Assessing the Flowering Genetic Regulatory Network in Neotropical Orchids †

Yesenia Madrigal 1, Diego Ospina-Zapata 1, Jessica A. Ramírez-Ramírez 1, Juan Fernando Alzate 2, and Natalia Pabón-Mora 1,*

1 Facultad de Ciencias Exactas y Naturales, Instituto de Biología, Universidad de Antioquia, Medellín 500010, Colombia; yesenia.madrigal@udea.edu.co (Y.M.); diego.ospinaz@udea.edu.co (D.O.-Z.); jessicaa.ramirez@udea.edu.co (J.A.R.-R.)
2 Centro Nacional de Secuenciación Genómica, Sede de Investigación Universitaria, Facultad de Medicina, Universidad de Antioquia, Medellín 500010, Colombia; jfernando.alzate@udea.edu.co
* Correspondence: lucia.pabon@udea.edu.co; Tel.: +57-321-772-0164
† Presented at the 1st International Electronic Conference on Plant Science, 1–15 December 2020; Available online: https://iecps2020.sciforum.net/.

Abstract: During the reproductive transition in flowering plants, a vegetative apical meristem (SAM) transforms into an inflorescence meristem (IM) that forms bracts and flowers. In grasses such as rice, a genetic regulatory network (GRN) controlling reproductive transitions has been identified. It includes the integration of promoters and repressors from different gene lineages with active duplication events during angiosperm diversification. With the objective to understand the evolution and expression of flowering GRN in Orchidaceae, we performed comprehensive phylogenetic analyses of all genes from the flowering GRN and analyzed by RT-PCR the expression of targeted homologs in key developmental stages. Our ML results indicate that the FT/FTL1, FD, FLC/FUL, SOCI and AGL24/SVP gene lineages have been subject to multiple duplications in monocots, as well as in Orchidaceae. Conversely, FLC genes are lost in Orchidaceae, suggesting major changes in the repression of flowering. Our studies also show active expression of many target genes in Elleanthus auranticus (Orchidoideae) in the SAM and in IM, indicating important functions in the reproductive transition. We describe how the flowering GRN in orchids has significant variations in copy number and expression patterns when compared to the canonical rice flowering GRN.

Keywords: AGAMOUS LIKE 24; FLOWERING; FLOWERING LOCUS T; FLOWERING LOCUS C; FLOWERING LOCUS D; gene evolution; genetic regulatory network; orchidaceae; SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1

1. Introduction

The floral transition is one of the most important developmental switches in the plant life cycle, resulting in the change from vegetative to reproductive phase. In Arabidopsis, the reproductive transition occurs when the vegetative apical meristem (SAM) forming leaves becomes an inflorescence meristem (IM) that forms bracts and flowers. This process is regulated by endogenous and environmental factors, which merge into four main pathways: photoperiod (light response), vernalization (cold response), autonomous and hormonal signaling [1,2]. In the model monocot, Oryza sativa, the core flowering genetic regulatory network (GRN) relies on the early activation of Heading date 3a (Hd3a, a FLOWERING LOCUS T-FT homolog) on short days (SD) [3,4]. The complex between Hd1 (a CO, CONSTANS homolog) and Hd3a plays a critical role in mediating the photoperiod flowering signal [5]. While on SD Hd1 activates FT expression in rice, on long days (LD), Hd1 is converted into a transcriptional repressor [5]. However, on LD rice cultivars, RICE FLOWERING LOCUS T1 (RFT1), an Hd3a paralog, is responsible for floral induction [3]. On SD, after the first FT signaling, FLOWERING LOCUS D (FD, a bZIP homolog) transcription factor in rice, OsFD1,
interacts with Hd3a via the 14–3–3 proteins to form a florigen activation complex (FAC) [6]. The FAC induces the transcription of OsMADS14 and OsMADS15 (the AP1/FUL homologs) in the shoot apex during floral transition [6–8]. On the other hand, OsMADS50 and OsMADS51 (the SOCI homologs), OsMADS22, OsMADS47 and OsMADS55 (the AGL24/SVP homologs) control floral meristem identity, but only OsMADS55 represses flowering [9–11]. A relatively similar flowering GRN is in place across grasses [12–14]. However, crown poodles like wheat (Triticum monococcum, Triticum aestivum) and barley (Hordeum vulgare), also have vernalization responsiveness determined by allelic variation at the VERNALIZATION1 (VRN1, an AP1/FUL homolog) and/or VRN2 (a CO-like homolog) loci [15–17]. VRN2 alleles repress flowering by direct or indirect repression of VRN1 alleles under LD [18]. In addition, during vernalization and/or exposure to SD, VRN2 transcription is reduced, resulting in an up-regulation of VRN1 and triggering flowering [15,17].

Although the flowering GRN has been well studied in grasses, little is known about the genetic mechanisms of flowering in non-model monocots, including orchids. The isolation and characterization of some flowering controlling transcription factors have been done in commercial, mostly temperate orchids like Cymbidium, Dendrobium, Oncidium and Phalaenopsis: here, homologs of FT or SOCI genes play an important role in promoting flowering [19,20]. Nevertheless, comprehensive phylogenetic analyses for all gene lineages involved in the flowering GRN are lacking, and as a consequence, few homologs have been studied, sometimes with unclear affiliation to a specific clade. This is particularly problematic considering that whole genome duplication (WGD) events are abundant in monocots. In turn, gene copy number and homology for all copies needs to be established prior to the expression and functional characterization of the flowering GRN. Our goal is to evaluate the evolution of the flowering GRN in the Orchidaceae (ca. 25,000 species), one of the most diverse groups of ornamental angiosperms. Here we use reference transcriptomes from 13 neotropical orchid species to find homologs from the transcription factors known to control flowering and perform comprehensive ML phylogenetic analyses to understand the evolution of all gene lineages involved in the reproductive transition. Our ML results indicate that FT/TFL1, FD, FLC/FUL, SOCI and AGL24/SVP gene lineages have been subject to multiple duplications in monocots, as well as in Orchidaceae. We also show that FLC genes are lost in orchids. Finally, we evaluate the expression of all target genes in Elleanthus aurantiacus, a tropical and terrestrial member of the Epidendroideae (Orchidaceae), and show the active expression of several factors in the SAM and IM, indicating important functions in the reproductive transition. We show that the flowering GRN in orchids has significant variations in copy number and expression patterns when compared to the canonical rice flowering GRN.

2. Experiments
2.1. Phylogenetic Analyses of Flowering Candidate Genes

In order to analyze the evolution of flowering-related gene lineages FD, FLC/FUL and SOCI and identify putative duplication events, we performed searches for gene homologs of all candidate genes using tBLASTX tools. Searches were done in our own reference transcriptomes, as well as in the Orchidstra and OrchidBase, which serve as repositories for orchid genomes and transcriptomes [21,22]. The queries were FD, FUL and SOCI homologs from Arabidopsis, orchids and rice. Detailed methodology for phylogenetic analyses can be found in [23–26].

2.2. Morpho-Anatomical Characterization of the Flowering Transition in Orchidaceae

In order to establish changes in size, and the initiation of lateral organs as well as new morphological features occurring during flowering transition in Elleanthus aurantiacus, light and scanning electron microscopy were used. Detailed steps for sample processing follow [24].
2.3. RT-PCR Expression Analysis of GRN Candidate Genes

RT-PCR using cDNA from dissected parts in *Elleanthus aurantiacus* was performed to evaluate the expression patterns of flowering gene homologs. Dissections follow [23]. For the amplification of each homolog, specific primers were designed for each copy, avoiding conserved domains and sometimes including either the 3' or 5' UTRs (Appendix A Table A1). Amplification reactions were done following [25]. ACTIN was used as a positive control.

3. Results

3.1. Flowering GRN Genes Have Undergone Multiple Duplication Events

The BLAST search resulted in the recovery of FT, FD, FLC/FUL, SOC1 and AGL24/SVP homologs in all orchid repositories, including our own reference transcriptomes from neotropical orchids (Table 1), as well as other publicly available angiosperm databases used. All sequences were evaluated using ML phylogenetic analyses and resulted in a comprehensive assessment of the flowering GRN evolution in Orchidaceae.

Table 1. Neotropical orchid species with available reference transcriptomes used in this study and their number of GRN homologs included in ML phylogenetic analyses.

| Species                        | FT | FD | FUL | SOC1 | AGL24/SVP |
|--------------------------------|----|----|-----|------|------------|
| Cattleya trianae               | 2  | 1  | 1   | 0    | 3          |
| *Elleanthus aurantiacus*       | 3  | 2  | 1   | 0    | 2          |
| Epidendrum fimбриatum          | 3  | 5  | 1   | 4    | 0          |
| Gomphichis scaposa             | 2  | 3  | 0   | 2    | 0          |
| Masdevvalia cocinea “Alba”     | 3  | 2  | 2   | 1    | 0          |
| Masdevvalia wendlandiana       | 5  | 3  | 1   | 1    | 0          |
| Maxillaria aurea               | 9  | 3  | 1   | 1    | 0          |
| Miltonia roezli                | 6  | 0  | 2   | 1    | 0          |
| Oncidium “Gower Ramsey”        | 1  | 1  | 1   | 5    | 0          |
| Oncidium “Twinkle”             | 2  | 4  | 6   | 1    | 0          |
| Stelis pusilla                 | 3  | 4  | 2   | 2    | 0          |
| Tolumnia “Cherry red × Ralph yagh” | 2  | 2  | 3   | 0    | 1          |
| Vanilla aphylla                | 12 | 3  | 2   | 2    | 0          |

1 Contig statistics for reference transcriptomes available in [23]. 2 Species selected for expression analysis in this study.

A total of 349 PEBP homologs were included to assess the evolution of the FT/TFL1 genes in Orchidaceae. The *Amborella trichopoda TFL1 (AmtrTFL1)* homolog was used as an outgroup. The topology shows a duplication event prior to angiosperm diversification, resulting in the FT and TFL1 clades [23]. TFL1 genes are either lacking or found scarcely in monocots when compared to eudicots [23]. Conversely, more copies of FT are found when compared to TFL1. FT genes show a duplication prior to angiosperm diversification, which generates clades FT1 and FT2. In monocots, the MonFT1 genes form a monophyletic group and have undergone at least two rounds of duplication, resulting in the MonFT1A, MonFT1B and MonFT1C clades, respectively. On the other hand, the FT2 genes appear to be exclusive to monocots, being absent in the other angiosperm lineages. These genes were duplicated at least twice in monocots, resulting in the MonFT2A, MonFT2B, and MonFT2C (Figure 1a) [23].
genes were duplicated at least twice in monocots, resulting in the MonFT2A, MonFT2B, and MonFT2C (Figure 1a) [23].

Figure 1. ML analyses of the flowering GRN in angiosperms with expanded sampling in Orchidaceae. (a) FT genes (PEBP), (b) FD genes (bZIP), (c) FLC/FUL genes (MADS-box), (d) SOC1 genes (MADS-box), (e) AGL24/SVP genes (MADS-box). All trees represent summary topologies with the terminal names removed for better visualization. Tree branch colors follow the conventions on the bottom. Stars point to duplication events. Scale: 0.2. FT and AGL24/SVP trees were modified from [23,26].

The FD genes (belonging to bZIP family) were analyzed in a matrix of 156 sequences including diverse angiosperm taxa (Figure 2b). The Amborella trichopoda FD homolog (AmtrFD) was used as an outgroup. These genes have undergone specific duplication in Brassicales and Solanales inside core eudicots. In monocots, these genes have undergone
at least three duplication events prior to the diversification of the Orchidaceae, forming the OrchFD1, OrchFD2a and OrchFD2b clades. Finally, local duplications have also occurred in Poales.

Figure 2. Morpho-anatomical observations and landmarks for developmental stages during flowering transition in *Elleanthus aurantiacus*, which grows at 1700–2400 m in the Andes and flowers during the rainy seasons twice a year. (a–d) Plants and apices during vegetative growth; (e–j) plants and meristems undergoing reproductive transition. (k) RT-PCR expression patterns of the flowering GRN genes in *E. aurantiacus* dissected organs. Actin was used as positive control. FT and AGI24/SVP gene expression were taken from [23,26] B: Bract; FB: Floral buttons; IM: Inflorescence meristem; L: leaves; P: Plastochron; S: Sepal; SAM: Vegetative meristem; -C: negative control. Scale d = 50 µm; e–g = 20 µm; h = 10 µm; i–j = 100 µm.
ML analyses for FLC/FUL (belonging to MADS-box family) were also performed to understand the evolution and the homology of FLC genes in orchids (Figure 1c). An exhaustive search was done across angiosperms resulting in a matrix with 273 putative homologs. The resulting phylogenetic tree shows that FLC genes are lacking in orchids, while they are still present in Poales. FLC homologs however have extensively diversified in eudicots. In addition, FUL genes have undergone at least two duplication events in monocots, resulting in the MonFUL1 (also called VRN1 clade), MonFUL2 and MonFUL3 clades. Interestingly, orchids lack homologs in the VRN1 clade and only have FUL2 and FUL3 homologs.

SOC1 gene evolution (belonging to MADS-box family) was also analyzed. The complete matrix comprised 268 angiosperm sequences (Figure 1d). The Amborella trichopoda SOC1 homolog (AmtrSOC1) was used as an outgroup. The ML resulting topology shows at least three duplications prior to the diversification of eudicots resulting in the Eu-didiAGL42/71/72, EudiAGL14/19, and EudiSOC1 clades. In monocots, there are three independent duplications prior to the diversification of the Orchidaceae, resulting in the OrchSOC1-1a, OrchSOC1-1b and OrchSOC1-2 clades.

Finally, the AGL24/SVP genes (belonging to the MADS-box family) were analyzed using a matrix of 363 sequences (Figure 1e) [26]. The Amborella trichopoda SVP homolog (AmtrSVP) was used as an outgroup. The topology shows a duplication prior to the diversification of eudicots, resulting in the AGL24 and SVP clades. Additional duplications have occurred for AGL24 in eudicots, resulting in the Core-eudi_AGL24a/b clades. Early diverging angiosperms and monocots only have pre-duplication copies. However, at least one independent duplication has occurred in monocots, resulting in the MonSVPLa and MonSVPLb clades, and two additional duplications have occurred in MonSVPLa, generating the orchid-specific OrchSVPLa and OrchSVPLb clades.

3.2. The Flowering Transition in Orchidaceae Recruits Several Flowering GRN Genes, Actively Expressed in the SAM and the IM

Morpho-anatomical analyses in Elleanthus aurantiacus (Epidendroideae, Orchidaceae) show that vegetative growth can occur until plants reach ca. 1.5 m tall (Figure 2a). The IM starts to differentiate during the rainy seasons (Figure 2e,f), blooming two times per year and yielding inflorescences of 4 to 10 cm long. Light and scanning electron microscopy show that the SAM is ca. 150 \( \mu \text{m} \) in diameter, forming in its flanks alternate enveloping leaves (Figure 2b–d). During the floral transition, the IM narrows down to ca. 100 \( \mu \text{m} \) in diameter and shifts to forming bracts in its flanks with axillary floral meristems (FM) (Figure 2g–j). Each racemose inflorescence forms up to 22–24 flowers.

Expression analyses were performed in dissected organs to understand the possible contribution of the flowering GRN homologs in E. aurantiacus. RT-PCR analyses show a homogeneous expression of the SOC1 genes in vegetative and inflorescence meristems and greater expression of FD in SAM (Figure 2k). It is noteworthy that copies of SOC1 are also expressed in leaves. None of these genes are expressed in fully differentiated floral buds. Additionally, FT1 genes are expressed in the IM, while FT2 genes have wide expression patterns in all tissues analyzed [23]. Finally, from the 7 AGL24/SVP copies, only two are expressed; specifically, MonSVPLa is active in the SAM, and OrchSVPLa is expressed in leaves, SAM and IM [26].

4. Discussion

Most expression and functional analyses of selected flowering genes have been done in model orchids like Cymbidium, Dendrobium and Phalaenopsis [19,20,27]. However, little is known about the evolution of each gene lineage across angiosperms in general and Orchidaceae in particular, as well as about their contribution to flowering in neotropical orchids. Our exhaustive phylogenetic analyses of all flowering genes, taking advantage of private and public databases (Figure 1), highlight that the FT, FD, FUL, SOC1 and AGL24/SVP gene lineages have been subject to multiple duplication events in monocots, contrary to what is established in eudicot model species [28–31]. Also, although the
Orchidaceae share some duplications with other monocots [32–35], there are additional family exclusive duplications, and, in turn, orchids have a greater number of gene copies than grasses. It is possible that the increase in copy number is linked to changes in protein structure and, as a consequence, to functional diversification across homologs [23]. One of the major differences we were able to find is the absence of canonical flowering repressors. Contrary to the other lineages, FLC genes have only been found in eudicots and Poales [36–39] and are lost in orchids (Figure 1c). The lack of FLC indicates a profound shift in the vernalization pathway for all orchids, temperate and tropical. It is possible that other genes are being recruited to fill that repressive function when needed.

The observations in E. aurantiacus allow us to conclude that: 1. Rainy seasons control flowering for this terrestrial orchid species in native environments. 2. The transition from the SAM to the IM triggers the reduction in size of the meristems concomitant with a shift in gene expression. 3. There is overlapping expression in the SAM and in the IM for the following copies: ElauSOC1-1-3, ElauFD1-2, ElauSVP2, ElauFT1A, ElauFT1C2, ElauFT2A2 and ElauMFT. Our results suggest important functions for these transcription factors in the reproductive transition in orchids. Endogenous functional analysis have only been standardized in Dendrobium, wherein the overexpression of DOFT (one of many FT homologs) [40] and DOSOC1 (one of three SOC1 homologs) [41] exhibits earlier flowering than wild-type orchids. These results suggest that both FT and SOC1 genes play an important role in promoting flowering in the Orchidaceae. However, the increase in the gene copy number and our findings about their expression in SAM and IM imply that functional studies from GRN are necessary to find the floral integrator genes with determining functions in flowering transition in Orchidaceae.

Based on our data, we propose two important assessments about the flowering GRN in Orchidaceae: (1) the genes of interest in orchids have undergone different evolution pathways in comparison with grass model species, due to independent duplication events in each group; (2) the increased number of homologs in orchids makes it difficult to assign a promoter or repressor function, and, for that, directed RNA-seq, as well as functional analyses, are a clue to understand the flowering mechanisms employed by the Orchidaceae.

5. Conclusions

Due to several independent WGD that have occurred inside both Orchidaceae and grasses, the flowering GRN has a remarkable increase in the gene copy number with unknown functions in orchids. Functional and comparative analyses are necessary to understand the role of the different homologs in flowering. It is probable that some of the GRN genes would be conserved in orchids, but the other ones have probably changed in function related to flowering repression.

Supplementary Materials: The poster presentation is available online at https://www.mdpi.com/article/10.3390/IECPS2020-08576/s1, the video presentation is available online at https://sciforum.net/event/IECPS2020/keynote/d571147d1bf822ebb04e027a7ce1d/presentation_video/Sciforum_2020_Madrigal_et_al_V2.mp4.

Author Contributions: Y.M. and N.P.-M. conceived and designed the experiments; Y.M., D.O.-Z. and J.A.R.-R. performed the Maximum Likelihood analyses; J.F.A. assembled the reference transcriptomes. All authors analyzed the data, wrote and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not Applicable.

Informed Consent Statement: Not Applicable.

Data Availability Statement: Genbank numbers: MZ673141–MZ673215; MN968819–MN968822, MN968823–MN968828, MN968829–MN968836, MN968837–MN968849, MN968850–MN968854, MN968855–MN968863, MN968864–MN968875, MN968876–MN968886, MN968887–MN968888, MN968889–MN968890, MN968891–MN968897, MN968898–MN968901, MN968902–MN968907, MN968908–MN968909, MN968910–MN968911, MN968912–MN968922, and MN968923–MN968926.
Acknowledgments: We thank Markus Günther from the Technische Universität Dresden for technical assistance at the SEM facilities. This research was funded by Estrategia de Sostenibilidad 2018–2019 from Universidad de Antioquia, the Convocatoria COLCIENCIAS 808-2018 (código 110180863819 CT 192-2019), the Convocatoria Programáticas 2017-16302 and the 2019 BSA Graduate Student Research Award from the Botanical Society of America.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Abbreviations
The following abbreviations are used in this manuscript:

- AGL24/SVP: AGAMOUS LIKE 24/SHORT VEGETATIVE PHASE
- FD: FLOWERING LOCUS D
- FLC: FLOWERING LOCUS C
- FT: Flowering Locus T
- FUL: FRUITFULL
- IM: Inflorescence Meristem
- GRN: Genetic Regulatory Network
- ML: Maximum Likelihood
- SAM: Shoot Apical Meristem
- SOC1: SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1
- WGD: Whole Genome Duplication

Appendix A

Table A1. Primers used for gene expression analyses. Fwd indicates forward primer. Rev indicates reverse primer.

| Primer Name     | Sequence                                      | Amplicon Size (bp) |
|-----------------|-----------------------------------------------|--------------------|
| ACTIN7a_fwd     | GCATTGTGCTTGATCCCGGTGATGTTGTCACCTAATCTCATC   | 450                |
| ACTIN7a_rev     | CACCTTAATCTTCATGCTGATGGTGT                  |                    |
| ElauSOC1-3_fwd  | GGAAAGACGGAGATGAGAC                         | 534                |
| ElauSOC1-3_rev  | CTTATGCTGATGATTGTCAT                       |                    |
| ElauSOC1-1_fwd  | GAAGGACGGAGATGAGAC                         | 555                |
| ElauSOC1-1_rev  | CAGTTCGGTGCTCTACATC                        |                    |
| ElauSOC1-2_fwd  | CCGAGATGAAAGCTATAGA                        | 457                |
| ElauSOC1-2_rev  | CATCCCTATAGTGAGTAC                        |                    |
| ElauFD2_Rev     | AGCGGATGAGGTTCTTTGAA                       | 425                |
| ElauFD2_FWD     | CCACCGTGCTTACCCTTAA                       |                    |
| ElauFD1_Rev     | ATACGTTGATCGCTTCTCTG                      | 357                |
| ElauFD1_FWD     | CCCAAAACACCTAAGCGTAA                      |                    |

References

1. Levy, Y.Y.; Dean, C. The Transition to Flowering. Plant Cell 1998, 10, 1973–1989. [CrossRef] [PubMed]
2. Parcy, F. Flowering: A Time for Integration. Int. J. Dev. Biol. 2005, 49, 585–593. [CrossRef] [PubMed]
3. Komiya, R.; Ikegami, A.; Tamaki, S.; Yokoi, S.; Shimamoto, K. Hd3a and RFT1 Are Essential for Flowering in Rice. Development 2008, 135, 767–774. [CrossRef] [PubMed]
4. Tamaki, S.; Matsuo, S.; Wong, H.L.; Yokoi, S.; Shimamoto, K. Hd3a Protein Is a Mobile Flowering Signal in Rice. Science 2007, 316, 1033–1036. [CrossRef] [PubMed]
5. Kojima, S.; Takahashi, Y.; Kobayashi, Y.; Monna, L.; Sasaki, T.; Araki, T.; Yano, M. Hd3a, a Rice Ortholog of the Arabidopsis FT Gene, Promotes Transition to Flowering Downstream of Hd1 under Short-Day Conditions. Plant Cell Physiol. 2002, 43, 1096–1105. [CrossRef]
6. Taoka, K.; Ohki, I.; Tsuji, H.; Furuita, K.; Hayashi, K.; Yanase, T.; Yamaguchi, M.; Nakashima, C.; Purwestri, Y.A.; Tamaki, S.; et al. 14-3-3 Proteins Act as Intracellular Receptors for Rice Hd3a Florigen. Nature 2011, 476, 332–335. [CrossRef]
36. Ruelens, P.; De Maagd, R.A.; Proost, S.; Geuten, K.; Kaufmann, K. FLOWERING LOCUS C in Monocots and the Tandem Origin of Angiosperm-Specific MADS-Box Genes. *Nat. Commun.* **2013**, *4*, 2280. [CrossRef] [PubMed]

37. Chen, F.; Zhang, X.; Liu, X.; Zhang, L. Evolutionary Analysis of MIKCC-Type MADS-Box Genes in Gymnosperms and Angiosperms. *Front. Plant Sci.* **2017**, *8*, 895. [CrossRef] [PubMed]

38. Ling, A.C.K.; Rozano, L.; Bakar, U.K.A.; Svp, P. Isolation and Phylogenetic Characterisation of LdSVP, SHORT VEGETATIVE PHASE (SVP) Homologous Gene from Lansium Domesticum. *J. Trop. Agric. Food Sci.* **2018**, *46*, 75–89.

39. Jiao, F.; Pahwa, K.; Manning, M.; Dochy, N.; Geuten, K. Cold Induced Antisense Transcription of FLOWERING LOCUS C in Distant Grasses. *Front. Plant Sci.* **2019**, *10*, 72. [CrossRef] [PubMed]

40. Ding, L.; Wang, Y.; Yu, H. Overexpression of DOSOC1, an Ortholog of Arabidopsis SOC1, Promotes Flowering in the Orchid Dendrobium Chao Parya Smile. *Plant Cell Physiol.* **2013**, *54*, 595–608. [CrossRef] [PubMed]

41. Wang, Y.; Liu, L.; Song, S.; Li, Y.; Shen, L.; Yu, H. DOFT and DOFTIP1 Affect Reproductive Development in the Orchid Dendrobium Chao Praya Smile. *J. Exp. Bot.* **2017**, *68*, 5759–5772. [CrossRef]