘Momiji No. 3’ are intermediate-day cultivars. Seeds were sown on September 11, 2018, and September 12, 2019, in plug trays with 288 cells (20 × 20 × 40 mm) filled with nursery soil containing 700 mg·L⁻¹ of nitrogen, 3,000 mg·L⁻¹ of P₂O₅, 400 mg·L⁻¹ of K₂O (H-700; Yanmar Co., Ltd., Osaka, Japan), and cultivated in a greenhouse until transplanting. Seedlings of each cultivar were transplanted by hand in triplicate plots on November 9, 2018, and November 6, 2019. The andosol field was fertilized with nitrogen, phosphate, and potassium at 0.064, 0.196, and 0.064 kg·m⁻².

Plant growth surveys

Plant growth surveys of each cultivar were conducted for five plants per plot from approximately 30 days after transplanting to plant lodging (i.e., leaves had fallen to the ground). In this study, plant lodging was used as an indicator of bulb maturity and harvesting time. Plant growth surveys were conducted every two weeks from the middle of December to the beginning of March, and every week from the middle of March to the end of cultivation.

Total leaf number (i.e., the integrated number of leaf blades from emergence), leaf area, plant height, leaf sheath, bulb diameter, and fresh and dry weights were measured for every plant before plant lodging. Plants of each cultivar were harvested within seven days after 50% of plants had lodged and naturally dried for more than a week in a dark greenhouse with a ventilator. Dried leaf blades and roots were removed, and the bulbs were cured in a cool and dark room. Subsequently, the final bulb size (diameter, fresh and dry weights, and dry matter content) was measured. Bulbs for dry weight and dry matter content analyses were cut and dried for more than two weeks at 70°C using a ventilation dryer. Leaf area calculation was completed using Image J software (NIH, Bethesda, MD, USA) from scanned images of leaf blades using an image scanner (CanoScan LiDE 400; Canon Inc., Tokyo, Japan). The bulbing ratio was calculated as the proportion between the diameter of the bulb and the leaf sheath of the onion (Brewster, 1982; Kedar et al., 1975; Mondal et al., 1986). Temperature and precipitation data were obtained from the Automated Meteorological Data Acquisition System (AMeDAS) in Tochigi, Moka, Japan (36.28° N, 139.59° E). The day length was calculated from the sunrise and sunset data for Moka, Tochigi, Japan from the National Astronomical Observatory of Japan (<http://eco.mtk.nao.ac.jp/cgi-bin/koyomi/koyomix_en.cgi>).

Expression analysis of AcFT1, AcFT4, and AcGI

Expression analysis of AcFT1, AcFT4, and AcGI was conducted every month for five plants per plot for each of the four cultivars from about five weeks after transplanting (5 WAT; December 13, 2018) to about 29 WAT...
(May 29, 2019). Leaf blades from five plants were sampled from triplicate plots at 1:00 PM on the sampling day. Samples in each plot were composited, homogenized, and stored at −80°C before use. Total RNA was extracted from leaf blades using an RNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands). Genomic DNA was removed from the RNA samples, and reverse transcription was performed using a ReverTra Ace qPCR RT Kit (Toyobo, Co., Ltd., Osaka, Japan). Quantitative real-time PCR (qPCR) was performed using a LightCycler 96 Real-Time PCR System (Roche, Basel, Switzerland) with a THUNDERBIRD SYBR qPCR Mix (Toyobo). The following program parameters were performed: 95°C for 30 s; 40 cycles of 95°C for 15 s, 62°C for 20 s, and 72°C for 60 s. A melting curve was used to confirm the presence of single products. The gene-specific primer sets of AcFT1, AcFT4, and Acβ-tubulin (which was used as a reference for normalization of gene expression) were obtained from Lee et al. (2013). Primer sequences for GIGANTEA (AcGI) were designed using sequences derived from GenBank (accession no. GQ232757) and Primer 3 (version 4.1.0) software (<https://bioinfo.ut.ee/primer3/>). Primer sequences for AcGI expression analysis were 5’-GCCT ACGGTCTTCTCCCTCTAACC-3′ and 5′-AGCAGGGTG GTGTAAAGGAGGTG-3′. The fold changes in gene expression between the three cultivars were determined using the ΔΔCt method of relative quantification.

Statistical analysis
All statistical analyses were performed using Excel (Microsoft, Redmond, WA, USA) and Bell Curve for Excel Version 3.20 (Social Survey Research Information Co., Ltd., Tokyo, Japan). The statistical significance of the results was analyzed with the t-test at the 5% and 1% levels with Bell Curve for Excel Version 3.20.

Results

Ambient temperature and day length during plant growth
The maximum, minimum, and average temperatures, monthly precipitation, and day length from transplanting (November 9, 2018, and November 6, 2019) to the end of cultivation (June 18, 2019, and June 5, 2020) are shown in Figure 1. Precipitation in both years showed differences compared to the average year in November and January; however, the environmental conditions of both years in the experimental fields were almost the same as the average year except in these months.

Plant growth and bulb size at harvest
Plant lodging, harvest and investigation dates, plant growth, and bulb size at harvest are shown in Table 1. The same pattern of lodging was observed in both years; the four commercially used onion cultivars were lodged in the following order: ‘Sonic’, ‘Turbo’, ‘Kei’, and ‘Momiji No.3’. The total leaf number of early maturing cultivars was smaller than that of later maturing cultivars, and the order of plant lodging, while the total leaf number was almost identical. In contrast, there was no relationship between the order of plant lodging, leaf area, and plant height. Bulb diameter and fresh and dry weight were almost the same among cultivars and were not related to cultivar maturity. However, the dry matter content of early maturing cultivars tended to be lower than that of later maturing cultivars.

Changes in plant growth and bulb size in the field
Plant growth and bulb development of ‘Sonic’, ‘Turbo’, ‘Kei’, and ‘Momiji No.3’ during growth in 2018–2019 were observed from transplanting to lodging (Fig. 2). The total leaf number (Fig. 2A), leaf area (Fig. 2B), plant height (Fig. 2C), bulb diameter (Fig. 2D), bulb fresh (Fig. 2E), and dry weights (Fig. 2F) were almost the same among the cultivars. Approximately 27 WAT (May 15), most early maturing ‘Sonic’ cultivars stopped initiating new leaf blades (Fig. 2A). Leaf area (Fig. 2B) and plant height (Fig. 2C) were almost the same among cultivars in the middle phase of growth; however, the final sizes were not relat-

<table>
<thead>
<tr>
<th>Year</th>
<th>Cultivar</th>
<th>Lodging(^z)</th>
<th>Harvest and investigation(^y)</th>
<th>Plant size (including bulb)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total leaf number(^a)</td>
<td>Height (cm)</td>
</tr>
<tr>
<td>2018–2019</td>
<td>‘Sonic’</td>
<td>May 20</td>
<td>May 27</td>
<td>12.0 ± 0.2 c</td>
</tr>
<tr>
<td></td>
<td>‘Turbo’</td>
<td>May 28</td>
<td>Jun. 6</td>
<td>15.0 ± 0.3 b</td>
</tr>
<tr>
<td></td>
<td>‘Kei’</td>
<td>Jun. 1</td>
<td>Jun. 10</td>
<td>15.1 ± 0.3 b</td>
</tr>
<tr>
<td></td>
<td>‘Momiji No. 3’</td>
<td>Jun. 10</td>
<td>Jun. 18</td>
<td>16.4 ± 0.3 a</td>
</tr>
<tr>
<td>2019–2020</td>
<td>‘Sonic’</td>
<td>May 8</td>
<td>May 15</td>
<td>14.3 ± 0.4 c</td>
</tr>
<tr>
<td></td>
<td>‘Turbo’</td>
<td>May 22</td>
<td>May 28</td>
<td>16.6 ± 0.3 ab</td>
</tr>
<tr>
<td></td>
<td>‘Kei’</td>
<td>May 28</td>
<td>Jun. 5</td>
<td>16.5 ± 0.5 b</td>
</tr>
<tr>
<td></td>
<td>‘Momiji No. 3’</td>
<td>May 30</td>
<td>Jun. 5</td>
<td>17.8 ± 0.3 a</td>
</tr>
</tbody>
</table>

\(^z\) Lodging date is defined as when leaves have fallen on more than half of the plants in the experimental field of a cultivar.

\(^y\) Plant growth and bulb size were investigated within 7 days after lodging.

\(^a\) The integrated number of leaf blades from emergence.

\(^*\) Values indicate means ± SE (n = 10), and values with the same letter were not significantly different at P < 0.01 (Tukey-Kramer test).

Changes in AcFT1, AcFT4, and AcGI expression during plant growth

AcFT1 and AcFT4 expression in leaf blades was determined during plant growth in 2018–2019 (Fig. 3). The expression of AcFT4 was high until 13 WAT (February 7) in ‘Sonic’, and until 21 WAT (April 3) in other cultivars. Thereafter, the expression of AcFT4 in ‘Sonic’ gradually decreased from 17 WAT onward (March 7), whereas the expression of AcFT1 increased gradually from 13 WAT (February 7) (Fig. 3A). The expressions of AcFT4 were evident by 21 WAT (April 13) in ‘Turbo’ and ‘Kei’, by 25 WAT (May 1) in ‘Momiji No. 3’; however, these expressions were barely detectable from the next stage, and the expression of AcFT1 gradually increased (Fig. 3B–D). The expression of AcFT1 in ‘Sonic’ reached a peak at 25 WAT (May 1) and in ‘Turbo’, ‘Kei’ and ‘Momiji No. 3’ at 29 WAT (May 29). The expression of AcGI in the leaf blades was also determined in 2018–2019 (Fig. 4). AcGI expressions were almost the same from 5 WAT (December 13) to 25 WAT (May 1), and increased in ‘Kei’ and ‘Momiji No. 3’ at 29 WAT (May 29); however, no distinct correlation between changes in the same pattern of plant growth and bulb development was also observed in 2019–2020 (Fig. S1).

Fig. 2. Total leaf number (A), leaf area (B), plant height (C), bulb diameter (D), bulb fresh weight (E), and dry weight (F) of four onion cultivars in 2018–2019.
Fig. 3. Relative levels of transcripts of AcFTs during plant growth of ‘Sonic’ (A), ‘Turbo’ (B), ‘Kei’ (C), and ‘Momiji No. 3’ (D). AcFT1 and AcFT4 expression were determined by quantitative real-time PCR and normalized against β-tubulin expression. Values indicate means ± standard error (SE; n = 3). The bulbing ratio was defined as the proportion between the diameter of the bulb and leaf sheath; a bulbing ratio exceeding 2 indicates bulbing. ‘Sonic’ (A) in 29 WAT were not sampled for gene expression analysis.

Fig. 4. Relative levels of transcripts of AcGI genes during plant growth of ‘Sonic’ (A), ‘Turbo’ (B), ‘Kei’ (C), and ‘Momiji No. 3’ (D). AcGI expression was determined by quantitative real-time PCR and normalized against β-tubulin expression. Values indicate means ± standard error (SE; n = 3). The bulbing ratio was defined as the ratio between the diameter of the bulb and leaf sheath; a bulbing ratio exceeding 2 indicates bulbing. ‘Sonic’ (A) in 29 WAT were not sampled for gene expression analysis.

bulbing ratio and AcGI expression was observed for ‘Sonic’.

Correlation between gene expression and plant growth

Correlation analysis between AcFT1 expression and plant growth (i.e., total leaf number and leaf area) was performed to reveal the important growth factors for bulb development. AcFT1 expression increased linearly with increasing total leaf number and leaf area, suggesting strong positive correlations (Fig. 5). The correlations in ‘Sonic’ and ‘Momiji No. 3’ were lower than in the other cultivars, whereas the AcFT1 expression level was positively correlated with leaf area. Correlation analyses between AcFT1 expression level and leaf number in all cultivars were also conducted, and highly positive correlations were observed (Fig. S2A). However, the correlation between AcFT1 expression level and leaf area was lower than that for leaf number (Fig. S2B).

Discussion

In the Northern Hemisphere, onion cultivation is roughly divided into two types: autumn- and spring-sowing. Some studies have investigated changes in plant and bulb sizes during growth. Kato (1963) and Brewster (1982) investigated bulb development during growth, but they used only one or two cultivars. Ikeda et al. (2020) investigated leaf number and area, plant height, leaf sheath and bulb diameter, and bulb fresh and dry weights throughout the growth period; however, these onions were sown in spring. Therefore, we evaluated plant growth and bulb development of different maturity types of four cultivars throughout the growing period to understand growth in the autumn-sowing (overwinter) cultivation.

In this study, the total leaf blade number of early maturing cultivars was smaller than that of later maturing cultivars (Table 1), as also shown by Ikeda et al. (2020) in spring-sowing cultivation. The number of total leaves and inner leaves that constituted the bulbs was lower in early maturing cultivars than later maturing cultivars (Yamasaki et al., 2015). While the number of inner leaves was not investigated in this study, the...
total leaf number of early maturing cultivars was lower than later maturing cultivars (Table 1), in agreement with Yamazaki et al. (2015). Therefore, early maturing cultivars may have fewer scale leaves, including inner leaves, than later maturing cultivars. Bulb diameter and fresh weight in each cultivar were nearly identical, and the dry matter content of early maturing cultivars was lower than that of later maturing cultivars (Table 1). This may be attributed to differences in the pattern of bulb development between early maturing and later maturing cultivars, with early maturing cultivars absorbing more moisture and later maturing cultivars forming more scale leaves.

When bulb development is induced, leaf blade formation stops and switches to bulb scale formation at the onion shoot apex (Brewster, 1982, 2008a). Therefore, plant growth was observed throughout the cultivation period. In this study, most short-day cultivars ‘Sonic’ stopped initiating new leaf blades, and leaf number, leaf area, and plant height reached a plateau by early May (26 WAT) (Fig. 2A–C). Subsequently, exponential increases in bulb size were observed (Fig. 2D–F). This was followed by the other cultivars in which plant growth stopped in mid to late May, and shifted to bulb formation. A similar growth pattern has also been observed in spring-sowing cultivation (Ikeda et al., 2020), indicating that the plant growth of onion cultivars was uniform irrespective of cultivation method. Plant growth almost stopped by early April in overwinter cultivation; however, no such growth stagnation has been reported in spring-sowing cultivation (Ikeda et al., 2020). This may be explained by the low temperature in winter inhibiting plant growth, and increasing temperatures in spring stimulating plant re-growth.

Onion bulb development is controlled by day length and promoted by long-day conditions. During the process of changing from vegetative growth to bulb development, onions stop leaf blade initiation and transition to bulb scales (Brewster, 1982). FT-like proteins in onions play a key role in bulb development; long-day lengths that are longer than the critical day length for onion bulb development enhance the expression of AcFT1, and downregulate the expression of AcFT4, which initiates leaf blades and inhibits bulb formation (Lee et al., 2013; Rashid et al., 2019). A subsequent study demonstrated that AcFT1 and AcFT4 in three cultivars grown in the field with spring-sowing cultivation were expressed following cultivar maturity, and revealed that onion responds to long-day length for bulb development with a bulbing ratio smaller than 2; a bulbing ratio exceeding 2 indicates bulbing (Ikeda et al., 2020). Although onion cultivation is roughly divided into two types, the expression patterns of AcFTs in overwinter cultivation remain unclear. Therefore, we examined the expression of AcFT1 and AcFT4 in several onion cultivars throughout the growing period of overwintering cultivation.

After transplantation to the field and while the bulbing ratio was smaller than two, the expression of AcFT4 was high in all four cultivars (Fig. 3). Expression of AcFT4 is induced by short-day length, initiates leaf blades, and inhibits bulb development (Lee et al., 2013). Therefore, in this study, AcFT4 expression may have been induced by a day length shorter than the day length for bulb development according to a previous study conducted by Lee et al. (2013), and this high expression of AcFT4 may inhibit bulb formation. During spring, the temperature and day length increase, and AcFT1 expression also gradually increases in contrast to AcFT4 expression (Fig. 3). A previous study also investigated the gene expression for different maturity types of three onion cultivars in spring-sowing cultivation and showed that the AcFT4 expression was in accordance with the maturity of the cultivars (Ikeda et al., 2020). Therefore, our results showed that AcFT1 was expressed following cultivar maturity, irrespective of the cultivation method. In addition, AcFT1 expression started before the bulbing ratio exceeded 2 (Fig. 3), and this phenomenon was also observed in spring-sowing cultivation (Ikeda et al., 2020). Thus, our study supports the previous study that onion responds to a critical day length for bulb development, starts bulb development before the bulbing ratio exceeds 2, and the maturity of cultivars and initiation of bulb formation can be determined by AcFT1 expression, irrespective of cultivation methods.

AcGI expression in leaf blades was examined, and it
increased in ‘Turbo’, ‘Kei’, and ‘Momiji No. 3’ at 29 WAT (May 29) when the bulbing ratio greatly exceeded 2 (Fig. 4B–D). Rashid and Thomas (2020) reported that AcGI showed diurnal expression patterns consistent with photoperiod sensing and regulation of AcFT1, and their findings suggested the involvement of AcGI in the daylength regulation of bulb development. Therefore, our results support the hypothesis that AcGI may be associated with day-length responses and bulb development in onion cultivars. However, no distinct correlation between changes in bulb development and AcGI expression was observed in the ‘Sonic’ cultivar (Fig. 4A). The interval of sampling times (four weeks) may have affected the results; AcGI expression in ‘Sonic’ may have been highest at 28 WAT (May 22). Therefore, further studies on the mechanisms affecting AcFT1, AcFT4, and AcGI expression are necessary to understand the genetic pathway of onion bulb development.

Several studies, including ours, have shown that AcFTs are expressed in leaf blades (Ikeda et al., 2020; Lee et al., 2013; Rashid and Thomas, 2020; Rashid et al., 2019) and that leaf area may be important for onion bulb development (Kato, 1965). Accordingly, to identify the important vegetative parts for bulb development, we conducted a correlation analysis of AcFT1 expression and total leaf number and area. Here, AcFT1 expression increased linearly with increasing total leaf number and area, showing strong positive correlations (Fig. 5), and these positive correlations were observed regardless of the cultivar (Fig. S2). Therefore, it is important to maintain a suitable leaf number and area for each cultivar to induce bulb development that follows an increase in AcFT1 expression. Indeed, leaf number is closely related to bulb size (Ikeda et al., 2019), and our present study supports this result genetically. The correlation between AcFT1 expression and total leaf number and area appears to be related to the lodging date. In our study, total leaf number and area in 2018–19 at harvest were greater than in 2019–20 (Table 1). We did not investigate the AcFT1 expression in 2019–20; however, the increase in total leaf number and area in this year may have induced AcFT1 expression and bulb formation. Increases in total leaf number and area in 2019–20 were higher than in 2018–19 (Fig. S3), and this difference may have resulted in the variation in lodging date (Table 1).

In this study, we investigated the changes in plant growth and bulb development of four onion cultivars to understand their growth during overwinter cultivation. A comparison of the cultivars throughout the growth period revealed the basic growth pattern of overwintering cultivation. Gene expression analysis revealed that AcFT1 was expressed following cultivar maturity, as shown in the spring-sowing cultivation. Correlation analysis of AcFT1 expression and total leaf number or leaf area indicated that leaf number and area are important for AcFT1 expression, which induces bulb development. We also assessed AcGI expression, and a correlation of changes in bulb development and AcGI expression was observed to some extent; however, further studies are necessary to reveal the genetic pathways involved in onion bulb development. Recently, CONSTANS-LIKE (COL), FLAVIN-BINDING, KELCH REPEAT, F-BOX PROTEIN 1 (AcFKF1), and TERMINAL FLOWER1 (TFL1) were newly identified in onion (Dalvi et al., 2019; Rashid and Thomas, 2020), and expression analysis of these genes will probably provide a breakthrough in the study of onion bulb development.

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