Introduction

Flowering time is a complex trait shaped by internal and external signals that interplay to determine reproductive success [1,2]. On the one hand, selection acts against mutations that cause flowering to occur during sub-optimal times of the year, such as in drought, freezing temperatures, or when pollinator abundances are low. On the other hand, plants that rarely experience optimal conditions must chance flowering eventually, or find alternative reproductive strategies (e.g. clonal reproduction and apomixis). Thus, flowering time pathways have evolved to both respond to, and buffer against, environmental variation. These seemingly opposing forces can be achieved either by integrating signals from parallel genetic pathways or by differentially utilizing related genes within the same pathway.

In the annual rosid species Arabidopsis thaliana (Brassicaceae) long day induced flowering is controlled by CONSTANS (CO)-mediated upregulation of the integrator protein FLOWERING LOCUS T (FT) in leaves [3–5]. FT protein is translocated through the phloem to the shoot apical meristem (SAM) where it binds with FLOWERING LOCUS D (FD) to induce the expression of floral promoters such as SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), APETALA1 (API), FRUITFULL (FUL), and LEAFY (LFY) [6–8]. Although largely functionally conserved across both long and short day-induced flowering plants [9–11], alterations of SOC1-like gene function have been implicated in flowering time and plant habit evolution [11]. For example, in perennial short day strawberry (Fragaria vesca, Rosaceae) SOC1 represses flowering through a novel regulatory interaction with the flowering repressor TERMINAL FLOWER1 (TFL1) [12]. Thus, fine-scale characterization of SOC1-like genes in diverse species has great potential to illuminate our understanding of flowering phenology and its evolution.

Petunia (Petunia hybrida, Solanaceae) is a short-lived perennial in the asterid lineage of core eudicots that is induced to flower by long day photoperiods [13]. The petunia genome contains three SOC1 homologs – UNSHAVEN (UNS)/FLORAL BINDING PROTEIN 21 (FBP21), but not FBP28, are positively correlated with developmental age. In contrast to A. thaliana, petunia SOC1-like gene expression did not increase with longer photoperiods, and FBP28 transcripts were actually more abundant under short days. Despite evidence of functional redundancy, differential spatio-temporal expression data suggest that SOC1-like genes might fine-tune petunia flowering in response to photoperiod and developmental stage. This likely resulted from modification of SOC1-like gene regulatory elements following recent duplication, and is a possible mechanism to ensure flowering under both inductive and non-inductive photoperiods.

Abstract

Flowering time is strictly controlled by a combination of internal and external signals that match seed set with favorable environmental conditions. In the model plant species Arabidopsis thaliana (Brassicaceae), many of the genes underlying development and evolution of flowering have been discovered. However, much remains unknown about how conserved the flowering gene networks are in plants with different growth habits, gene duplication histories, and distributions. Here we functionally characterize three homologs of the flowering gene SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) in the short-lived perennial Petunia hybrida (petunia, Solanaceae). Similar to A. thaliana soc1 mutants, co-silencing of duplicated petunia SOC1-like genes results in late flowering. This phenotype is most severe when all three SOC1-like genes are silenced. Furthermore, expression levels of the SOC1-like genes UNSHAVEN (UNS) and FLORAL BINDING PROTEIN 21 (FBP21), but not FBP28, are positively correlated with developmental age. In contrast to A. thaliana, petunia SOC1-like gene expression did not increase with longer photoperiods, and FBP28 transcripts were actually more abundant under short days. Despite evidence of functional redundancy, differential spatio-temporal expression data suggest that SOC1-like genes might fine-tune petunia flowering in response to photoperiod and developmental stage. This likely resulted from modification of SOC1-like gene regulatory elements following recent duplication, and is a possible mechanism to ensure flowering under both inductive and non-inductive photoperiods.

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flowering similar to Arabidopsis thaliana SOC1 [18]. However, the relative importance of UNS, FBP21, and possibly FBP28, in flowering time regulation and plant architecture has yet to be rigorously tested.

Here we use virus-induced gene (co)-silencing (VIGS) and gene expression analyses to functionally characterize the petunia SOC1-like genes. Our data suggest a conserved role for all three genes in the promotion of flowering. However, the contribution of each gene to flowering likely varies under different photoperiods and in an age-dependent manner.

Materials and Methods

Plant Materials and Growth Conditions

Petunia ‘Fantasy Blue’ (2PET131) seed from Seedman.com were grown and selfed for at least two generations under standard greenhouse conditions. For the photoperiod experiment, plants were either grown under long day (16 h light: 8 dark) or short day (8 h light: 16 h dark) conditions at 22 °C in controlled growth chambers. For VIGS experiments, plants were grown from seed in two batches at the University of Vermont greenhouse from September 2012 and August 2013 with supplemental light to mimic long days (16 h light: 8 h dark). Diurnal temperatures ranged from 20 to 24 °C. Significant differences in days to flowering and leaf number at flowering were determined using the analysis of variance (aov) and posthoc Tukey test functions in R.

Phylogenetic Analysis

SOC1-like gene sequences of representative angiosperms were downloaded from Genbank after a BLAST search with SOC1 from A. thaliana. The closest A. thaliana SOC1 paralogs AGL14, AGL19, AGL42, AGL72, and AGL71 were also downloaded for reference and to root the tree. Amino acid sequences were aligned manually in Mesquite version 2.75 [19] (File S1). Nucleotide sequences were then subjected to maximum likelihood phylogenetic analysis in GARLI under a GTR+I+C model of evolution with 200 bootstrap replicates [20].

Vector Construction and Plant Transformation

Previous results suggest functional redundancy of petunia SOC1-like genes under standard greenhouse conditions [15–17]. Thus, in order to target more than one petunia SOC1-like gene for silencing, but without affecting the expression of more distantly related paralogs, two VIGS constructs were designed based on approximately 200 bp sequences spanning part of the coiled-coil keratin-like and C-terminal domains of FBP21 and FBP28. Gene fragments were amplified from petunia floral cDNA with gene specific primers containing restriction fragment ends (Table S1), sequence verified, and cloned into the tobacco rattle virus 2 (TRV2) vector. Each VIGS vector was transformed into Agrobacterium tumefaciens strain EHA105. A 194 bp fragment of the petal pigment gene CHALCONE SYNTHASE (CHS) was also amplified, cloned, and ligated into TRV2 for use as an experimental control as previously described [21].
and Hileman et al. (2005 [22,23]). Twenty plants at the four-leaf pair stage for each silencing target were infiltrated in half their leaves with a 1:1 ratio of TRV1 and TRV2 using a needleless syringe for a total of 60 plants (CHS-, FBP21-, and FBP28-TRV2). To target all three SOC1-like genes for silencing, a batch of 20 plants was also infiltrated with TRV1, FBP21-TRV2, and FBP28-TRV2 in a 2:1:1 ratio.

Gene Expression and Phenotyping

RNA was extracted from leaves, SAMs, and nodes on the main stem of wild type plants at different developmental stages three hours post-zeitgeber, and in leaves at different times post-zeitgeber. Total RNA was extracted using TriReagent (Life Technologies) according to the manufacturer’s instructions, DNA was degraded using TURBO DNase (Life Technologies), and cDNA was synthesized using 1 μg RNA and iScript reverse transcriptase (BioRad). Primers for quantitative PCR (qPCR) of the housekeeping genes EF1alpha and UBQ5, UNS, FBP21, and FBP28 were designed in Primer3 [24] and tested for efficiency using Fast SYBR Green Master Mix (Life Technologies) as previously described [25]. qPCR expression of UNS, FBP21, and FBP28 was compared between ten plants positive for FBP21-TRV2 or FBP28-TRV2, and CHS-TRV2. Each VIGS positive plant was phenotyped for number of days from germination to anthesis, leaf number at anthesis, and petal whitening. Significant differences in gene expression were determined using the analysis of variance (aov) and posthoc Tukey test functions in R.

Results and Discussion

Petunia SOC1-Like Gene Function

Petunia SOC1-like Genes are Partially Redundant in the Control of Flowering Time

Infiltration of petunia seedlings with Agrobacterium resulted in a total of 24, 21, 37, and 4 plants positive for the CHS-, FBP21-, FBP28-, and FBP21/28-TRV2 constructs, respectively (Fig. 2A). Relative expression analyses on vegetative SAMs of flowering individuals revealed significant silencing of UNS and FBP21, but not FBP28, by the FBP21-TRV2 construct (Fig. 2B). Although the FBP28-TRV2 construct had high sequence similarity to UNS, FBP21, and FBP28, only FBP21 and FBP28 were silenced using this construct. This disparity might be explained by the number of
contiguous 21 bp matches between the three target SOC1-like genes, which is the minimum recognition length for RNA interference in plants. Thus, all three genes were targeted for silencing when plants were positive for FBP21- and FBP28-TRV2, and different combinations of two genes were silenced when positive for one construct alone.

In order to test the hypothesis that SOC1-like genes positively regulate flowering in petunia, days to flowering were calculated for each infected group of plants (Fig. 3). Data were compared between CHS-infected control plants, which all showed loss of anthocyanin accumulation in petals (Fig. 3A), and SOC1-like infected VIGS plants (Fig. 3B–E). The flowering response varied across plants as expected for incomplete and variable silencing, and between treatment blocks as predicted based on differences in ambient greenhouse light. Despite this, SOC1-like silenced plants in every category were later flowering on average than CHS-TRV2 control plants grown at the same time (Fig. 3D). The most extreme late flowering phenotypes were observed for the triple silenced plants positive for both FBP21- and FBP28-TRV2 (p < 0.001).

Moreover, plants silenced for UNS and FBP21 (FBP21-TRV2 vector) were later flowering (p < 0.001) than plants silenced for FBP21 and FBP28 (FBP28-TRV2 vector) (p = 0.015) when grown under the same conditions (Fig. 3D).

Petunia SOC1-like Genes are Differentially Regulated by Photoperiod and Age

Heterogeneous protein function is often correlated with differences in the spatiotemporal pattern of underlying gene expression. In order to determine if petunia SOC1-like genes are differentially regulated diurnally, during development, and in response to different photoperiods, qPCR was conducted on wild type tissues. No significant differences in SOC1-like gene expression levels were found across 16 hours of daylight. This result is in contrast to A. thaliana SOC1 expression, which peaks 12 hours post-zeitgeber in long day photoperiods [26]. Thus, petunia SOC1-like genes could potentially promote flowering in light under both long and short day conditions.

Transcript levels of UNS and FBP21 significantly increased in leaves (p < 0.05 and < 0.01, respectively) and SAMs (p < 0.05, respectively) during vegetative development (Fig. 4D and 4E). However, expression was not significantly different for FBP28, or any of the SOC1-like genes between vegetative and inflorescence apices (Fig. 4D–F). With the exception of FBP28, these data are consistent with reports of A. thaliana SOC1 expression [18, 27]. Following the transition to flowering at day 56, FBP21 decreased significantly (p < 0.001) in nodes from the plant base to the SAM (Fig. 4E). In contrast, no significant differences in nodes expression were detected for UNS and FBP28 (Fig. 4D and 4F).

Although petunia is a long day plant, flowering earlier with increasing daylight hours, FBP28 was significantly (p < 0.05) more
strongly expressed in the fully expanded top leaf 12 days post-germination when grown under short versus long days (Fig. 5). In contrast, there was no significant photoperiod effect on the expression of UNS and FBP21 (Fig. 5). Taken together, data from this experiment suggest subtle differences in the spatiotemporal regulation of petunia SOC1-like genes during vegetative development and by photoperiod, with FBP28 expression being the most divergent compared to A. thaliana SOC1 [18,27]. We hypothesize that FBP28 has been recruited to ensure flowering under short days in petunia, and that the regulatory elements of all three SOC1-like genes have evolved following their recent duplication.

Conclusions

Our data support the hypothesis that petunia SOC1-like genes have retained their function as flowering time pathway integrators following duplication [15,17]. This is in contrast to the closely related SOC1-like gene GhSOC1 in Gerbera hybridra (Asteraceae) that appears only to function in later flower development [28]. However, differential expression of UNS, FBP21, and FBP28 in response to photoperiod and age suggests subtly different developmental roles to both ensure and fine-tune flowering under variable environmental conditions. A similar mechanism has been assigned to duplicated FLOWERING LOCUS C (FLC)/MADS AFFECTING FLOWERING (MAF) genes in A. thaliana that respond to different low and high temperature cues [29]. Future studies determining the effect of co-silencing SOC1-like genes under variable photoperiod and temperature conditions will be needed to further test this hypothesis.

Supporting Information

File S1 SOC1-like gene nucleotide alignment. (NEX)

Table S1 Primer pairs used for VIGS and qPCR. (DOCX)

Author Contributions

Conceived and designed the experiments: JCP. Performed the experiments: JCP SAJ SGJ. Analyzed the data: JCP. Contributed reagents/materials/analysis tools: JCP. Wrote the paper: JCP SAJ SGJ.

References

1. Samis KE, Murren CJ, Bossdorf O, Donohue K, Fenster CB, et al. (2013) Longitudinal trends in climate drive flowering time clines in North American Arabidopsis thaliana. Ecol. Evol. 2: 1162–1180.

2. Colautti RI, Barrett SC (2013) Rapid adaptation to climate facilitates range expansion of an invasive plant. Science 342: 364–366.

3. Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, et al. (1999) Activation tagging of the floral inducer FT. Science 286: 1962–1965.

4. Suárez-López P, Whelan K, Robson F, Onouchi H, Valverde F, et al. (2001) CONSTANS mediates between the circadian clock and the control of flowering in Arabidopsis. Nature 410: 1116–1120.

5. Valverde F, Mouradov A, Soppe W, Ravencroft D, Samach A, et al. (2004) Photoreceptor regulation of CONSTANS protein in photoperiod flowering. Science 303: 1005–1006.

6. An H, Roussot C, Suárez-Lopez P, Corbesier L, Vincent C, et al. (2004) CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. Development 131: 3615–3626.

7. Huang T, Bohlenius H, Eriksson S, Parcy F, Nilsson O (2005) The mRNA of the late flowering Arabidopsis gene FT moves from leaf to shoot apex and induced flowering. Plant Cell 16: 1490–1505.

8. Andres F, Coupland G (2012) Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. Science 3296–3310.

9. Zhou C-M, Zhang T-Q, Wang X, Yu S, Lian H, et al. (2013) Molecular basis of age-dependent vernalization in Cardamine-flexuosa. Science 340: 1097–1100.

10. Mouhui K, Kuokura T, Kookota EA, Albert VA, Elenaa P, et al. (2013) The Fragaria vesca homolog of SUPPRESSOR OF OVEREXPRESSİON OF CON-
23. Hileman LC, Drea S, Martino G, Litt A, Irish VF (2005) Virus-induced gene silencing is an effective tool for assaying gene function in the basal eudicot <i>Papaver somniferum</i> (opium poppy). Plant J. 44: 334–341.

24. Rozen S, Skalesky H (2000) Primer3 on the WWW for general users and for biological programmers. In Bioinformatics Methods and Protocols. Methods in Molecular Biology. Edited by Krawetz S and Misener S. New Jersey: Humana Press; 365–386.

25. Preston JC, Hileman LC (2010) SQUAMOSA-PROMOTER BINDING PROTEIN 1 initiates flowering in <i>Antirrhinum majus</i> through the activation of meristem identity genes. Plant J. 62: 704–712.

26. Blázquez MA, Trénor M, Weigel D (2002) Independent control of gibberellin biosynthesis and flowering time by the circadian clock in Arabidopsis. Plant Physiol. 130: 1770–1775.

27. Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, et al. (2007) An “electronic fluorescent pictograph” browser for exploring and analyzing large-scale biological data sets. PLoS One 2: 718.

28. Ruokolainen S, Ng YP, Alberti VA, Eloemaa P, Teeri TH (2011) Over-expression of the <i>Gerbera hybrida</i> <i>At-SOC1-like</i> gene <i>Gh-SOC1</i> leads to floral organ identity deterioration. Annals Bot. 107: 1491–1499.

29. Pöö D, Verhage L, Ott F, Mathieu J, Angenhent GC, et al. (2013) Temperature-dependent regulation of flowering by antagonistic FLM variants. Nature 503: 414–417.

30. Becker A, Thieussen G (2003) The major clades of MADS-box genes and their role in the development and evolution of flowering plants. Mol. Phyl. Evol. 29: 464–489.