Isolation and characterisation of oil palm LEAFY transcripts

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ABSTRACT
LEAFY (LFY) is an important regulator of plant’s reproductive system, regulating the transition from vegetative growth to flowering, and also acts as an upstream regulator of floral homeotic MADS-box genes in Arabidopsis. Owing to its importance in flower development, oil palm LFY transcripts were isolated and the expression pattern was characterised to elucidate the role of LFY in oil palm. In addition, the gene expression pattern was analysed to determine a possible correlation of LFY to a type of floral abnormality in clonal palms termed mantling. In this study, three LFY transcripts were isolated from oil palm inflorescence tissue. The isolated cDNAs, OpLFY1, OpLFY2 and its splice variant, OpLFY2v were predicted to encode proteins each consisting of 430, 451 and 181 amino acids, respectively. OpLFY1 and OpLFY2 share 90% and 94% amino acid sequence identity to two different LFYs from Phoenix dactylifera, respectively. OpLFY1 was localised in all floral organ primordia. Quantitative polymerase chain reaction (qPCR) analysis revealed that OpLFY genes are expressed in shoot apical meristem and inflorescence tissues with the highest expression level. The expression of the OpLFY1 gene was significantly lower in stage 1 of inflorescence development in mantled palms compared with that of normal inflorescences, suggesting possible association of the gene with this somaclonal variation.

Introduction
The African oil palm, Elaeis guineensis, originates from West Africa and is classified in the palm family Arecaceae and subfamily Arecoideae [1]. Oil palm is the most efficient oil crop in terms of oil productivity per hectare. Two types of oil are produced from the fruit, i.e. palm oil and palm kernel oil, which differ in their fatty acid composition [1]. Palm oil and palm kernel oil are extracted from the pulp and kernel of the fruit, respectively [1]. The oil palm is a monoecious species where male and female inflorescences are produced separately in alternate bearing, and also acts as an upstream regulator of floral homeotic MADS-box genes in Arabidopsis. Owing to its importance in flower development, oil palm LFY transcripts were isolated and the expression pattern was characterised to elucidate the role of LFY in oil palm.

To improve the oil productivity per hectare, planting of clonal material is desirable as the yield is reported to be 30% higher compared with that of the Dura × Pisifera cross [3]. Micropropagation enables large-scale multiplication of elite palms. Besides that, clonal materials also demonstrate a higher degree of uniformity than the conventionally produced palms. However, a type of somaclonal variation, termed mantling, affects the reproductive development in approximately 5% of clonal palms, resulting in reduced oil yield [4,5]. The mantled somaclonal variation is characterised by the feminisation of male counterparts in the flower, resulting in abnormal fruits containing supplementary carpel structures. Furthermore, excessive parthenocarpy is observed on severely mantled bunches [6].

A recent discovery on mantling pointed towards the hypomethylation of Karma retrotransposon located in the intron of a B-class MADS-box gene, DEFICIENS (DEF) [7]. The loss of methylation of Karma retrotransposon enabled the expression of an alternatively spliced transcript of DEF, termed kDEF. The expression of kDEF is restricted only to the mantled inflorescence at stages 3–5 [7], coinciding with initiation of reproductive organs at stage 3. This specific spatiotemporal expression pattern of kDEF possibly involves the action of alternative splicing (AS) regulators. Regulators such as serine/arginine-rich (SR) proteins, chromatin remodeling complexes and histone modifying enzymes modulate the AS event in response to endogenous and environmental signals or stresses [8–11].

LEAFY (LFY), a plant-specific transcription factor, functions as an important gene which regulates the
reproductive development in plants. In non-flowering plants, LFY orthologs have been found to play important roles in moss, fern and gymnosperms [12–14]. For example, the LFY ortholog PpLFY regulates the first cell division of the zygote in *Phycomitrella patens* [12]. In *Pinus radiata*, which is a gymnosperm, expression of two LFY orthologs, NEEDLY and PrFLL, was observed in reproductive structures of female and male cones, respectively [14,15]. Furthermore, NEEDLY was able to complement an *Arabidopsis* ify-mutant plant, demonstrating its importance in reproductive development [14].

Expression and functional studies in several species have identified diverse roles played by LFY in regulating genes involved in floral meristem and floral organ development in angiosperms [16,17]. In the model plant *Arabidopsis thaliana*, LFY promotes the transition from inflorescence to floral meristem by activating APETALA1 (AP1) expression. In Ify mutant plants, AP1 expression is delayed and, consequently, the transition from the vegetative to the flowering phase is delayed [18]. Similarly, a delay in the flowering time has also been observed in Ify mutant rice, maize and tomato plants [19–21]. In several species, LFY activates members of floral homeotic MADS box transcription factors, namely AP1, APETALA 3 (AP3) and AGAMOUS (AG) and, hence, indirectly regulates the floral organ patterning [16,22]. This idea was confirmed by the formation of abnormal flower structures in Ify mutant plants such as in *A. thaliana*, *Zea mays* and *Pisum sativum* [20,23–25]. Collectively, LFY functions as a master regulator of plant’s reproductive system in both flowering and non-flowering plants. Besides regulating the reproductive development, LFY is also involved in controlling the leaf shape in *Medicago truncatula* and *Lotus japonica* [26,27]. However, less information is available on the LFY gene activity in the palm family.

In this study, isolation and molecular characterisation of LFY transcript was carried out to elucidate the roles of LFY in oil palm flower development. We compared the expression pattern in normal and mantled inflorescences to determine the possible association of LFY with the mantled somaclonal variation in oil palm.

**Materials and methods**

**Plant materials**

The plant materials from *E. guineensis* oil palm clones were collected from Pusat Penyelidikan Pertanian Tun Abdul Razak (PPPTR), Malaysia. Root, meristem and spear leaf samples were obtained from 1-year-old oil palm seedlings and were dissected into thin sections. Immature inflorescences which were still enclosed in the fibrous spathe structure were collected from clonally propagated *tenera* palms. Particularly, inflorescences located at leaf axils between the order of +4 and +18 were collected. Hereinafter, a set of inflorescences harvested from a palm shall be referred to as a *series of inflorescence*. The prophyll and peduncular bract structures which enclosed the inflorescence were removed, followed by dissection of the inflorescence samples. The dissected inflorescences and other plant organs were immersed in RNAlater® solution (Thermo Fisher Scientific, USA) and stored at −20 °C until further processing. The developmental stage of the inflorescence was determined through histological studies according to the guidelines published in Adam et al. [6].

**RNA extraction and first-strand cDNA synthesis**

Samples immersed in RNAlater® solution were homogenised in liquid nitrogen with a mortar and pestle. Total RNA extraction was carried out using RNasy Plus Universal Kit (QIAGEN, Germany) according to the manufacturer’s instructions. Genomic DNA was effectively removed using the qDNA eliminator solution from the kit. First-strand cDNA was synthesised with 1 μg of total RNA as template using M-MLV Reverse Transcriptase and Oligo(dT) primers from SuperScript® III First-Strand Synthesis System kit (Invitrogen, USA).

**Isolation of OpLFY transcripts**

A partial OpLFY1 sequence was amplified using first-strand cDNA template synthesised from young female inflorescence tissue. Gene-specific primers for the amplification were designed based on oil palm LFY expressed sequence tag deposited in the National Centre for Biotechnology Information (NCBI) database (Accession number: EL682469). Subsequently, 5’ and 3’ rapid amplification of cDNA ends (RACE) polymerase chain reactions (PCRs) were carried out to obtain the full-length coding sequence of *OpLFY1*. A final concentration of 5% dimethyl sulfoxide (DMSO) was added to the RACE PCR reaction to facilitate the amplification of the GC-rich *LFY* template. All amplified PCR fragments were purified, cloned and sequenced to verify the identity of the inserts. *LFY2* and *LFY2* _V_ sequences were obtained from an in-house reference inflorescence transcriptome and amplified with HotStarTaq Polymerase (QIAGEN, Germany) using gene-specific primers. The primer sequences are indicated in Table 1.

**Phylogenetic analysis of LFY/FLO homologs**

The amino acid sequence of *OpLFY1* and *OpLFY2* was predicted using ORF finder software (https://www.ncbi.
Protein sequences from other angiosperm species which have homology with OpLFY were retrieved from the NCBI database (http://www.ncbi.nlm.nih.gov/). Gblocks software (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) was used to remove poorly aligned positions prior to phylogenetic tree construction. The phylogenetic relationship of these genes was inferred by MAFFT software (http://mafft.cbrc.jp/alignment/server/) using the neighbour-joining method with 1000 bootstrap replicates.

### Quantitative PCR (qPCR) expression analysis of OpLFY

Expression analysis was conducted to quantify the abundance of OpLFY1, OpLFY2 and OpLFYv2 transcripts in different organs of oil palm. Quantitative PCR (qPCR) primers were designed using PerlPrimer software (http://perlprimer.sourceforge.net/index.html), with the exception of two housekeeping gene sequences, Cyclophilin 2 (Cyp2) and GvHK1, which were obtained from Yeap et al. [28]. The PCR efficiency was determined using the formula [efficiency = \(10^{1/S_{\text{slope}}\text{ of standard curve}}\)], based on a standard curve generated by plotting the \(C_p\) value of each dilution against the log input of cDNA template amount. Real time qPCR was performed in a total volume of 10 \(\mu\)L reaction mixture containing 1x Sybr Green Master mix from LightCycler® 480 SYBR Green I Master Kit (Roche Applied Sciences, Germany). The primer sequences.

| Primer Name          | Forward 5’ to 3’ sequence | Reverse 5’ to 3’ sequence |
|----------------------|---------------------------|---------------------------|
| **Partial CDS primer** |                           |                           |
| OpLFY1_EL82469       | CACCTCTCTTGTGGGTCTCATG    | GCTCCCTAGACAAGGCTCCTCA    |
| **RACE PCR primer**  |                           |                           |
| GSP1 5’ RACE         | TCCACGACGACAGACAGGCTGCG   | CTCAAAATTACCCACGAGCAGC    |
| GSP2 3’ RACE         | CCAAGAGCTTGGTGGAGGCGGG    | CAGTCTCTGCCCCAGATTCAGC    |
| **CDS primer**       |                           |                           |
| OpLFY1_FL            | GAAAGCGAACAGATCCAGAATGAC  | CGACGACCCGATGAGGCCAAGC    |
| OpLFY2v FL           | GAGAAGGATCCGACAGATTGGA    | CAGTCTCTGCCCCAGATTCAGC    |
| **qPCR primer**      |                           |                           |
| Rt_OpLFY1            | CCACCCGCCAGCAGTTTATTG     | GTGGCTCTCACAAGCAGATTG     |
| Rt_OpLFY2            | CACGACCAAACACCATACCAACC  | ACATGCTCTCTTGGGAGGAGG     |
| Rt_OpLFYv            | TCCCGGATTCATGGAAGAAAAA    | AAGATGTGTGGTGTTGGTACCTCA  |
| Rt_Ubiquitin         | CCACCTAAGTACGATCTGAGG     | CAAAGAAGAACAGATTGAAACG    |
| Rt_Cyp2             | CTGCTCTGATGTCGTCTCA       | CTGCTGGAATCTGACCTCA       |
| Rt_GvHK1             | CCACCTCCTGCCATACGCTC      | ATATAGACATTTCTGGACTAC     |

*Primer sequence obtained from Yeap et al. [28] *
the normal and mantled inflorescence at the same stage of development at $p < 0.05$.

**RNA in situ hybridisation (ISH)**

Locked Nucleic Acid (LNA) RNA probe (Exiqon, Denmark) was used in the ISH reaction. The probe sequences were designed online at [https://www.exiqon.com/mRNA-probes](https://www.exiqon.com/mRNA-probes) and synthesised by Exiqon, Denmark (Table 2). Fixation, dehydration and embedding of the inflorescence samples were performed according to the modified method from Ramachandran et al. [30]. The embedded samples were subsequently cut into 7 µm sections and affixed onto positively-charged ProbeOn Plus (Fisher Scientific, USA) microscope slides. Pre-treatment of tissue section, probe hybridisation, post-hybridisation washes and colour reaction development were performed according to Ooi et al. [31]. Oil palm elongation factor-α probe and commercially available scrambled ISH probe (Exiqon, Denmark) were used as positive and negative controls in the ISH reaction, respectively. The signal was viewed and captured with AxioCam ERC 5 s camera (Zeiss, Germany) attached to Axio Lab.A1 microscope (Zeiss, Germany).

**Results and discussion**

**OpLFY1, OpLFY2 and its splice variant, OpLFY2v from oil palm**

Two homologs of the LFY gene were isolated from oil palm inflorescence tissues, termed as OpLFY1 and OpLFY2. The LFY gene is generally present in a low gene copy number in plants, where in angiosperms, LFY exists mostly as a single gene copy. However, there are a few exceptions where two LFY gene copies are observed, such as in the apple tree family in dicot plants as well as in lily and maize, which are monocots [20,32,33]. Nucleotide search using the blastn program with the isolated OpLFY variants as queries against the oil palm genomic sequences indicated that the corresponding genes are present in different genomic scaffolds, where the OpLFY1 sequence matches with scaffold p5_sc00057_638 (Genbank accession: ASJS01029820.1) and the OpLFY2 sequence matches with scaffold p5_sc00046_562 (Genbank accession: ASJS01025661.1). A schematic representation of the OpLFY genes and the isolated transcripts is shown in Figure 1. The two identified LFY genes in oil palm possibly arose through segmental duplication, as this incident is widespread in the Elaais genus [34]. The open reading frame (ORF) of OpLFY1 is 1349 bp in length and was predicted to encode a protein consisting of 430 amino acids. OpLFY2 ORF is comprised of 1393 bp encoding a protein of 451 amino acids. OpLFY1 and OpLFY2 have 80.4% identical amino acid composition. Interestingly, a splice variant of OpLFY2, termed OpLFY2v was isolated. The OpLFY2v transcript has retained the first intron in the mature mRNA due to possible inhibition in the splicing event. The intron inclusion results in encountering a premature stop codon during translation of OpLFY2v mRNA and therefore it is predicted to encode a truncated protein with 181 amino acids in length. A part of the retained intron one, which will be translated into protein, is denoted as OpLFY2v-specific exon (Figure 1). Intron retention is the most common form of AS which occurs in plants, represented by approximately 40% of AS events in *A. thaliana* [35].

The predicted LFY protein possesses two conserved domains; N-terminal and C-terminal domains which are

| Probe       | 5' to 3' sequence                                      |
|-------------|-------------------------------------------------------|
| Ish_OpLFY1  | /3DigN/ TGCTTTATAGAAACAGGCTGCA/5Dig_N/              |
| Ish_OpElf-a | /3DigN/ TCATGCGCATCTCAACAGAT/5Dig_N/                |
| Ish_Scrambled | /3DigN/ GTTAACACGTCTATAGCCCA/5Dig_N/              |

Figure 1. Schematic representation of the intron-exon boundary in OpLFY genes and the isolated OpLFY1, OpLFY2 and OpLFY2v transcripts. Note: Colour-coded boxes represent exons, OpLFY2v-specific exon and untranslated region (UTR). The connecting lines represent introns. The inverted triangle denotes the premature stop codon in OpLFY2v.
Figure 2. Amino acid sequence alignment of predicted OpLFY proteins with LFY from other species. Note: Accession numbers are as follows: LFY (Arabidopsis thaliana, AAM27941.1), FLO (Anthirrinum majus, AAA62574.1), FAL (Solanum lycopersicum, AAF66101.1) and MaLFY (Musa acuminate, XP_009405247.1). Two conserved domains in LFY proteins, N-terminal and C-terminal domain (represented by 2 α-helices and 7 β-sheet secondary structures), are annotated by coloured boxes, based on Sayou et al. [36] and Hamés et al. [37].
depicted in Figure 2. The N-terminal domain contains a Sterile Alpha Motif, which is necessary for the LFY protein to self-associate and form oligomers [36]. Oligomerisation promotes co-operative binding of LFY at low affinity binding sites and enables LFY to access closed chromatin regions [36]. The DNA-binding and dimerisation motifs in the C-terminal domain of LFY are made up of two β-sheets and seven α-helices [37]. Similar to the full-length LFY protein, OPLFY2, OpLFY2v has the conserved N-terminal domain. However, it lacks the C-terminal region due to AS (Figure 2). AS of the LFY gene has been reported in garlic, where two transcripts, GaLFYs and GaLFYl, are produced and both variants have an intact C-terminal domain [38]. The spliced GaLFYs transcript is expressed in all developmental stages of the plant. Conversely, the GaLFYl transcript accumulates only at the flower differentiation stage. Temporal expression exhibited by GaLFYl indicates the importance of GaLFYl in the floral transition process of garlic [38].

In oil palm, the isolated OpLFY2v lacks the conserved C-terminal region which encodes for the DNA-binding domain, and hence the protein is hypothesised to be unable to bind and regulate its downstream target gene activities. However, strong interaction between truncated LFY protein lacking the C-terminal region and full-length LFY protein from Arabidopsis has been demonstrated in vitro [39]. Furthermore, overexpression of this truncated LFY protein from Arabidopsis also displayed lfy-like mutant abnormalities [39]. Therefore, OpLFY2v may be able to form oligomers with OpLFY1 and 2 proteins and subsequently reduce the functional protein complexes which are required to carry out the necessary function in oil palm.

**OpLFY1 and OpLFY2 clade together with other LFY proteins from monocotyledon family**

Phylogenetic analysis of LFY proteins showed three distinct clades for gymnosperm, dicots and monocots species, respectively (Figure 3). OpLFYs clustered together with LFY proteins from Liliales, Asparagales, Zingiberales and Poaceae, which form the monocot clade. Within monocots, OpLFY1 and OpLFY2 clustered together with two predicted LFY proteins from Pheonix dactylifera, which is in the Arecales order. This is consistent with the fact that P. dactylifera contains most of the segmental duplications that are found in oil palm [34] and also suggests that the duplication most likely occurred prior to the speciation event.

**OpLFY1 is highly expressed in reproductive organs and apical meristem compared with OpLFY2 and OpLFY2v**

OpLFY transcripts were detected in several organs (Figure 4). Among the OpLFY transcripts, OpLFY1 showed the highest expression in the apical meristem and floral tissues compared with OpLFY2 and OpLFY2v. In the

Figure 3. Phylogenetic tree of predicted LFY protein sequences, constructed with the neighbour-joining method.

Note: Accession numbers are as follows: NEEDLY(AAB686601.1), LFY(AAM27941.1), UNI(AAC49782.1), FLO(AAA62574.1), FAL(AAF66101.1), NFL1(AFY06673.1), LiLFY1(ABR13015.1), OrcLFY(BAC54955.1), AcLFY(AFR67541.1), HoLFY(AAS00458.1), MaLFY(XP_009405247.1), PdLFY1(XP_008797515.1), PdLFY2(XP_008775161.1), JeLFY1(AAF77077.1), ZFL1(NP_001105 201.1), ZFL2(AAV68202.1), ShLFY1(AGT16538.1), RFL(BAE78663.1). Bootstrap values are indicated at nodes based on 1000 bootstrap replicates.
meristem and inflorescence tissues, the OpLFY2 and OpLFY2v transcript accumulation was about 12%–14% and 16%–44% of OpLFY1, respectively, in the calibrator tissue. OpLFY transcripts were not observed in the root and leaf tissues, as it has been found for LFY homologs in *Arabidopsis*, *Antirrhinum* and tobacco [40]. A high abundance of OpLFY transcripts in male and female inflorescence implies participation of OpLFY in the oil palm floral development pathway. The reduced expression of OpLFY2 compared with OpLFY1 might be due to several reasons. In mammals and yeasts, reduced expression of one of the duplicated genes is necessary in order to maintain the optimum level of the total gene product which co-regulates a particular process [41]. This is because overproduction of protein is detrimental due to wastage of energy and raw material and, furthermore, formation of additional misfolded proteins that are cytotoxic [41]. A study in *Arabidopsis* showed that low expression of a gene compared to its paralog may indicate a possible pseudogenisation of the gene through promoter disablement [42].

**Figure 4.** Quantitative PCR of OpLFY1, OpLFY2 and OpLFY2v transcripts in leaf, apical meristem, root, male and female inflorescence of oil palm.
Note: Target gene expression level was normalised with Cyp2 and Ubiquitin endogenous references. Fold-change value was calculated by calibrating the normalised expression of all transcripts against OpLFY1 in apical meristem tissue. Error bars indicate standard deviations for three technical replicate values.

**Figure 5.** Quantitative PCR of OpLFY1, OpLFY2 and OpLFY2v transcripts in female inflorescence series obtained from a normal clonal palm.
Note: Cyclophilin2 and GVHK1 were used to normalise target gene expression levels. The developmental stage from youngest stage 1 to late stage 4 of the inflorescence samples are as indicated. Target gene expression level was normalised with Cyp2 and GVHK1 endogenous references. Fold-change value was calculated by calibrating the normalised expression of all transcripts against OpLFY1 in stage 1 inflorescence tissue. Error bars indicate standard deviations for three technical replicate values.
The qPCR results showed expression of OPLYF transcripts in the shoot apical meristem (SAM). The OplyF transcripts are predicted to be localised at the peripheral zone of the SAM, where the cells divide and differentiate into lateral organs, similar to the expression of NFL1, a LFY ortholog, in tobacco [40]. Further investigation on its spatial localisation through in situ hybridisation will enable the identification of zone-specific expression of OplyF at the SAM.

**OplyF transcripts are highly expressed at the early inflorescence development stage**

Similar to the transcript accumulation results in oil palm organs (Figure 4), the highest transcript accumulation of OplyF1 was observed in all stages of the developing female inflorescence series. At stage 1 inflorescence, the transcript accumulation of OplyF2 and OplyF2v was only 3.9% and 15.7% of that of OplyF1. The OplyF1 transcript abundance gradually decreased in the subsequent stages (Figure 5). The transcript accumulation of OplyF1 was only 5% of that at stage 1. According to the histological characterisation of oil palm flower development by Adam et al. [6], the floral meristem is formed at stage 1, followed by initiation of the perianth organs consisting of sepal and petal structures at stage 2. The perianth organs then develop, and reproductive organs including stamen and carpel structures are initiated at stage 3. At stage 4, the reproductive organs develop, and stage 5 corresponds to mature flower [6]. The qPCR expression results suggest that OplyF transcripts were required during formation of the floral meristem and subsequent floral organ patterning steps in oil palm.

**OplyF1 is highly expressed at stage one of normal female inflorescence compared with mantled inflorescence**

At developmental stage 1, the OplyF1 transcript accumulation in mantled inflorescence was only 75% of that of normal inflorescence (Figure 6). Student’s t-test indicated that the difference in expression was statistically significant ($p < 0.05$). The differences in the accumulation of the OplyF1 transcript decreased between normal and mantled inflorescence in subsequent developmental stages. Functional studies are required to further elucidate the role of OplyF1 in mantled flower development. In Arabidopsis, abnormal flowers were observed in plants with a severe Ify mutant allele, where petal and stamen structures were absent and intermediate floral organ structures such as sepalloid carpels were produced [43]. Although the reproductive organs in the oil palm flower physically start to appear only at stage 3 [6], the pathway that leads to the initiation of the organs may have begun at earlier developmental stages. Therefore, it may be too early to rule out the possibility that low accumulation of OplyF at the early developmental stages of flower development contributes to the mantled somaclonal variation. There are insufficient results to associate kDEF expression in mantled inflorescence to OplyF1; however, it has been reported in Arabidopsis that LFY is able to interact with SPLAYED, an ATPase component of the chromatin remodeling complex, to regulate B-class MADS box gene expression [17,44].

**Uniform localisation of OplyF1 observed in floral organ primordia**

OplyF1 was localised in the emerging floral primordium in inflorescence at which floral organs such as sepal, petal, stamen and carpel structures develop subsequently (Figure 7). This coincides with the known function of LFY gene, which regulates AP1, AP3 and AG from A, B and C classes of MADS-box genes, respectively, to specify the floral organs formation [18,43]. As the OplyF1 expression is uniform throughout the floral primordia, the whorl-specific regulation of these MADS-box genes is speculated to be dependent on the spatio-temporal expression pattern of LFY co-regulators. For example, in Arabidopsis, petal and stamen specific expression of B-class genes requires the UFO co-factor action, and the stamen and carpel specific expression of AG requires the WUS co-factor activity in combination with LFY expression [45].
Conclusions

Three transcripts of the oil palm LFY gene, *OpLFY1*, *OpLFY2* and its splice variant, *OpLFY2v*, with a premature termination codon, were isolated. *OpLFY1* was predominantly expressed in the SAM and inflorescence tissues compared with *OpLFY2* and *OpLFY2v*. Functional studies are required to understand the expression of *OpLFY2v* in flower development. A significant difference in the expression of *OpLFY1* between normal and mantled inflorescence at stage 1 was observed. It would be worthwhile to conduct further investigations to verify the potential involvement of *OpLFY1* in mantling phenomena.

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