RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
54 (1) 88-98, 2022
http://doi.org/10.54910/sabrao2022.54.1.9
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

CHARACTERIZATION AND EXPRESSION OF THE PARTIAL *FLOWERING LOCUS T*-2 GENE IN THE SHALLOT (*ALLIUM CEPA* VAR. *AGGREGATUM*) CULTIVAR 'LOKANANTA'

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SUMMARY

Shallot (*Allium cepa* var. *Aggregatum*) is an economically important nutritive vegetable and medicinal plant. Given their low seed production, shallots are vegetatively propagated by using bulb material. Flowering is essential for transferring important traits, such as resistance to *Fusarium oxysporum* and tolerance to salinity. However, the flowering abilities and times of shallot cultivars are very diverse. Therefore, studying the mechanism and regulatory genes of flowering is mandatory. The *AcFT*2 gene has a significant correlation with flowering in shallots. The present research aims to obtain information on the gene sequence, relative expression, and correlation of *AcFT*2 with flowering in shallots under vernalized and nonvernalized conditions. This study was conducted from August 2019 until September 2019 at the Center for Tropical Horticulture Studies, IPB University, Tajur, Bogor, Indonesia. Gene isolation and expression analyses were conducted from October 2019 to July 2020 at the Horticulture Laboratory, Gifu University, Japan. The shallot cultivar 'Lokananta' was subjected to vernalization at 8 °C for 6 weeks under nonvernalization treatment and then planted for 30 days for gene isolation. The *AcFT*2 gene sequence generated from the shallot cultivar 'Lokananta' was analyzed by using Geneious, MUSCLE, and Molecular Evolutionary Genetics Analysis. Gene expression was analyzed via qRT-PCR. Results showed that the *AcFT*2-like gene obtained from shallot had high homology with other FT genes from other plants, especially plants in the *Allium* genus. The shallot cultivar 'Lokananta' showed relatively similar expression as the partial shallot *AcFT*2-like gene under vernalization and nonvernalization treatments given that the number of umbel flowers did not significantly differ between both treatments.

Keywords: Vernalization and nonvernalization, qRT-PCR, *AcFT*2, umbel flower

Key findings: In the shallot cultivar 'Lokananta', the shallot *AcFT*2-like gene contains key amino acids for flowering activity. Its expression patterns are similar under vernalization and nonvernalization conditions. This discovery is useful for further research work on the functions of other *AcFT* genes under different flowering conditions.

Communicating Editor: Dr. Aris Hairmansis
Manuscript received: July 6, 2021; Accepted: January 4, 2022.

To cite this manuscript: Fairuzia F, Sobir, Ochiai M, Maharijaya A, Yamada K (2022). Characterization and expression of the partial *flowering locus t*-2 gene in the shallot (*Allium cepa* var. *Aggregatum*) cultivar 'Lokananta'. *SABRAO J. Breed. Genet.* 54(1): 88-98. http://doi.org/10.54910/sabrao2022.54.1.9

INTRODUCTION

Shallot (*Allium cepa* var. *aggregatum*) belongs to the Allium family. It has smaller bulbs than the common onion. It is an essential nutritional and medicinal vegetable crop that is consumed and cultivated in Asia and Africa but is less commonly cultivated worldwide (Assefa *et al.*, 2016; Major *et al.*, 2018). China, India, and Pakistan are the largest onion (common onion, shallot, and green onion) producers. These countries produce more than 20 million tons of onions and account for 84% of the total onion production. Other onion-producing countries include Japan, South Korea, Bangladesh, Indonesia, and New Zealand (FAOSTAT, 2020).

Shallot has high resistance to several diseases, such as anthracnose and *Fusarium* basal rot, and tolerance to salinity (Aprilia *et al*., 2020; Syamsiah *et al*., 2020). Farmers mostly use bulbs for shallot propagation. Propagation with true shallot seed (TSS) has many uses and advantages over vegetative propagation. These advantages include disease-free seed, low seed volume needed, highly accessible transportation, low production costs, high production, and large and pathogen-free bulbs (Palupi *et al*., 2017; Askari-Khorasgani and Pessarakli, 2019). However, some shallot cultivars, such as 'Rubaru' and 'Palasa', do not produce flowers (Idhan *et al*., 2015). Other shallot cultivars need different treatments to initiate flowering (Marlin *et al*., 2018). These treatments include the addition of GA3, vernalization, and exposure to long photoperiods (Sopha *et al*., 2014; Pramukyana and Den-Respatijarti, 2018; Nemtinov *et al*., 2021). Differences in flowering abilities and times complicate crossing genotypes with each other and introgressing new traits through TSS production in breeding programs. Therefore, knowledge on the flowering mechanism and genes in various shallot cultivars is important for shallot breeding and TSS production.

Flowering transition is the change in phase from vegetative to reproductive through a mechanism involving flowering regulators that are driven by endogenous and exogenous factors. These factors, such as photoperiod, ambient temperature, and vegetative growth, promote the transition of flowers from a vegetative shoot apical meristem into a flowering meristem that forms flower buds during plant development (Liu *et al*., 2015; Pasriga *et al*., 2019). The five main flowering pathways in *Arabidopsis thaliana* have been identified, i.e., the photoperiod, vernalization, autonomic, gibberellin (GA), and age pathways (Liu *et al.,* 2015). The genes involved in the flowering pathways in *A. thaliana* have also been identified on the basis of signal transduction sequences, i.e., *MAD-box*, *F-box*, *constant* (CO), *flowering locus T*, *flowering locus D* (FD), *suppression of constant 1* (SOC1) or *agamous like*, *PETALA*, *LEAFY* (LFY), and *flowering locus C* (FLC) (Yang *et al.,* 2016).

The *FT* gene encodes for the flowering hormone in *A. thaliana*, and its orthologs are found at almost all plant levels (Lee *et al.,* 2013; Zheng *et al.,* 2016). The *FT* gene is part of the phospatidylethanolamine-binding protein domain (*PEBP*) family, which mainly acts as a flowering activator; it is produced in the leaves (phloem) and transported to the shoots to produce flowering shoots (Pasriga *et al.,* 2019). In transgenic plants, the overexpression of the *FT* gene causes early flowering under short- and long-day conditions. Mutants of the *FT* gene cause a delay in flowering. These mutants include *RFT*1 and Heading3a (*Hd3a*) mutants in rice (Zhu *et al.,* 2017; Pasriga *et al.,* 2019) and ZCN in maize (Castelletti *et al.,* 2020). The *FT* gene in *A. thaliana* (*AtFT*) works in the presence of light to produce *FT* transcripts under long days and low temperature (vernalization), as well as the involvement of other factors in the leaves (Adeyemo *et al.,* 2017).

The *FT* genes that have been discovered in the common onion (*A. cepa*) line CUDH2150 are *AcFT*1, 2, 3, 4, 5, 6, and 7. *AcFT*1 is known to participate in bulb formation. Bulb formation is suppressed by preventing the up-regulation of *AcFT*1, and the *AcFT7* gene is involved in bulb formation and maturation (Lee *et al.,* 2013; Tagashira and Kaneta 2015; Manoharan *et al.,* 2016; Lyngkhoi *et al.,* 2019). In the double-haploid onion line CUDH2150, exposure to cold temperatures (vernalization) triggers the increased activity of *AcFT*2 gene expression, leading to flowering initiation. *AcFT*2 is highly expressed on long and short days (Lee *et al.,* 2013).

However, some other past studies reported different functions for *AcFT*2 (Lee *et al.*, 2013). Dalvi *et al.* (2016) stated that in the short-day onion cultivar 'JISL-5', the *AcFT*2 gene is strongly expressed in the final stages of tuber formation. Research on onions planted under short- and long-day conditions showed that *AcFT*2 gene expression is low until 60 days after transplanting (DAT) and increases at 75 DAT (Lyngkhoi *et al.,* 2019). The said gene was not detected in the long-day onion cultivar 'Renate'. *AcFT*2 was detected in the short-day cultivar 'Hojem', in which its expression was

similar to that during tuber development and maturity under long-day conditions; however, the function of the *AcFT*2 gene remains unknown (Rashid *et al.,* 2019).

The vernalization treatment to trigger flower production has been conducted on several shallot cultivars, and the flowering gene has been identified as *LFY* (Yang *et al.,* 2016; Palupi *et al.,* 2017; Marlin *et al.,* 2018). *AcFT* genes, especially *AcFT*2, have been studied in other *Allium* families, such as *Allium cepa* or the common onion (Lee *et al.* 2013; Manoharan *et al.* 2016; Lyngkhoi *et al.* 2019). However, this gene in local Indonesian shallot cultivars, especially in the shallot (*Allium cepa* var. *aggregatum*) cultivar 'Lokananta', a local Indonesian shallot cultivar that easily produces flowers and has a large bulb size (Saidah *et al.,* 2019; Wijoyo *et al.,* 2019), needs further investigation. Therefore, understanding the bulb and flowering gene, namely, *FT* 2 locus, and its expression in the shallot cultivar 'Lokananta' is essential for breeding purposes. The present study aims to obtain information on the existence of the *AcFT*2 gene sequence and to study the quantitative expression of the *AcFT*2 gene in the shallot cultivar 'Lokananta' under vernalization and nonvernalization treatments.

MATERIALS AND METHODS

Plant material and growth conditions

The shallot cultivar 'Lokananta' used in this work was from the collection from the Center of Tropical and Horticulture Study (PKHT), IPB University, Tajur, Bogor Indonesia. The shallot bulbs were subjected to nonvernalization treatment or to vernalization treatment at 8 °C for 6 weeks in cold storage (Marlin *et al.,* 2018). Shallot cultivation was conducted at the experimental garden of PHKT, IPB University, Tajur, Bogor, Indonesia from August until September 2019. Gene isolation and gene expression analysis were conducted from October 2019 to July 2020 at the Horticulture Laboratory, Gifu University, Japan. Shallot samples for gene isolation and quantitative real-time polymerase chain reaction (qRT-PCR) analysis were taken from the basal part of the leaves close to the apical meristem area or the central bud in plants that have not flowered yet (Lee *et al.,* 2013).

Samples were taken from leaves near the central bud. The leaf samples were confirmed to not produce inflorescence at 30 days after planting (DAP) because *FT* genes are expressed in the leaves and transferred to the shoot apical meristem (Shimazaki *et al.,* 2007; Taoka *et al.,* 2013). The leaves were immediately preserved in RNA-later solution for 2 days (Sigma Aldrich, Japan), then stored in a freezer at −50 °C before isolation to maintain the activity of the *FT*2 gene as a florigen in protein transport from the phloem to the shoot apex. The average temperature and photoperiodicity (day length) during planting are presented in Table 1. The *FT2* genes in the shallot cultivar 'Lokananta' under nonvernalization and vernalization treatments were isolated and subjected to gene expression analysis. The flowering rate and umbel number were counted at 30 DAP.

Table 1. Weather conditions during shallot cultivation in Bogor, Indonesia.

Parameter (average)	2019 (Year)	
	August	September
Temperature (°C)	21.00	21.70
Day length (h)	11.53	12.31

Source: BMKG, 2020.

Isolation of the partial *FT*-2 gene in the shallot cultivar 'Lokananta'

The total RNA from the shallot cultivar 'Lokananta' was extracted by using TRIzol® reagent in accordance with the manufacturer's protocol (Invitrogen, USA). RNA concentration and quality were measured by Nanovue (GE Healthcare, UK). RNase-free DNase treatment was carried out by using a recombinant DNase I (RNase-free) kit (Takara Bio Inc., Japan) to remove DNA contamination. cDNA synthesis was conducted with PrimeScriptTM RT Master Mix Perfect Real-Time (Takara Bio Inc., Japan). Gene isolation was performed by utilizing KOD FX Neo reagent (Toyobo, Japan) through PCR. The stages of PCR were performed by applying a TAKARA PCR machine (Takara Bio Inc., Japan) and included predenaturation at 94 °C for 2 min, 40 cycles of denaturation at 98 °C

Table 2. Primer pairs for gene isolation and qRT-PCR assay.

Primers	Sequences (5'–3')	Target (bp)
Gene isolated		
AcFT2	F:CGGTTGGGTAGAGTAGTGGG R:TAAAGAGCAGCAACCGGAGA	455
qRT-PCR		
*AcFT*2-like shallot	F:GAATTGAAATTGGCGGCGGT R:CTGGAAGCACAAGTGCAAGC	143
Ac Tubulin	F:GTCTTCAGAGGCAAGATGAGCAC R:TCAGTCCAGTAGGAGGAATGTCG	138

for 10 s, annealing 60 °C for 30 s, and extension at 68 °C for 10 s. The primers used and their target products are presented in Table 2. The primer pairs were designed with Primer 3.0 online software (http://primer3.ut.ee/) on the basis of the conserved sequence of the *FT*2 genes. Electrophoresis was conducted for 30 min with 2.5% 100 V agarose gel in 1× TBE solution. The gel's target product was purified by using Nucleospin gel PCR Clean-up (Macherey-Nagel GmbH & Co, Germany). The sequences were sent to the Genomic Research Center, Gifu University. The size of the target product was 455 bp. Sequence data from forward and reverse primers were edited and assembled with Geneious 11.0.4 software. Ambiguous bases were adjusted manually by comparing chromatogram data. Partial sequence results were BLASTed with NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Retrieved data were then aligned by using MUSCLE (https://www.ebi.ac.uk/Tools/msa/clustalo/), and phylogenetic trees were constructed through the neighbor-joining method with Molecular Evolutionary Genetics Analysis software. A total of 1000 bootstrap repetitions were used to evaluate the degree of agglomeration in phylogenetic trees.

Expression studies on the shallot *AcFT*2-*like* gene

qRT-PCR assays

The target gene primer pair was designed with Primer 3.0 online software (http://primer3.ut.ee/) from the shallot *AcFT2*-like sequence resulting from the previously isolated *AcFT*2 gene. The primer pair for the housekeeping genes, namely, *AcTUB* (AA451529), was used in accordance with Rashid *et al.* (2019) as an internal reference to normalize expression data. Both primer pairs are presented in Table 2. The qRT-PCR assay was performed with a 12.5 µL mixture of Prime script SYBR Green I master mix (Takara Bio Inc., Japan) consisting of 6.5 µL of SYBR Premix Ex Taq II (2×), 0.5 µL of 10 µM primer mix (final concentration, 0.2 µM), 2.5 µL of cDNA solution, and 3 µL of dH_2O. A Takara Dice Real-Time machine (Takara Bio Inc., Japan) was used to perform qRT-PCR with an initial denaturation step at 95 °C for 3 min, followed by 40 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and extension at 72 °C for 5 min. Gene expression data were evaluated by using standard deviations with three biological replicates (Zhou *et al.,* 2018). The data were used only when the melting curve produced one peak, and the standard curve's efficiency was between 80%–120%.

RESULTS

Bioinformatics analysis of the partial putative shallot FT-2 gene

The 447 bp specific DNA fragments were amplified from the cDNA samples of the shallot cultivar 'Lokananta' (Table 3). However, the partial sequences of the shallot *AcFT2*-like gene were obtained without a start codon (methionine). In the data retrieved from NCBI blastn, the partial *AcFT2*-like shallot gene nucleotides shared 100% homology with the reference gene from the *A. cepa* line CUDH2150 (KC485349.1) and 72%–95% homology with the genes from another *Allium* genus and other plants (Table 4). Therefore, the partial nucleotides of the shallot *AcFT2*-like genes are highly conserved among different *Allium* genera. A partial *AcFT*2-like gene was identified and named in accordance with the shallot's cultivar identity (Figure 1, Table 4).

The further analysis of the phylogenetic tree showed that the shallot *AcFT*2-like gene from 'Lokananta' grouped with the *AcFT*2 gene of the *A. cepa* CUDH5120 line (Figure 2). The said findings were supported by the high homology between the *AcFT*2 nucleotide

Table 3. Partial *AcFT*2 gene obtained from the shallot cultivar 'Lokananta'.

Partial *AcFT*2 gene	Nucleotide (bp)	Amino acid (aa)
'Lokananta'	447	148

Table 4. Sequence identity between the predicted amino acid sequence of the partial shallot *FT*2 gene and the *FT*2 amino acid sequence in Genebank.

Partial *AcFT*2-like shallot gene	Accession in GeneBank			
	FT2 *A. cepa* CUDH5120 line (AGZ20208.1)	Flowering locus T *A. sativum* (AKJ54481.1)	Hd3A *A. officinalis* (XP_020267911.1)	FT-like protein *A. cepa* (AGO81838.1)
'Lokananta'	100%	93.24%	75.51%	72.79%

Figure 1. Nucleotide alignment of the putative partial *AcFT*2 gene from the shallot cultivar 'Lokananta' and the *AcFT*2 gene from the common onion line CUDH5120.

sequence of the shallot cultivar and that of the reference gene *AcFT*2 of the *A. cepa* CUDH5120 line. The involvement of the shallot *AcFT*2-like gene in flowering activity was revealed by its amino acid sequence alignment with the amino acid sequences of five shallot cultivars and plants from the genus *Allium*, such as *A. cepa* and *Allium sativum*, and *Hd3*a from *Asparagus officinalis*.

Amino acid alignment also revealed that the shallot *AcFT*2-like gene has key conserved protein residues and motifs related to *FT* gene function and a residual 14-3-3 protein that links to the PEBP protein (Figure 3). The conserved amino acid motif present in flowering genes, such as DPDxP, GxHR, and L/IYN, were present in the amino acid sequence of the shallot *AcFT*2-like gene from 'Lokannata'. The *FT*-like gene key amino acids that determine function were also found. They were tyrosine (Y) at 85 bp and glycine (Q) at 140 bp.

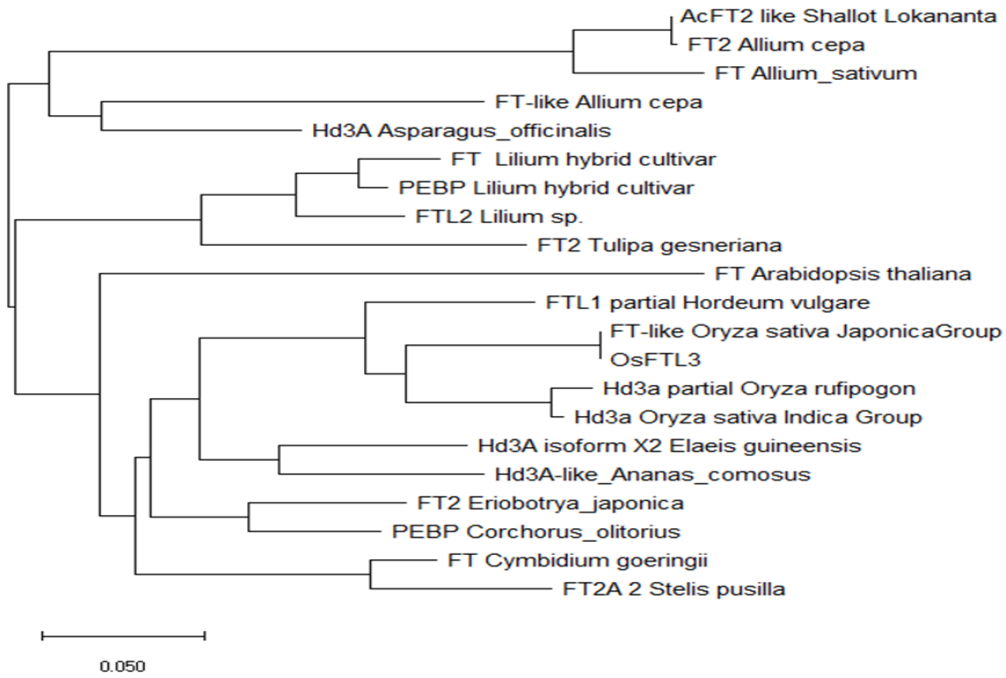

Figure 2. Phylogenetic tree of the shallot *AcFT2*-like proteins with other *FT*, *FTL*, *PEBP*, and *Hd3a* proteins related to flowering.

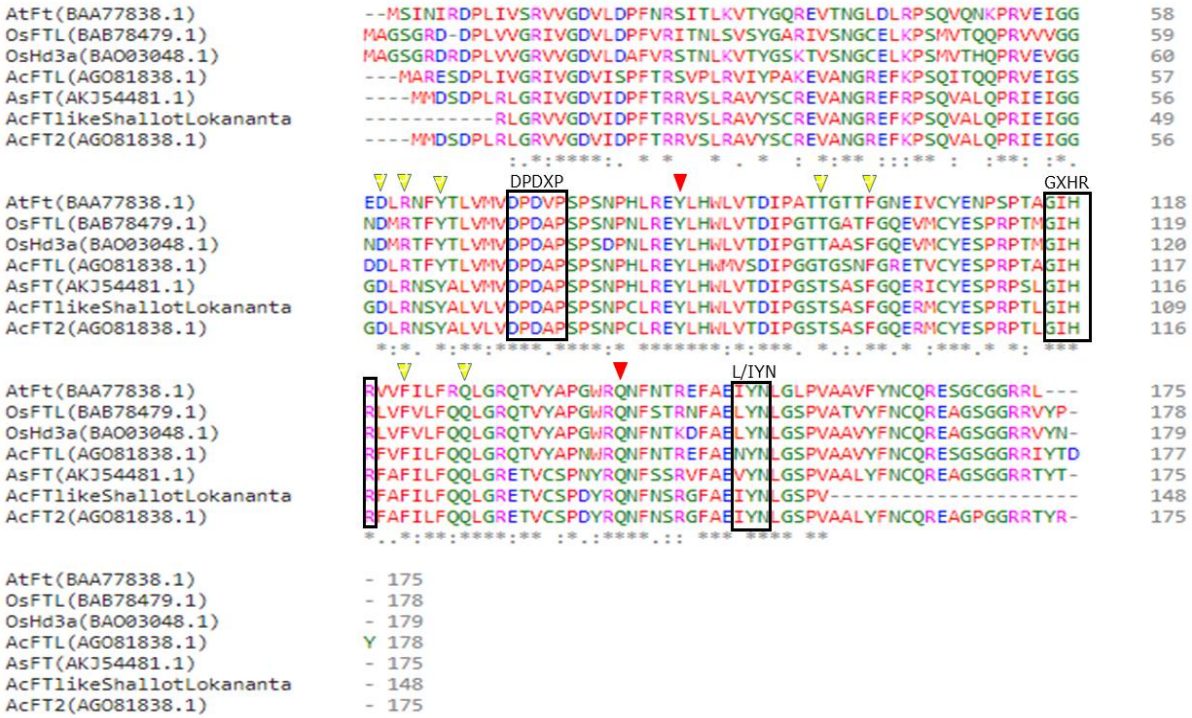

Figure 3. Comparison of the sequence of the shallot *AcFT2*-like protein with other flowering proteins. Amino acid alignment of shallot *AcFT2*-like with *PEBP* family proteins from other plants, including the model plant *A. thaliana* (At*FT*) and *O. sativa* (Os*FT*). Red triangles indicate key amino acid residues that determine *FT*-like function. Yellow triangles indicate amino acid residues that interact with the 14-3-3 protein (Yang *et al.*, 2019). Red boxes represent the conserved DPDxP, GxHR, and L/IYN motifs.

Relative expression of the shallot AcFT2-like gene

The relative expression analysis of the shallot *AcFT*2-like gene revealed that this gene was highly up-regulated in the shallot cultivar 'Lokananta' (Figure 4). Although the relative expression levels of the shallot *AcFT*2-like gene under the vernalization treatment (43.91) and nonvernalization treatment (87.44) differed, they were relatively similar (Figure 4).

The similarity of the relative expression levels of the shallot *AcFT*2-like gene under nonvernalization and vernalization treatments was reflected by the number of umbel flowers. The nonsignificant differences in the number of emerging flower umbels between the two treatments (Figure 5) indicated that the shallot *AcFT*2-like gene participated in the flowering mechanism of shallot plants under both treatments.

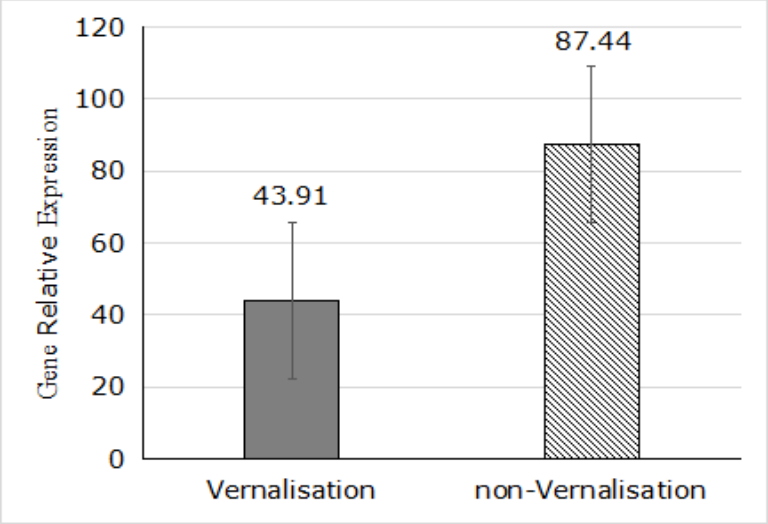

Figure 4. Relative expression of the shallot *AcFT*2-like gene. Each value represents standard deviation (n = 6).

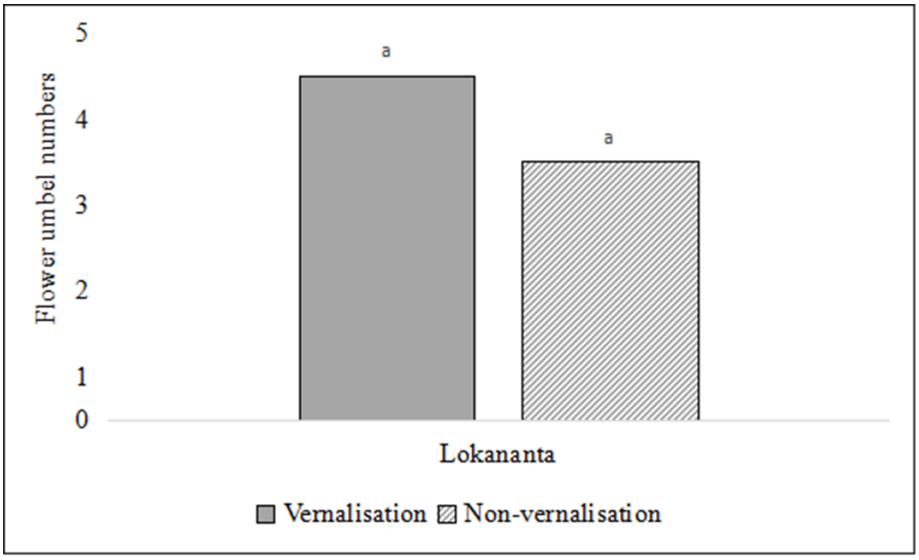

Figure 5. Flower umbel number of the shallot cultivar 'Lokananta'. The same letter presented on the stem diagram indicates nonsignificant difference by LSD test at the 5% level.

DISCUSSION

The possible involvement of the *AcFT*2 gene of the shallot cultivar 'Lokananta' in flowering activity was indicated by the high homology of the nucleotide sequence of the partial *AcFT*2 gene of shallot cultivar 'Lokananta' with that of the reference gene *AcFT*2 of the common onion CUDH5120 line (Table 4). This high homology indicated that in flowering activity, the function of the partial *AcFT*2 nucleotide sequence from the shallot cultivar 'Lokananta' is similar to that of the reference gene. The same flowering gene function is indicated by the same key amino acid for flowering activity even though the complete sequence of *the AcFT*2 gene was not obtained, which prevented the discovery of new alleles in the cultivar 'Lokananta'. The high homology between the partial *AcFT*2 nucleotide sequence of the shallot cultivar 'Lokananta' and the reference *AcFT*2 nucleotide sequence of the common onion *A. cepa* CUDH5120 line resulted from the same key amino acids shown by the alignment of the partial putative gene proteins of the partial shallot *AcFT*2-like gene cultivar 'Lokananta'; the CUDH5120 line; long-day and short-day plants; *A. thaliana*; other *Allium* genus family plants, namely, *A. cepa* and *A. sativum*; and Hd3a in *A. officinalis* (Figure 3). The sequence alignment of the shallot *AcFT2*-like gene from 'Lokananta' with other *PEBP* genes from other plants revealed the key amino acid residues that determine *FT*-like function. The conserved Tyr85 and Gln140 amino acids were present in the shallot *AcFT2*-like gene in 'Lokananta'. In *Arabidopsis*, the amino acids at position AA85 (Tyr, Y) are critical for determining *FT* function (Hanzawa *et al.,* 2005). The identified amino acids Y and Q are the key amino acids that differentiate between *FT* and TFL genes. In *Arabidopsis*, the amino acids at positions AA85 (Tyr, Y) and AA140 (Gln, Q) showed residues that determine the function of the *FT*-like gene (Wang *et al.,* 2017; Zhou *et al.,* 2018).

The *FT* gene belongs to the *PEBP* family, among which the homologous rice *FT* gene *Hd3a* interacts with 14-3-3 and OsFD1 proteins to form the florigen activation complex (Karlgren *et al.*, 2011). The 14-3-3 protein is important because of its attachment to the *PEBP* protein (Yang *et al.,* 2019). The 14-3-3 protein acts as a key regulator of the liaison of cellular networks with different signaling pathways and translates and integrates various hormonal signals to regulate physiological processes (Camoni *et al.,* 2018). The conserved DPDxP and GxHR motifs are the *PEBP* domains that conform between ligand binding sites (Karlgren *et al.,* 2011). The conserved 14-aa sequence loop known as the B segment and the C segment LYN/IYN in the *FT* homolog are required for *FT* gene activity (Bi *et al.,* 2019; Yang *et al.,* 2019). The partial *AcFT2*-like gene of the shallot cultivar 'Lokananta' has conserved DPDxP and GxHR LYN/IYN motifs. The *FT* gene in the shallot cultivar 'Lokananta' has the same function or pathway as the *FT* genes from other plants given its conserved amino acid motif. The *FT* gene is the main flowering component, and its expression is initiated by photoperiodism and vernalization (Adeyemo *et al.* 2017). The overexpression of the *FT*2 gene in dicotyl and monocotyl plants induces flowering in short-day, long-day, and day-neutral photoperiodism (Lee *et al.* 2013; Freiman *et al.* 2015; Wolabu *et al.* 2016).

The relative expression of the partial *AcFT*2-like gene in the shallot cultivar 'Lokananta' was relatively similar under both treatments; this similarity was supported by the nonsignificant differences in the number of the flower umbels of the cultivar 'Lokananta' under nonvernalization and vernalization treatments (Figure 5). This result further revealed that the partial shallot *AcFT*2-like gene is related to the flowering mechanism and may be not regulated through the vernalization pathway. The expression results obtained in this work contradicted the findings of Lee *et al*. (2013), who stated that the *AcFT*2 gene in the common onion functions mainly because of vernalization. The influence of the expression of the partial shallot *AcFT2*-like gene in 'Lokananta' on flowering activity should be further investigated under other factors, such as photoperiodism, besides vernalization to understand the correlation among flowering initiation factors.

The vernalization treatment could activate (up-regulate) *FT* gene activity by suppressing the function of the *FLC* gene that suppresses the *FT* system. However, *FT* genes require exposure to light in *A. thaliana*, which is a long-day model plant that requires long-day photoperiodicity to control the *FT* gene system. By contrast, *Hd3a*, the *FT* gene ortholog in rice, a short-day plant, induces flower initiation under short-day conditions, and *RFT*1 is not induced under long-day conditions (Itoh and Izawa, 2013; Liu *et al.,* 2015). Even though shallot requires short days of approximately 10 h for tuber initiation (Marlin *et al.*, 2018), it needs long days for flowering initiation (Sopha *et al.,* 2014). Therefore, exposure to long-day light is needed even after vernalization in shallot cultivars.

In addition to low-to-moderate temperatures, long-day photoperiodicity determines flower formation. Temperature and light intensity greatly affect flowering initiation after vernalization. The temperatures for the fastest opening of flower umbels and the blooming of shallots are 17 °C–19 °C; however, inflorescence is fastest at high temperatures (20 °C–30 °C) and on long days (14–16 h) (Khokhar, 2014; Sopha *et al.,* 2014; Nemtinov *et al*., 2021). The results of this work revealed that the temperatures during cultivation were approximately 20.0 °C–21.7 °C, which initiated flowering in 'Lokananta' under nonvernalization and vernalization treatments (Table 1). A recent study on the genes associated with the flowering component of *A. thaliana* found that long-day conditions increase the light-induced opening of stomata, *SOC*1, the *FT* downstream transcription factor, their expression via *FT*, and the expression level of the PM H^+ATPase isoform *AHA*5 in guard cells (Aoki *et al.,* 2019). This mechanism is enhanced by the light-induced long-day-dependent enhancement of stomatal opening and H3K4 trimethylation in *SOC*1, and its action is suppressed in the *FT*-2 mutant.

The *FT* gene is a positive regulator of H^+ATPase, and blue light induces stomatal opening (Wang *et al.*, 2014). The increased stomatal opening in guard cells is due to the overexpression of this gene, whereas the FT mutant suppresses H^+ATPase activity. Transcriptional regulation is involved in the activation of H^+ATPase in the downstream FT and TSF genes. In this mechanism in *A. thaliana*, the *FT* gene associates with the *GI-CO* gene through the photoperiod pathway transcribing the gene that determines AP1 flowering (Liu *et al.,* 2015). The *GI*-mediated *CO* gene regulates the transcription of the *FT* gene, which accumulates at the end of the day and acts naturally under long day conditions. *CO* genes not only induce flowering through the transcriptional activation of FT but also through the induction of the *FT* transporter, which is required to transport *FT* proteins from the leaves to the shoot tips of plant meristems (Shim *et al.,* 2017; Kinoshita and Richter, 2020). In addition, the low expression of the partial shallot *AcFT*2-like gene under vernalization provides evidence that this gene works directly in the photoperiod pathway.

The data obtained from BMKG (2020), Citeko Meteorological Station, Bogor, Indonesia, during the planting period from August 2019 to September 2019 showed that photoperiodicity ranged from 11.53 h to 12.31 h and that the average air temperature ranged from 21.0 °C–21.7 °C. The condition during cultivation after vernalization was suitable for flowering initiation. The occurrence of flowering proved that vernalization occurred in the cultivar 'Lokananta'. Khokhar (2019) reported that in contrast to low temperatures (15 °C–20 °C), temperatures over 20 °C accelerate the growth of emerged inflorescences. However, the flowering process can be inhibited by exposure to high temperatures during the day (29 °C) and night (21 °C) after induction with low temperatures (Brewster, 2008).

The photoperiod during cultivation was approximately 11.53–12.31 h, which is the intermediate photoperiod category (Bosekeng and Coetzer, 2015). However, shallot needs a long-day photoperiod for flowering initiation (Sopha *et al.,* 2014). The cultivar 'Lokananta' could produce flowers, and the flower umbel number under the nonvernalization treatment did not significantly differ from that under vernalization treatment. The shallot cultivar 'Lokananta' exhibited high responsiveness to flowering. Therefore, the cultivar 'Lokananta' can be a model genotype for the further study of shallot *AcFT2*-like genes, flowering mechanism, and other genes that work under various photoperiodicity conditions. It can also be used as a genetic source to transfer the high flowering responsiveness trait to other shallot cultivars, for example, the cultivar 'Rubaru', which has several superior traits but lacks the flowering trait, through conventional breeding, such as hybridization, or nonconventional breeding, such as protoplast fusion.

CONCLUSIONS

This work obtained a partial 447 bp *FT* gene from the Indonesian shallot cultivar 'Lokananta'. This gene was named as the partial shallot *AcFT*2-like gene. It contained the functional amino acids in the *FT* gene, including two key amino acids: the Tyr amino acid at 85 bp and Gln at 140 bp linked to the 14-3-3 protein, the key regulator of liaisons among cellular networks. The functional amino acids for FT were conserved in the partial *AcFT* 2-like gene in the shallot cultivar 'Lokananta'. The gene showed similar expression levels under vernalization and non-vernalization treatments have. Field observations confirmed that no significant differences in umbel flower number were observed under both treatments. These findings still need to be confirmed again in several shallot cultivars and under treatment with different factors that promote flowering

transition, such as photoperiodism, other than vernalization

ACKNOWLEDGMENTS

We are grateful to the Center of Tropical and Horticulture Study, IPB University, Bogor, Indonesia, and 2019–2020 six-month sandwich program at Gifu University, Gifu, Japan, for funding this research.

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