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Title Page

Expression of coffee florigen *CaFT1* reveals a sustained floral induction window associated with asynchronous flowering in tropical perennials

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Running title

CaFT1 is the *FLOWERING LOCUS T* ortholog in *Coffea* sp. L.

Highlight

Coffee florigen CaFT1 and related regulators revealed an extended floral induction window clarifying the asynchronicity and influence of environment for flowering in tropical perennial crops, providing perspectives to its control.

32 **Abstract**

33

34 The behavior of florigen(s) and environment-influenced regulatory pathways that control
35 flowering in tropical perennials with complex phenological cycles is poorly understood.
36 Understanding the mechanisms underlying this process is important for food production in the
37 face of climate change. To explore this, homologs of *Arabidopsis* florigen *FLOWERING LOCUS*
38 *T* (*CaFT1*) and environment-related regulators *CONSTANS* (*CO*), *PHYTOCHROME*
39 *INTERACTING FACTOR 4* (*PIF4*) and *FLOWERING LOCUS C* (*FLC*) were isolated from
40 *Coffea* sp. L. (Rubiaceae). Overexpression of *CaFT1* in *Arabidopsis* showed typical early-
41 flowering and yeast two hybrid studies indicated CaFT1 binding to bZIP floral regulator, FD,
42 demonstrates that CaFT1 is a coffee orthologue of florigen. Expression of *CaFT1* and floral
43 regulators were evaluated over one year using three contrasting genotypes: two *C. arabica* and
44 one *C. canephora*. All genotypes showed active *CaFT1* transcription from February until
45 October, indicating a potential window for floral induction. *CaCO* expression, as expected,
46 varied over the day period and monthly with day length, whereas expression of temperature-
47 responsive homologs, *CaFLC* and *CaPIF4*, did not correlate with temperature changes. Using
48 coffee as a model, we suggest a continuum of floral induction that allows different starting points
49 for floral activation, which explains developmental asynchronicity and prolonged anthesis events
50 in tropical perennial species.

51

52 **Keywords** - Floral induction and development; *FLOWERING LOCUS T* (*FT*); Asynchronous
53 flowering; *Coffea* sp. L. (Rubiaceae); Tropical perennial crops; Environment.

54

55 **Abbreviations**

56

57 BFT – BROTHER OF FT

58 CO – CONSTANS

59 FLC – FLOWERING LOCUS C

60 FM – Floral meristem

61 FT – FLOWERING LOCUS T

62 GA – Gibberellin

- 63 LD – Long day
64 MFT – MOTHER OF FT
65 PEBP - Phosphatidylethanolamine-binding protein
66 PIF4 - PHYTOCHROME INTERACTING FACTOR 4
67 SAM – Shoot apical meristem
68 SFT – TWEEN SISTER OF FT
69 SD – Short day
70 TFL1 – TERMINAL FLOWER 1
71 VM – Vegetative meristem

72
73

INTRODUCTION

74

75 In flowering plants, the transition of vegetative meristems (VMs) to a reproductive state
76 is mainly controlled by members of the phosphatidylethanolamine-binding protein (PEBP)
77 family that act as transcriptional coactivators (Cao *et al.*, 2016). The *Arabidopsis* *FLOWERING*
78 *LOCUS T* (*FT*) gene is the first PEBP member identified to encode a florigen; that is, FT
79 translocation via phloem from source leaves to VMs induces floral meristem (FM) production
80 (Wigge *et al.*, 2005; Corbesier *et al.*, 2007). To ensure proper reproductive timing, the FT
81 pathway integrates inductive exogenous stimuli, such as photoperiod, temperature, gibberellins
82 (GA) and vernalization, together the endogenous autonomous pathway (Amasino, 2010; Song *et*
83 *al.*, 2008; Kim *et al.*, 2009; Andrés and Coupland, 2012). The flowering process is very well
84 explored in model plants such as *Arabidopsis*, however, it is poorly understood in perennial
85 plants that have more complex reproductive cycles, such as *Coffea* sp. (Rubiaceae).

86 An example of this complexity is that perennial plants must maintain simultaneously in a
87 branch the shoot apical meristem (SAM) in a vegetative state to guarantee the continuity of plant
88 vegetative growth and reproduction in subsequent years, whereas older meristems at the base of
89 branches form FMs and then flourish (Amasino, 2009; Albani and Coupland, 2010). Thus, this
90 mode of reproduction is intrinsically connected with plant architecture indicating a precise
91 molecular control to determine the different forms of meristems (Wang *et al.*, 2009; Albani and
92 Coupland, 2010). The coffee phenological cycle is biennial, which means that it takes two years
93 to produce fruits (Teketay, 1999; Camargo and Camargo, 2001). The first year is characterized

94 by the floral induction and floral development until anthesis (or bloom) which, in *Brazil*, occurs
95 from January to March and in September, respectively (Camargo and Camargo, 2001;
96 Majerowicz and Söndahl, 2005; Morais *et al.*, 2008). Importantly, the floral induction period is
97 based on morphological observations, when vegetative and floral buds are distinguishable (de
98 Oliveira *et al.*, 2014), but without precise molecular characterization of the inductive floral
99 pathway (reviewed by López *et al.*, 2021 in press).

100 Organogenesis during coffee floral development was previously characterized
101 (Majerowicz and Söndahl, 2005; de Oliveira *et al.*, 2014) and shown to be affected by
102 environmental conditions that result in uneven development and sequential flowering (Camargo
103 and Camargo, 2001; DaMatta and Ramalho, 2006; DaMatta *et al.*, 2007). For example, in the G4
104 stage coffee buds enter a latent state during the cold and dry period of Brazilian winter in June to
105 August (Wormer and Gituanja, 1970; Camargo and Camargo, 2001; Morais *et al.*, 2008). After
106 this period, bud growth resumes in a quick passage from the G4 to G6 following the anthesis that
107 coincides with the rain period around September. Thus, this suggests that coffee flowering is
108 controlled by different factors, such as low temperatures and plant water potential (Crisosto *et*
109 *al.*, 1992; Majerowicz and Söndahl, 2005; DaMatta and Ramalho, 2006), in addition to
110 photoperiod, as commonly observed in angiosperms. Nevertheless, from the molecular point of
111 view, the mechanism involved in the perception of these environmental stimuli and its
112 relationship with the *FT*-dependent inductive pathway has never been explored in coffee.

113 The PEBP family of proteins can be divided into five subgroups defined by *Arabidopsis*
114 family members: *FLOWERING LOCUS T (FT)*, *TWIN SISTER OF FT (TSF)*, *TERMINAL*
115 *FLOWER 1 (TFL1)*, *BROTHER OF FT AND TFL (BFT)* and *MOTHER OF FT AND TFL*
116 *(MFT)*. These phosphatidyl ethanolamine-binding protein (PEBP) genes have important roles in
117 plant development, most notably floral induction and inflorescence architecture (Karlgrén *et al.*,
118 2011; Zhu *et al.*, 2021). Accordingly, PEBP members are highly conserved between flowering
119 species, especially *FT* and *TFL1* sequences (Turck *et al.*, 2008; Wickland and Hanzawa, 2015).
120 Interestingly, *FT* and *TFL1* proteins are highly similar, yet they have opposite functions. That is,
121 *FT* promotes floral meristem formation whereas *TFL1* represses flowering and maintains the
122 inflorescence in an indeterminate state (Kaneko-Suzuki *et al.*, 2018; Nakamura *et al.*, 2019).
123 These antagonistic roles are due *FT* and *TFL1* competition for the bZIP transcription factor *FD*
124 and 14-3-3 proteins to create a floral activation complex (FAC) or a floral repression complex

125 (FRC), respectively (McGarry and Ayre, 2012; Kaneko-Suzuki *et al.*, 2018). Another difference
126 is that FT protein accumulates in leaves and then moves long distances to the SAM (Yoo *et al.*,
127 2013), whereas *TFL1* is expressed in a subdomain of the SAM (Bradley *et al.*, 1997).

128 In *Arabidopsis*, long-day (LD) induction of *FT* expression is largely dependent on the B-
129 box transcription factor CONSTANS (CO), which is modulated by the circadian clock and day
130 length (Amasino, 2010; Andrés and Coupland, 2012; Liu *et al.*, 2014). Under LD conditions, CO
131 protein accumulates to a threshold sufficient to activate *FT* expression, whereas in short days
132 (SD) CO is degraded via the 26S proteasome complex and does not activate *FT* expression
133 (Suárez-López *et al.*, 2001; Valverde *et al.*, 2004; Zuo *et al.*, 2011). Other key transcription
134 factors (TFs) regulating *FT* are PIF4 and FLC, both responsive to temperatures changes and
135 activity depending on epigenetic changes in chromatin (Helliwell *et al.*, 2006; Kumar *et al.*,
136 2012; Madrid *et al.*, 2021). PIF4 is responsive to warmer temperatures and positively regulates
137 *FT* expression, whereas FLC is a repressor that is inactivated by prolonged cold periods
138 (vernalization) allowing *FT* expression and flowering initiation (Michaels *et al.*, 2005; Amasino,
139 2010). *FLC* homologs together with other MADS-box genes were described in *C. arabica* (de
140 Oliveira *et al.*, 2010; 2014) and related to drought (Barreto *et al.*, 2012) that occurs in the cold
141 period of the Brazilian winter. However, the interplay between the FT pathway and
142 environmental signals through the identification and analysis of *CO*, *PIF4* and *FLC* and their
143 roles in modulating floral induction is poorly understood in perennials and has never been
144 explored in coffee.

145 In addition to environmental effects, another regulatory factor is sugar signaling, which
146 plays an important role in plant development, including flowering regulation (Corbesier *et al.*,
147 1998; Lastdrager *et al.*, 2014; Sheen, 2014). Day length directly affects sucrose and starch
148 accumulation as a consequence of photosynthesis, which has been correlated with reproductive
149 induction (Corbesier *et al.*, 1998). Accordingly, varying day length of different seasons is
150 directly associated with the transition from vegetative to reproductive time in plants (reviewed
151 by Andrés and Coupland, 2012). A possible role for sucrose as a signal for flowering is
152 implicated by its influence on *FT* expression (Moghaddam and Van den Ende, 2013). Sucrose
153 has been shown to act in leaves, downstream of *CO* and upstream to *FT*, to affect flowering
154 (Corbesier *et al.*, 1998; Ohto *et al.*, 2001). Moreover, transcriptomic studies of maize floral
155 regulator *indeterminate1 (id1)* shows a correlation between sugar and starch metabolism and

156 floral induction in this autonomously flowering plant (Coneva *et al.*, 2007, 2012; Minow *et al.*,
157 2018).

158 Florigen and *FT* orthologs in diverse plant species are key integrators of reproductive
159 meristem specification via interconnecting molecular pathways that determine the proper floral
160 induction window (Amasino, 2009; He *et al.*, 2020). Despite conservation across plant species,
161 variable expression patterns of *FT* homologs have been reported (Coelho *et al.*, 2014; Cao *et al.*,
162 2016; Wolabu *et al.*, 2016; Štorchová *et al.*, 2019). This variable expression likely reflects the
163 variety of flowering evolutionary strategies required to tailor flowering time and floral
164 architecture diversification to particular geographical locations (Pin and Nilsson, 2012; Wickland
165 and Hanzawa, 2015; Jin *et al.*, 2020). Very little research on the mechanisms underlying floral
166 induction and its interplay with environmental-dependent pathways has been carried out in
167 *Coffea* sp, a tropical perennial crop of considerable socio-economic importance (IOC, 2021).

168 Here we report the first characterization of homologs of the key floral regulators *FT*, *CO*,
169 *PIF4*, and *FLC* from *C. arabica*, *C. canephora* and *C. eugenioides*. Through transgenic analysis
170 and protein interaction assays we demonstrate that *CaFTI* from coffee is a functional floral
171 inducer (an *FT* ortholog). To determine the precise floral induction window and establish a
172 possible correlation with environmental signals, expression of *CaFTI* was determined during a
173 year at two daily time points together with *FT* regulators *CaCO*, *CaPIF4*, and *CaFLC*.
174 Expression was analysed in three coffee genotypes with contrasting flowering patterns, two *C.*
175 *arabica* cvs. Acauã and IAPAR59 and *C. canephora* cv. Conilon, to examine related intra- and
176 interspecific variation. In addition, to investigate a possible connection between sugar levels,
177 gene expression and floral development, carbohydrate content was determined at the same time
178 points for all genotypes. Based on these results we propose that an extended floral induction
179 window for coffee could explain the asynchronous development and sequential flowering in this
180 perennial species. Whereas *CaFTI* expression was correlated to short day photoperiod and cold
181 (Brazilian winter), a similar pattern was not detected in the expression profiles of *CaCO*,
182 *CaPIF4*, and *CaFLC*, indicating a more complex process regulating the flowering process. Thus,
183 this work contributes to our understanding of the floral transition in perennial species and could
184 support future strategies aimed at mitigating asynchronous flowering.

185
186

MATERIAL AND METHODS

187

188 **Plant material**

189

190 Plant material used for RNA extraction, cloning, gene expression, carbohydrate content
191 (total soluble sugar, sucrose, reducer sugar, and starch) was obtained from 4-year-old coffee
192 plants of three different cultivars established at the National Institute of Science and Technology
193 (INCT-Cafe) experimental field at the Federal University of Lavras, Brazil (21°23'S, 44°97'W):
194 *C. arabica* genotypes IAPAR 59 and Acauã, and *C. canephora* Conilon. All plants were
195 cultivated under nutritional and pest control conditions recommended for coffee (Vieira, 2008).
196 Three biological repetitions of each coffee cultivar were distributed randomly in the field. Each
197 sampling consisted of a mix of three completely expanded leaves collected and immediately
198 immersed in liquid nitrogen and stored at -80 °C until RNA extraction. Samples were collected
199 at five-time points (December 2016, February, April, June, and October 2017) over a two-day
200 period, at 6:00 am and 5:00 pm, considered the start and end of the day, totaling 90 samples.
201 *Arabidopsis thaliana* var. Landsberg *erecta* (Ler) was used for *CaFTI* heterologous expression
202 studies following Coelho *et al* (2014) plant growth conditions. Plants were grown in growth
203 chambers under 16 hrs light / 8 hrs dark at 22 °C and 60 % humidity.

204

205 ***In silico* and phylogenetic analysis**

206

207 Searches for coffee homologs of *FLOWERING LOCUS T* (*FT*), *CONSTANS* (*CO*), and
208 *PHYTOCHROME INTERACTING FACTOR 4* (*PIF4*) were performed by sequence comparisons
209 using the BLAST tool (Johnson *et al.*, 2008). First, the described genes *FT* (At1G65480), *CO*
210 (At5G15840), and *PIF4* (At2G43010), and *FLC* (At5g10140) from *A. thaliana* were used as
211 queries (Lamesch *et al.*, 2012) against the *C. arabica* (NCBI:txid13443) annotated genome
212 deposited at the National Center for Biotechnology Information (NCBI) and the Coffee Genome
213 Hub (Denoëud *et al.*, 2014). To enrich searches for new coffee sequences phylogenetic analysis
214 also was performed with different homologs from various species, including *Arabidopsis*
215 *thaliana*, *Solanum lycopersicum*, *Brassica napus*, *Jatropha curcas*, *Nicotiana tabacum*, *Glycine*
216 *max*, *Oryza sativa*, *Zea mays*, *Sorghum bicolor*, *Solanum tuberosum* and *Populus nigra*. The
217 sequence for coffee *FLC*, *CaFLC*, was retrieved from the previously reported sequence (de

218 Oliveira *et al.*, 2010).

219 Putative homologous sequences (>80% similarity and e-Value < 0,005) were aligned by
220 ClustalX2 (Larkin *et al.*, 2007), analyzed by Genedoc software (Nicholas and Nicholas, 1997),
221 and with the translated nucleotide sequence to protein, phylogeny was inferred with the nearest
222 neighbour joining method in MEGA-X (Kumar *et al.*, 2018). Duplicate sequences were deleted
223 from the first phylogenetic tree and only considered *A. thaliana* and *S. lycopersicum* sequences
224 for the final tree (Fig. S1). The IDs of the used sequences are presented in the legend of Fig. S1.
225 To provide further evidence of the putative coffee FT as a floral inducer, key amino acids were
226 identified in alignment with CETS members from *A. thaliana* and *S. lycopersicum* (Fig. S2). The
227 conserved amino acids that distinguish the related proteins FT and TFL1, a floral inducer and a
228 repressor respectively (Ahn *et al.*, 2006; Wickland and Hanzawa, 2015; Jung *et al.*, 2016), are
229 shown in Fig. S2. Similar analyses were made to show the conserved amino acids between
230 coffee proteins and homologs of CO and PIF4 (Fig. S3 and S4).

231

232 **Gene cloning**

233

234 Total RNA from coffee leaves was isolated using Concert™ Plant RNA Reagent
235 (Invitrogen) following the manufacture's recommendation. RNA concentration and purity were
236 measured by spectrophotometric analysis (*GE NanoVue*™ Spectrophotometer). All samples
237 were treated with Ambion DNase I (RNase-free) kit (Thermo Fisher) and cDNA synthesized
238 using High-Capacity cDNA Reverse Transcriptase Kit (Thermo Fisher) following the
239 manufacturer's recommendation. Primers were designed for gene isolation with the putative
240 *CaFTI* sequence previously identified by *in silico* analysis and including the restriction sites
241 *SpeI* in the forward primer and *BsrGI* in the reverse (5'Fw = ATGCCTAGAGGGGGAGGAGA;
242 5'Rv = TTATCGTCTTCTGCCTC). The Polymerase Chain Reaction (PCR) was carried out
243 using *iProof* High-Fidelity DNA Polymerase (Bio-Rad) following the manufacture's protocol.
244 PCR fragments were isolated from 1 % agarose gel after electrophoresis and purified by
245 *GeneJET Gel Extraction Kit* (Thermo Fisher). The fragment generated was inserted into the
246 PJET1.2/blunt cloning vector (Thermo Fisher) and transferred to pK2WG7 plasmid (Thermo
247 Fisher), both previously digested using restriction enzymes, and then ligated with T4 DNA ligase
248 (New England Biolabs – NEB). All procedures followed the manufacturer's instructions.

249

250 **Plant transformation**

251

252 *Arabidopsis* var. *Landsberg erecta* (*Ler*) wild type plants (WT) and *ft* loss-of-function
253 mutants were transformed to overexpress *CaFTI* using the *Agrobacterium tumefaciens* strain
254 GV3101::pMP90 and floral dip protocol (Clough and Bent, 1998), using the isolated and cloned
255 *CaFTI* fragment under the control of the CaMV 35S promoter (Gene cloning above section).
256 Seeds from different transformation events were harvested separately for each background
257 genotype. Positive transformed seeds were screened in selective growth media with Kanamycin
258 30 ug/mL and, after 2 days of incubation in darkness and at 4 °C, they were maintained in a
259 growth chamber under continuous light for one week. Twenty positive T1 plants were transferred
260 to soil and after two weeks, DNA was extracted from leaves and PCR analysis was conducted to
261 confirm insertions. Overexpression of *CaFTI* driven by 35S CaMV was analyzed in 9
262 independent lines from 24 in T2 both *Ler* wild type (*Ler*-WT) plants as well as *ft* mutants.

263

264 **Yeast two Hybrid assay**

265

266 Protein-protein interaction analysis by Yeast Two Hybrid assay with CaFT against AtFD
267 and At14-3-3 was performed with the Matchmaker Gold Yeast Two-Hybrid System (Chien *et*
268 *al.*, 1991). The *CaFTI* sequence was amplified by *iProof* High-Fidelity DNA Polymerase (Bio-
269 Rad) with restriction enzyme site tag insertion (Fw-*EcoRI* and Rv-*BamHI*), inserted to pBridge
270 plasmid by T4 DNA ligase and transformed in DH5α *E. coli* competent cells. Gold Yeast was
271 transformed with *CaFTI*-pBridge (Bait) and *AtFD*-pGADT7-RecAB (Prey); and *CaFTI*-pBridge
272 (Bait) and *At14-3-3*-pGADT7-RecAB (Pry). AtFD and At14-3-3 were screened from
273 *Arabidopsis* GoldY2H library.

274

275 **Gene expression analysis (RT-qPCR)**

276

277 The primers for Reverse Transcription quantitative Polymerase Chain Reaction (RT-
278 qPCR), shown in Table S1, were designed using as template the *CaFTI*, *CaCO* and *CaPIF4*
279 sequences identified in this work by *in-silico* analysis, whereas for *CaFLC*, the sequence was

280 previously characterized by de Oliveira *et al.* (2010). Primers were designed following
281 specifications for standard qPCR such as amplicon length (between 80 and 150 bases), having no
282 sequences at the conserved domain, 40% to 60% GC content, and others as suggested by MIQE
283 (Bustin *et al.*, 2009) and examined for hairpins and dimers with the Oligoanalyzer Tool IDT
284 (<https://www.idtdna.com/pages/tools/oligoanalyzer>). The genes *CaUBQ2* and *CaMDH* and their
285 described primers were used as reference genes (Martins *et al.*, 2017). RNA extraction, DNase
286 treatment, and reverse transcription reaction were performed as described above (Gene cloning
287 session). RT-qPCR analysis was performed using 15 ng of cDNA to a final volume of 15 μ L
288 reaction with Rotor-Gene SYBR® Green PCR Kit (Qiagen), in a Rotor Gene-Q (R)
289 thermocycler (Venlo, Netherlands). Mix reagents: 7.5 μ L of SYBR-green (QuantiFast SYBR
290 Green PCR Kit - Qiagen), 3.0 μ L of forward and reverse gene-specific primers, 1.5 μ L of cDNA
291 at 10 ng/ μ L, and 3 μ L of RNase-DNase-free water, resulting in at 15 μ L final volume. Three
292 biological repetitions were used, run in duplicate, and amplification was performed following
293 manufacturer's instructions. Relative expression differences were calculated by log₂ of fold
294 change and statistical analysis by Linear Mixed Model as described by Steibel *et al.* (2009).

295

296 **Carbohydrate Content Analysis**

297

298 Carbohydrate quantification was carried out following methods adapted by Meireles da
299 Silva *et al.* (2014) with modifications: 100 mg of fresh frozen tissue previously powdered with
300 liquid nitrogen were homogenized in 5 mL potassium buffer (100 mM, pH 7.0) followed by 30
301 min incubation in a water bath at 40 °C. Supernatant was collected after 10 min centrifugation at
302 10,000 xG and stored at -20 °C. Pellets were used for starch extraction by 5 mL potassium
303 acetate buffer (200 mM, pH 4.8) resuspended with 16 units of amyloglucosidase enzyme,
304 followed by two hours at 40 °C and 20 min of centrifuge at 10,000 xG for supernatant separation
305 and starch quantification. Starch, sucrose, and total soluble sugars were quantified according to
306 Dische (1962) and, reducing sugar levels according to Miller (1959).

307

308 **Statistical analysis**

309

310 A Linear Mixed Model was used for statistical analysis of biochemistry quantification by

311 the lme4 R package (Bates *et al.*, 2015), and adjustment parameters following Oliveira *et al.*,
312 (2020) statistical methods. RT-qPCR statistical analysis was carried out by log₂ of fold change
313 by Linear Mixed Model as described by Steibel *et al.* (2009).

314
315

RESULTS

316

317 Identification of *FT*, *CO* and *PIF4* homologs from *Coffea* sp.

318

319 To identify *FT* sequences and other genes related to floral regulatory pathways, such as
320 *CO* and *PIF4*, we searched for homologous genes in *C. arabica* (NCBI:txid13443) and *C.*
321 *canephora* (<https://coffee-genome.org/>) genome databases. Through phylogenetic analysis,
322 sequences were compared to their homologs in other species to identify the most similar
323 counterparts as putative orthologs (**Fig. S1**). Genome analysis of homologous sequences
324 identified seven *FT*-like genes, four *CO*-like genes and four *PIF4*-like genes. The PEBP family
325 members, which include FT-like (**Fig. S1-A**), were separated into three subgroups based on
326 previous reports: *FT-LIKE* (*FT* and *TSF*), *TFL1-like* (*TFL1* and *BFT*) and *MFT-like* (*MFT*)
327 (Karlgrén *et al.*, 2011; Nasim *et al.*, 2017).

328 Sequence alignments of PEBP homologs, including those from *Arabidopsis thaliana* and
329 *Solanum lycopersicum* (**Fig. S2**), revealed conserved amino acids in *Coffea* sequences related to
330 FT-like floral inducers. For example, the conserved Y85 amino acid residue reported to be a
331 hallmark of FT orthologs supports the identified coffee genes as a possible florigen (Hanzawa *et*
332 *al.*, 2005). These FT-like sequences used in further functional characterization were identical in
333 *C. arabica* and *C. canephora* and here both are referred to as *CaFTI*. Similar genomic analysis
334 was undertaken for CO-like (**Fig. S3**) and PIF4-like genes (**Fig. S4**), which showed conserved
335 motifs indicating they are putative *Coffea* sp. orthologs.

336

337 Overexpression of *CaFTI* in *Arabidopsis* and protein interaction analysis suggest that it is a 338 *Coffea* sp. *FT* ortholog

339

340 We explored *CaFTI* function by over-expressing this gene in *Arabidopsis thaliana*
341 Landsberg *erecta* (*Ler*) to examine the effects on flowering. In addition, yeast two hybrid (Y2H)

342 assays were performed to test conserved protein-protein interactions between CaFT1 and
343 components of the Arabidopsis floral regulatory complexes. In 24 independent lines from T2
344 generations of *CaFT1* overexpression driven by the 35S CaMV promoter, nine independent lines
345 caused a strong early flowering phenotype in both *Ler* wild type (*Ler*-WT) plants as well as *ft*
346 mutants. Transgenic plants flowered 12 days after germination (DAG) in contrast to *Ler*-WT
347 plants, which flowered at 28 days (**Fig. 1A**). Accelerated transition from vegetative to
348 reproductive development was also indicated by the reduced number of rosette leaves initiated in
349 both *Ler*-WT and *ft* mutant backgrounds (**Fig. 1B**).

350 Known conserved components of the *Arabidopsis* Floral Activation Complex (FAC)
351 include FT and FD together with a 14-3-3 protein (Kaneko-Suzuki *et al.*, 2018). Y2H assays with
352 CaFT1 were carried out to test whether it interacts with AtFD and a 14-3-3 protein. CaFT1
353 protein was shown to interact with AtFD (**Fig. 1C**) as expected, since both proteins are reported
354 as partners to form a protein complex (Abe *et al.*, 2005; Wigge *et al.*, 2005; Corbesier *et al.*,
355 2007; Jung *et al.*, 2016). However, despite the 14-3-3 protein being a component of the FAC
356 with FT and FD proteins, no interaction was detected between CaFT1 and At14-3-3, suggesting
357 diversification on 14-3-3 homologs and/or components of the FAC in *Coffea* sp. Together these
358 results strongly support a role for CaFT1 as a floral inducer and that the selected *CaFT1* gene is a
359 functional FT ortholog in *Coffea* sp.

360

361 ***CaFT1*, *CaFLC*, *CaCO*, *CaPIF4* show variable expression patterns over the course of a year**

362 Cultivars of the same perennial species can show differential floral development patterns,
363 especially flowering time (reviewed by Lopez *et al.*, 2021 in press). Such variation between
364 cultivars is important for breeding programs to derive different cultivar genotypes with
365 contrasting floral development times (Carvalho, 2008). Relevant to this study, variable flowering
366 patterns were observed for the *C. arabica* cvs. Iapar 59, Acauã and *C. canephora* cv. Conilon
367 cultivars (**Fig. S5**). To determine the floral induction window and explore a possible intra- and
368 inter-specific diversification of *CaFT1* related to the contrasting flowering patterns of coffee
369 cultivars, the expression of *CaFT1* in leaves of these genotypes were examined by RT-qPCR
370 over the course of a year (**Fig. 2**).

371 Despite quantitative differences, all coffee genotypes showed a clear “bell shaped”
372 pattern of *CaFT1* expression during the year for both morning and afternoon samples. *CaFT1*

373 expression was observed in leaf samples from February, and increased progressively to
374 maximum expression in June, followed by drastically reduced transcript levels in October and
375 December (**Fig. 2A**). This result was correlated with the precise floral induction window,
376 extending and encompassing the described emergence of floral buds (Majerowicz and Söndahl,
377 2005; de Oliveira *et al.*, 2014). Comparing genotypes, *C. arabica* cv. Iapar 59 and *C. canephora*
378 showed similar values for *CaFTI* expression throughout the year and independent of the time
379 period, whereas *C. arabica* cv. Acauã showed lowest expression levels in February and highest
380 in April and June (**Fig. 2A**). This result can be associated with phenotypic differences between
381 early- and late flowering coffee cultivars (**Fig. S5**).

382 Moreover, *FT* and florigen genes from diverse plant species have been shown to act as a
383 hub for floral meristem activation by integrating different interconnected pathways responsive to
384 environmental signals (Amasino, 2010; Andrés and Coupland, 2012). Thus, to gain insights into
385 the regulation of *CaFTI*, the co-expression expression of photoperiod and thermosensitive FT
386 regulators, as *CaCO*, *CaFLC* and *CaPIF4* (**Figs. S1 to S4**) were also determined at the same
387 time points (**Fig. 2B, 2C and 2D**). Expression analysis showed that *CaFTI* transcription is
388 detected first in leaves collected in February and increases gradually until it reaches a maximum
389 in June. This period coincides with the shorter day length photoperiod and colder temperatures,
390 typical of the winter in coffee-growing regions of Brazil (**Fig. 4A and S6**).

391 For all coffee genotypes, expression of *CaCO* was higher in the morning compared to
392 afternoon (**Fig. 2B**), in agreement with the photoperiod-dependent regulation of *CO* orthologs
393 reported in other plants (Suárez-López *et al.*, 2001; Zuo *et al.*, 2011). In the morning, *CaCO*
394 expression presents a “smile shape” (or inverted bell shape) pattern throughout the year and was
395 quite similar among genotypes, which means higher values in December and October and lower
396 in the middle timepoints. This result correlates with the photoperiod of longer day length (**Fig.**
397 **4A and S6-A**). In afternoon samples, *CaCO* expression was lower and stable with few
398 differences observed for genotypes, except in April and October. Comparing genotypes, in the
399 morning *CaCO* was expressed at lower levels in cv. Conilon than *C. arabica* both cvs. for all
400 time points throughout the year.

401 Regarding thermosensitive FT regulators, expression of *CaFLC* and *CaPIF4* was
402 detected in all months for all three coffee genotypes, with few differences during the year in the
403 morning, but varying in the afternoon (**Fig. 2C and 2D**). In the afternoon, *CaFLC* expression

404 presents different patterns and maximum levels depending on genotype, for *C. arabica* cv. Iapar
405 59 it is in December and cv. Acauã in April and *C. canephora* cv. Conilon June. In the morning,
406 no significant differences were found for *CaFLC* expression between *C. arabica* genotypes
407 while cv. Conilon were always expressed less. Similar to *CaFLC*, *CaPIF4* expression in leaves
408 showed little variation throughout the year, and no expression differences between coffee
409 genotypes were observed during the day or throughout the year, except for cv. Acauã in October
410 **(Fig. 2D)**.

411 The *CaFLC* and *CaPIF4* expression did not vary with the temperature changes in Brazil
412 **(Fig. 4A and S6-B)**, with the lowest values in June and highest in October and December,
413 except for *CaFLC* in June and afternoon for cv. Conilon. Moreover, and importantly, *CaFLC* and
414 *CaPIF4* expression did not correlate with the expression pattern of *CaFTI* since it is expressed at
415 higher levels in June when the putative negative regulator *CaFLC* is expressed and *CaPIF4* did
416 not change. In addition, no differences or expression correlations were found for precipitation
417 and humidity **(Fig. S6-C and D)**. Thus, these results suggest that the floral inductive *CaFTI*
418 pathway is not influenced, or at least to a lesser extent, by these thermosensitive floral FT
419 regulators.

420

421 **Analysis of carbohydrate content in coffee leaves shows correlation with *CaFTI* expression**

422

423 Sugars provide energy to all metabolic processes in plants and, with respect to the
424 preparation for reproductive development, possibly expression of florigens such as *FT* as well
425 (Corbesier *et al.*, 1998; Ohto *et al.*, 2001; Cho *et al.*, 2018). To examine a possible correlation
426 with the *CaFTI* expression pattern and the energy status of coffee leaves, the carbohydrate
427 content (total soluble sugar, sucrose, reducing sugar and starch) in adult plants was quantified at
428 two daily timepoints over the course of a year.

429 Sucrose, starch, total soluble sugar and reducing sugars were quantified from the same
430 samples used for expression analysis **(Fig. 2)**. Results show patterns of distribution for the
431 different coffee genotypes **(Fig. 3)**. In general, all genotypes showed higher values for Total
432 Soluble sugars (TS) and Sucrose (SC) in December and April **(Figs. 3A and B)**, the summer
433 period in Brazil, which is hotter and has more hours of daylight **(Figs. 4A, S6-A and B)**. Similar
434 results were observed for sucrose, but it was noted that cv. Iapar 59 had higher levels in all

435 months (**Fig. 3B**). For reducing sugars (Fig. 3C), all genotypes had similar levels throughout the
436 year, with *C. arabica* cv. Acauã showing higher values at all timepoints, except April. Finally,
437 the quantification of starch levels showed a contrasting pattern between coffee genotypes. For
438 example, the cv. Iapar 59 and cv. Conilon showed a clear pattern of increasing values from
439 December to October in the next year, differing only at the highest timepoints, June and April,
440 respectively. Whereas the cv. Acauã showed lower starch levels overall compared to other
441 genotypes and the highest levels in October (**Fig. 3D**).

442 These findings showed different patterns among the three coffee genotypes that could be
443 related to differences observed for *CaFTI* expression (**Fig. 2A**) and, consequently, flowering
444 time (**Fig. S5**). Thus, sucrose and other soluble sugars were the only types analysed whose levels
445 correlated with the period of higher *CaFTI* expression, in April and June (Fig. 2A). Since
446 sucrose is the main transport sugar (Lemoine, 2000) it is possible that *CaFTI* expression could
447 be responsive to the sugar state. Similarly, a correlation between the energy status and flowering,
448 as well as *FT* expression with trehalose-6-phosphate was established in *Arabidopsis* (Wahl *et al.*,
449 2013; Fichtner *et al.*, 2021).

450
451

DISCUSSION

452

453 ***CaFTI* function and expression revealed the floral induction window in coffee that could be**
454 **associated with the asynchronous flowering in perennial species**

455

456 Previous studies focused on understanding the transition from juvenile to reproductive
457 phase in perennial species have demonstrated that *FT*-related genes are involved in floral
458 induction and dormancy time in response to seasonal stimuli in trees (Böhlenius *et al.*, 2006).
459 However, the link between regulatory pathways of floral activation and asynchronous flowering
460 is still poorly understood. First, in this work we demonstrate that overexpressing *CaFTI* in
461 *Arabidopsis*, wild type and *ft-1* mutants, causes the typical early flowering phenotype and that
462 *CaFTI* interacts with AtFD (**Fig. 1**), a component of the floral activation complex (Kaneko-
463 Suzuki *et al.*, 2018). Similar results were found for other annual or perennial plants when *FT*
464 homologs were overexpressed in *Arabidopsis* and/or also in their respective species; for
465 example, Poplar and apple (Kotoda *et al.*, 2010; Tränkner *et al.*, 2010), tomato (Cao *et al.*, 2016),

466 *Eucalyptus* (Klocko *et al.*, 2016), *Medicago* (Laurie *et al.*, 2011) and blueberry (Gao *et al.*,
467 2016). Thus, CaFT1 can act as a florigen in *Arabidopsis* strongly suggesting same function in
468 coffee plants as observed in other perennials. Further studies demonstrating that CaFT1 can act
469 as a florigen in *Coffea* sp. will need to overcome the difficulty of transforming this species
470 (Ribas *et al.*, 2011).

471 In coffee, the meristem floral transition window is reported to occur from January to
472 March (Camargo and Camargo, 2001; Majerowicz and Söndahl, 2005). To understand the
473 molecular events occurring at this transition, here we characterize an FT homolog from coffee
474 that acts as a florigen and show that it is transcriptionally active for a longer period than the
475 previously described window (Fig. 2A). *CaFT1* expression extends from February to October,
476 reaching a maximum in June, which is a more precise characterization to indicate the potential
477 floral induction window. Such a window overlaps with the entire period of floral bud
478 development, which was interpreted as a continuum of induction (Fig. 4B). This extended
479 window explains the formation of floral buds at the base of branches (older buds) together with
480 induction of newly formed buds from the SAM at the distal end of branches (Fig. 4C). Floral bud
481 development starts at different times until anthesis in September, when *CaFT1* expression
482 decreases rapidly, followed by vegetative growth of new branches, restarting the cycle (**Fig. 4B**
483 **and C**). Moreover, our finding suggests that the developmental time is also different, or, in other
484 words, floral meristems induced early (at the base of the branch) develop slower than later ones
485 (those closer to SAM). Thus, the *CaFT1* expression patterns observed in this study suggest that
486 the prolonged expression window is associated with the asynchronous flowering behavior of
487 coffee (**Fig. 4B and C**). Moreover, due to the conserved nature of flowering mechanisms, we
488 speculate that this occurs in other tropical perennials plants.

489 In addition, the *CaFT1* expression pattern in coffee supports evidence that floral
490 induction occurs only in axillary meristems because the FT from the leaves is distributed along
491 the branch controlling floral induction to proximal meristems (McGarry and Ayre 2012). This
492 also may explain the asynchronous flowering behavior of *Coffea* sp L., in which floral bud
493 development occurs at axillary meristems from branches formed at different times and does not
494 produce terminal flowers from the SAM (**Fig. 4C**). Alternatively, from the expression results
495 (**Fig. 2 and 4B**), it is possible that *CaFT1* other functions in addition to floral induction, since it
496 is reported to have a role in a wide range of developmental processes such as fruit set, vegetative

497 growth, stomatal control and tuberization (Pin and Nilsson, 2012). Further study is required to
498 explore this possibility.

499

500 **Coffee floral transition and photoperiodic stimulus**

501

502 Despite the extended window of *CaFTI* expression, it is clear from the analysis with
503 three genotypes that there is a conservation of the maximum level in June (Fig. 2), raising the
504 question of whether there is a correlation with environmental cues during the period (Fig. 4). The
505 florigen encoded by *FT* and its orthologs from numerous plants has been shown to induce the
506 floral transition in response to temperature and photoperiod (Tsuji, 2017). With the isolation of
507 an *FT* orthologue in coffee, this analysis can be extended to floral induction in tropical species
508 that are subjected to high temperatures throughout the year. The expression profile of *CaFTI*
509 (**Fig. 2A**) may show less variation with respect to photoperiod effects, possibly because there is
510 less daylight variation in equatorial regions compared to temperate zones.

511 Examination of flowering control in trees provides evidence that CONSTANS (CO)
512 protein is responsible for *FT* ortholog expression induction by its accumulation in long days
513 (LD) (Böhlenius *et al.*, 2006). However, despite *CaCO* showing expression variation in response
514 to day length (**Fig 2B**), its expression over the course of the year showed an inverse relationship.
515 The *CaCO* showed a variable expression pattern for all three coffee genotypes with higher values
516 reached in the morning, according to the circadian clock that is mainly responsive to photoperiod
517 (Valverde *et al.*, 2004). Comparing expression over the year, *CaCO* was higher in December and
518 October (**Fig. 2B**), coinciding with summer in the Southern hemisphere and the period of
519 greatest solar light incidence (**Fig. 4 and S6-A**). This was opposite to the pattern since *CaFTI*
520 expression is higher during the Brazilian winter (Fig. 2A), with the shortest day length (**Fig. 4A**
521 **and S6-A**) and more highly expressed in summer in December and October (**Fig. 2A**). Other
522 factors might be involved in the CO regulatory pathway of *CaFTI* expression; for example,
523 Wenkel, *et al.*, (2006) showed that the protein HEME ACTIVATOR PROTEIN2 (HAP2) or
524 HAP3 can create a complex with CO that causes a reduction in the expression of *FT*.

525

526 **Expression of *CaFTI* is not correlated with *CaFLC* and *CaPIF4* expression, suggesting**
527 **alternative thermoregulatory pathways that are not influenced by temperature**

528

529 In regions where temperatures vary during the year, floral initiation and dormancy for
530 some species can be controlled by cold exposure or vernalization (Michaels *et al.*, 2005; Kim *et*
531 *al.*, 2009; Madrid *et al.*, 2021). In the *Arabidopsis* vernalization pathway, *FLC* expression is
532 repressed after prolonged exposure to colder temperatures, thus activating *FT* expression and
533 allowing floral meristem induction (Michaels and Amasino, 1999; Helliwell *et al.*, 2006; Aikawa
534 *et al.*, 2010). On the other hand, *PIF4* is responsive to warmer temperatures and positively
535 regulates *FT* expression (Kumar *et al.*, 2012). Thus, we hypothesized that *FLC* and *PIF4*
536 homologs in coffee could be related to thermal-sensitive pathways, possibly regulating *CaFTI*
537 expression. Here we use them as a probe to evaluate co-expression patterns that are integrated
538 with temperature changes during the growing season.

539 In this work, *FLC* homologs were found in coffee, which is interesting given that it is a
540 tropical plant that does not normally experience freezing temperatures. In line with this, *CaFLC*
541 expression was not associated with temperature variation, as might be expected. A similar result
542 was found for *CaPIF4* expression (Fig. 2C and D, Fig. 4A and S6-A). Over the course of the
543 experiment, when the lowest temperatures were registered in June and the highest occurred in
544 December, *CaFLC* and *CaPIF4* showed stable expression patterns or changes in different
545 periods not correlated with these temperature changes. Moreover, comparing *CaFTI* and *CaFLC*
546 expression patterns, there was no inverse co-expression correlation as would be expected if *FLC*
547 repressed *FT* activity, nor was there a positive correlation with *CaPIF4*, suggesting it is not an
548 *FT* inducer. Overall, expression of *CaFTI* was highest in June, during the Brazilian winter (Fig.
549 2A, Fig. 4A and S6-A), suggesting positive regulation by cold or even drought, typical
550 characteristics of the Brazilian winter. Further analysis under controlled conditions will
551 demonstrate whether *CaFTI* is responsive to cold and/or drought. In addition, *CaFLC* expression
552 varied between coffee genotypes, with Iapar 59 and Acauã showing similar expression, whereas
553 Conilon showed lower expression levels throughout the growth period and higher in afternoon
554 samples of June (Fig. 2C). These differential expression patterns suggest intra- and inter-specific
555 transcriptional differences, which might coincide with contrasting flowering behavior (DaMatta
556 and Ramalho, 2006), or it could be associated with greater phenotypic homeostasis of the
557 allotetraploid *C. arabica* than its diploid parents in response to different temperature conditions
558 (Bertrand *et al.*, 2015).

559 These results show no evidence that the *FT*-dependent floral transition pathway is
560 regulated by coffee *FLC* or *PIF4* homologs, thus the potential roles of *CaFLC* and *CaPIF4* in
561 flowering of tropical perennials awaits further study. Alternatively, despite tropical species such
562 *Coffea* sp. having sequences that are homologous to *Arabidopsis FLC* and *PIF4*, they may have
563 functions not necessarily related to floral control in response to temperature variation. The
564 finding that *CaFLC* expression varied widely in different coffee tissues such as, roots, leaf, SAM
565 at all floral and fruit developmental stages (de Oliveira *et al.*, 2014) suggests functions other than
566 flowering repression, such as coordinating organogenesis together with *SOC1* as reported in
567 *Arabidopsis* (Deng *et al.*, 2011). In support of a possible role in organogenesis, *CaFLC*
568 expression is upregulated in response to drought (Barreto *et al.*, 2012), which coincides with the
569 growth latency in the G4 stage interpreted as a dormant stage (Wormer and Gituanja 1970;
570 Majerowicz and Söndahl, 2005). At present, however, no direct mechanism for this has been
571 described yet (reviewed by Lopez *et al.*, 2021 in press).

572 Future research will decipher whether *CaFLC* and *CaPIF4* are involved in flowering, and
573 their relationship with environmental signals and the *FT*-regulatory pathways. In addition, both
574 genes are regulated by chromatin epigenetic changes in *Arabidopsis* (Helliwell *et al.*, 2006;
575 Kumar *et al.*, 2012; Madrid *et al.*, 2021), a very little explored field of research in *Coffea* sp. and
576 crop perennials in general.

577

578 **The role of carbohydrates in coffee floral induction as a model for perennials**

579

580 Sugars have been shown to be important chemical signals that affect flowering, as
581 strongly supported in model plants like *Arabidopsis* (Wahl *et al.*, 2013; Cho *et al.*, 2018;
582 Fichtner *et al.*, 2021). Since no correlation was found between *CaFTI* expression and possible
583 environment-related regulators, we examined sugar levels to assess their role as possible
584 regulators in tropical perennial species. Gene expression analysis showed association with Total
585 Soluble Sugar and Sucrose levels (**Fig. 3A and B**) in relation to temperatures during the five
586 analyzed months, showing an association with warmer periods of the year (**Fig. 4A and S6-A**).
587 This association is related to higher levels of TS and SC and coincided with *CaFTI* expression
588 levels (**Fig. 2A**). As previously described by Cho *et al.* (2018) and, in accordance with our
589 results (**Fig. 4**), sugar levels, a product of photosynthesis, can change according to seasons in

590 response to more hours of light and higher temperature.

591 Sugar variation is an important indicator of reproductive phase initiation, with evidence
592 that it affects the expression of floral integrators, such as *FT* (Rolland *et al.*, 2006; Moghaddam
593 and Van den Ende, 2013). In our study, sugar accumulation was higher in December and April,
594 the summer period in Brazil that is hotter and has more hours of daylight (**Fig. S6-A**).
595 Interestingly, these results coincide with two relevant periods associated with *CaFTI* expression
596 - in December, before the increase in *CaFTI* expression in February, and in April which is a
597 period before *CaFTI* expression peaks in June. Whether this pattern suggests that sugars act as a
598 stimulus for *CaFTI* expression requires further evidence (**Fig. 1A**). Association of *CaFTI* with
599 TS and sucrose levels suggest that its expression is responsive to carbohydrate signals, showing a
600 possible connection between sugars and floral induction in perennial species. Thus, it will be
601 interesting to determine whether energy status is a key regulator of *CaFTI* expression and
602 controls coffee floral development, further suggesting that environmental factors are indirectly
603 involved since photo-assimilate production is affected by photoperiod and temperature (Pego *et*
604 *al.*, 2000; Lastdrager *et al.*, 2014). Accordingly, we demonstrate that sugar content in coffee
605 plants changes in response to temperature regimens (de Oliveira *et al.*, 2020). These findings
606 support previous studies demonstrating a correlation between *FT* expression pattern and
607 carbohydrate content (Corbesier *et al.*, 1998).

608
609

CONCLUSIONS

610

611 Floral induction in perennial plants is a poorly understood process that integrates
612 endogenous and environmental factors. Elucidating the molecular components of this floral
613 regulatory process is a necessity in the face of imminent climate change to secure food
614 production (Howden *et al.*, 2007; Lobell *et al.*, 2011; Zhao *et al.*, 2017). In this work we
615 identified the coffee *FT* ortholog and determined its expression profile to describe the precise
616 and potential floral induction window. This analysis includes the analysis of key *FT* regulators
617 responsive to environmental signals, evaluation of climatic parameters and sugar content over
618 the period of one year. Together, our results indicate a continuum of florigen transcription,
619 conserved between contrasting coffee genotypes, that could underlie asynchronous floral
620 development and flowering. The environment-related floral regulators, *CaCO*, *CaFLC* and

621 *CaPIF4*, were not co-expressed as expected with *CaFTI*, whereas a correlation was found with
622 sugar content, which is affected by environmental changes over the course of a year. This
623 suggests that *CaFTI* is not directly regulated by these genes, but that there may be a connection
624 between sugar metabolism and florigen function in coffee. Thus, the present work contributes to
625 comprehending asynchronous flowering in tropical perennial plants and provides a basis for
626 targeting molecular components in crop breeding programs.

627

628 **SUPPLEMENTARY DATA**

629

630 Table S1 shows all the primers used in this work; figures S1 to S4 show the phylogenetic
631 analyses and amino acids alignments for the gene families *FT*, *CONSTANS*, *FLC* and *PIF4*;
632 Figure S5 shows a photo panel of the plagiotropic branches with floral meristems at different
633 stage of development comparing *C. arabica* cvs. Iapar 59 and Acauã and *C. canephora* cv.
634 Conilon in three different timepoints; Figure S6 shows the variation of photoperiod, temperature,
635 precipitation and relative humidity during the experiments at the experimental field of Federal
636 University of Lavras (UFLA, MG/Brazil).

637

638

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640

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650

651 **AUTHORS' CONTRIBUTIONS**

652

653 C.H.C., R.R.O. and A.C-J. conceptualized the project. C.H.C., conducted all experiments and
654 data analyses. L.P.P. participated in the collection of plant materials, RNA extractions and the
655 RT-qPCR execution. V.L. and C.F. participated in the yeast two hybrid and heterologous
656 expression analyses. T.H.C.R. assisted bioinformatic and statistical analyses. R.R.O., J.C. and
657 A.C-J. conceived of and supervised the experiments and data analyses. C.H.C. wrote the
658 manuscript. R.R.O., J.C and A.C-J revised the manuscript and contributed to writing.
659

660

661 **CONFLICT OF INTEREST**

662

The authors declare that there is no conflict of interest.

663

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670

671 **DATA AVAILABILITY**

672

Main data supporting the findings of this study are available within the paper and within its
673 supplementary materials published online. The raw data used for analyses and figures are
674 available from the corresponding author, Antonio Chalfun-Júnior, upon request.

675

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- 929

930

FIGURE LEGENDS

931

932 **Figure 1 – Functional analysis of *CaFTI*.** A – Heterologous overexpression of *CaFTI* in
933 *Arabidopsis thaliana* Ler ecotype causes early flowering. From left to right: Wild-type (*Ler*-
934 WT); 35S::*CaFTI* construct in *Ler*-WT background; *FT* loss-of-function (*Ler-ft*); 35S::*CaFTI*
935 construction with *Ler-ft* background. The overexpression of *CaFTI* driven by 35S CaMV was
936 analyzed in 9 independent lines from 24 in T2 both *Ler* wild type (*Ler*-WT) plants as well as *ft*
937 mutants. **B** – Rosette leaf number compared between *Ler*-WT / 35S::*CaFTI*, *Ler-ft* /
938 35S::*CaFTI*, *Ler*-WT, and *Ler-ft*. **C** - Yeast Two Hybrid Protein-protein interaction assay: Yeast
939 transformed with Empty plasmid, *AtFD*, and *At14-3-3* inserted into pGADT7-RecAB plasmid
940 (Prey), against *CaFTI* inserted into pBridge plasmid (Bait). Transformed yeast grown in SD
941 medium as negative control and SD/-LTH/X- α -Gal as select medium and, as positive control
942 *Arabidopsis* FT interacted with *Arabidopsis* FD in SD/-LTH/X- α -Gal select medium.

943

944 **Figure 2 - RT-qPCR expression analysis of putative floral regulators genes in leaves**
945 **collected over one year from three different coffee genotypes: *C. arabica* cvs. Iapar 59 and**
946 **Acauã and *C. canephora* cv. Conilon.** Expression analysis were performed for the genes
947 previously characterized (Fig. 1 and S1 to S4), *CaFTI* (A), *CaCO* (B), *CaFLC* (C) and *CaPIF4*
948 (D). Leaf samples of different coffee genotypes were used. *C. arabica* cvs. IAPAR59 (black
949 bars) and Acauã (white bars) and *C. canephora* cv. Conilon (gray bars). Samples collected at the
950 first hours of the day (morning) and at the last hours of the day (Afternoon) in December of 2016
951 (DEC) and February (FEB), April (APR), June (JUN) and October (OCT) of 2017. Relative
952 expression differences were calculated as log₂ fold Change by Linear Mix Model as described
953 by Steibel, *et al.* (2009). Letters at the top of the bars indicate the statistical differences, in which
954 lower case letters represent comparisons between genotypes in each month, and capital letters
955 compare the same genotype at different time points during the year.

956

957 **Figure 3 – Carbohydrate content quantification from coffee leaves in microgram per gram**
958 **of fresh weight.** Samples collected from two *Coffea arabica* cultivars, Iapar 59 (red line), Acauã
959 (blue line), and one *Coffea canephora* cultivar, Conilon (purple line), collected along five
960 months of a year (DECEMBER, FEBRUARY, APRIL, JUNE, and OCTOBER). **A** – Total

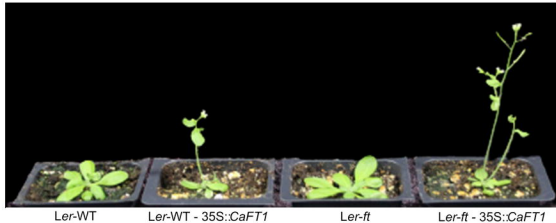
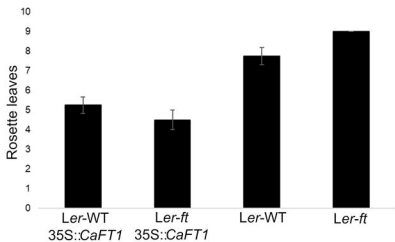
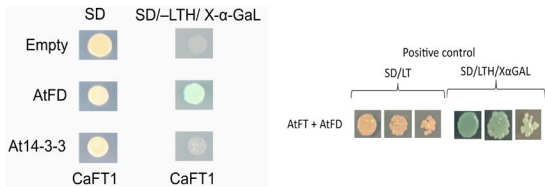
961 soluble sugar; **B** – Sucrose; **C** – Reducing sugar; **D** – Starch. Each sampling data set is
962 represented by a point in the line with the standard deviation. All cultivars in each timepoint
963 were represented by 3 biological samples and two technical replications.

964

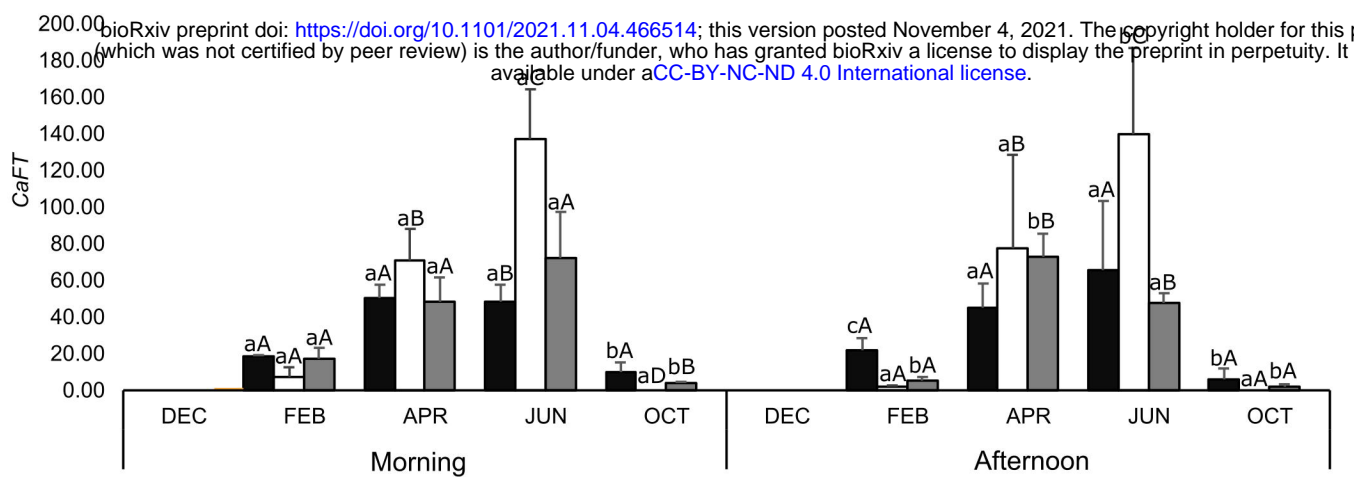
965

966 **Figure 4 – The floral induction window in coffee trees as a model for perennial species.**

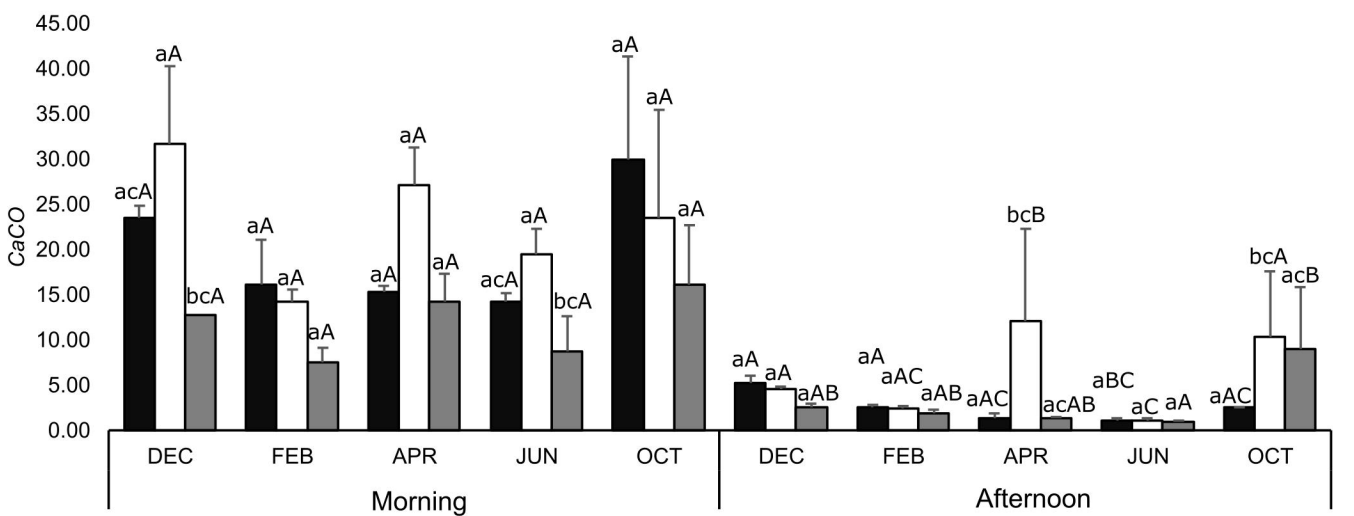
967 Figure **A**, left axis of the graph represents the daylight hours variation divided by 24 hours which
968 is separated in yellow color (day) and light blue color (night) in the graphic area. Right axis
969 represents degree values (Celsius) from minimum (blue line), mean (purple line), and max (red
970 line) temperature variation over the year; **B**, proposed floral induction window from February
971 until October based on variation of *CaFTI* expression levels, total soluble sugars (TSS) and
972 sucrose (SUC) content over the year. Gradients of color intensity (dark blue indicates higher
973 level, light blue lower levels and white not detectable) represents the content level in terms of
974 gene expression, total soluble sugar, and sucrose variation. The floral development stages
975 observed in plants when the leaves were collected for the five time points (months) are also
976 indicated in the figure; **C**, shows a plagiotropic branch with floral buds in the same development
977 in both parts, the early formed branch (brown and lignified stem) and the late formed branch
978 (green stem). Because the late formed branch originates from early nodes, the continuous
979 vegetative activity of the shoot apical meristem (SAM) along the year (details in Figs. S5 e S6),
980 in this figure suggests a need for extended florigen activity to induce axillary meristems
981 formation at different times. Interestingly, floral meristems induced at different times reach
982 flower buds at the same time.

A**B****C**

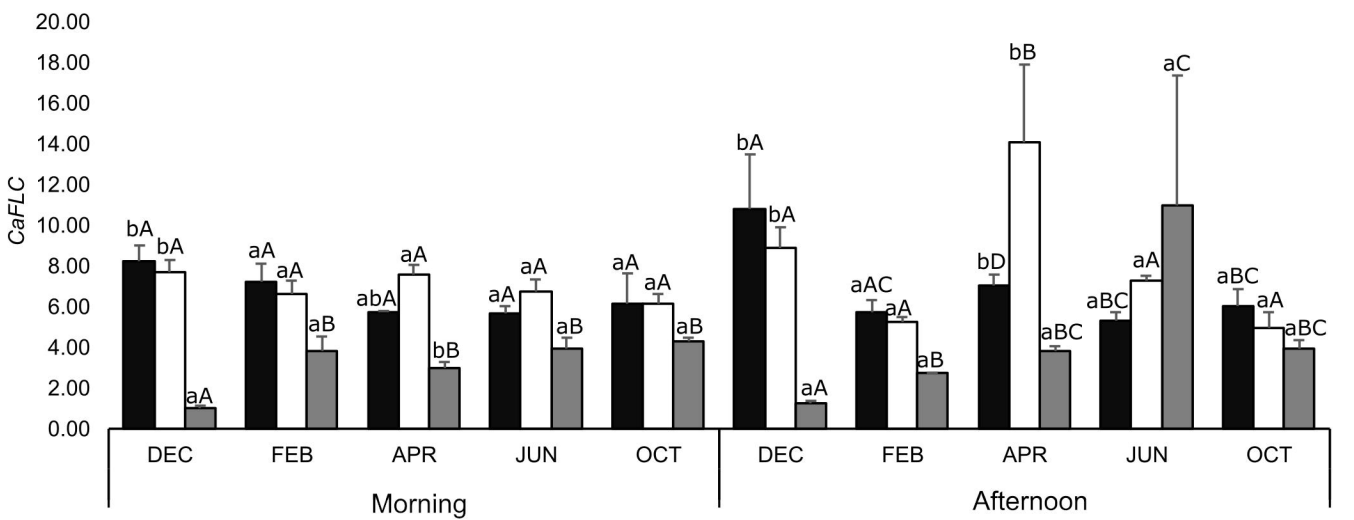
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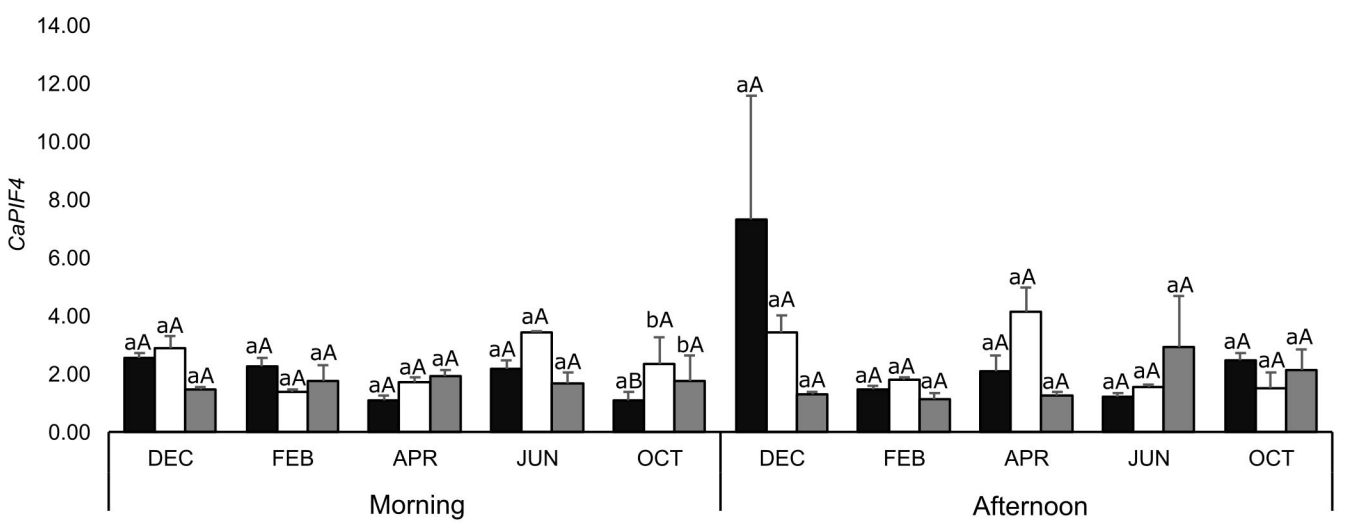
B



C

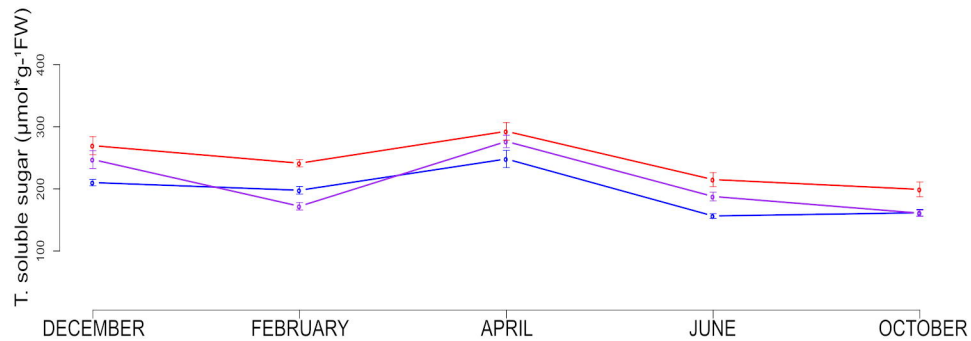


D

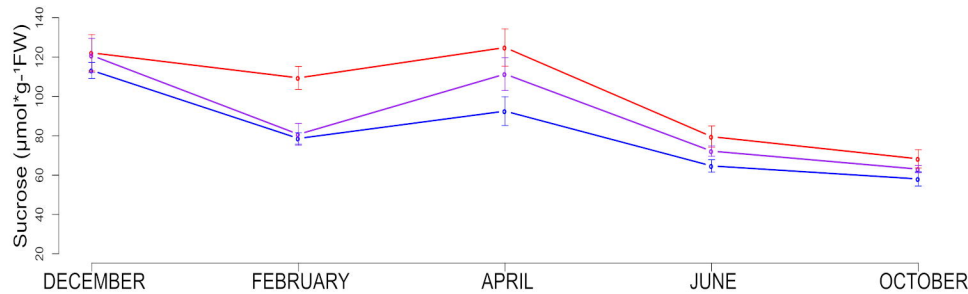


■ Iapar59 ■ Acauã ■ Conilon

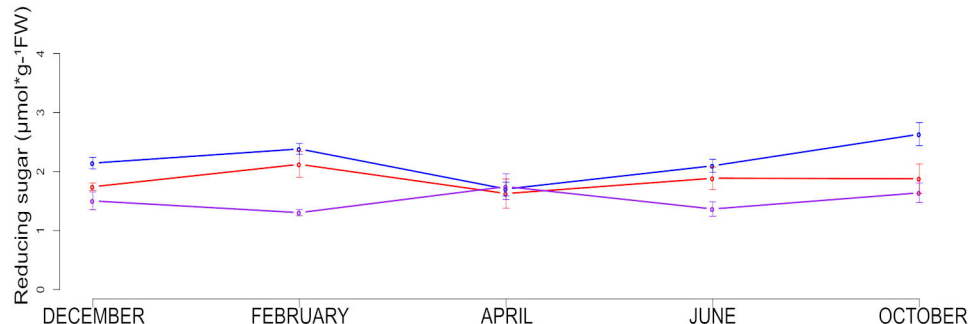
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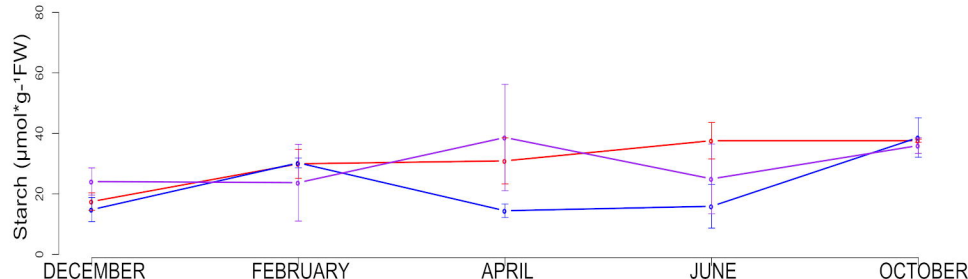
B

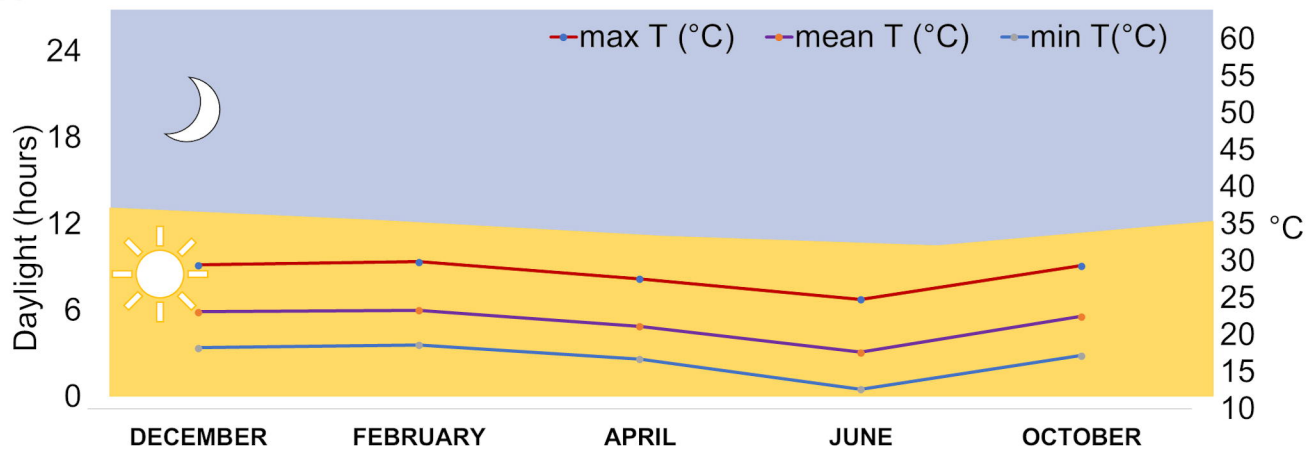
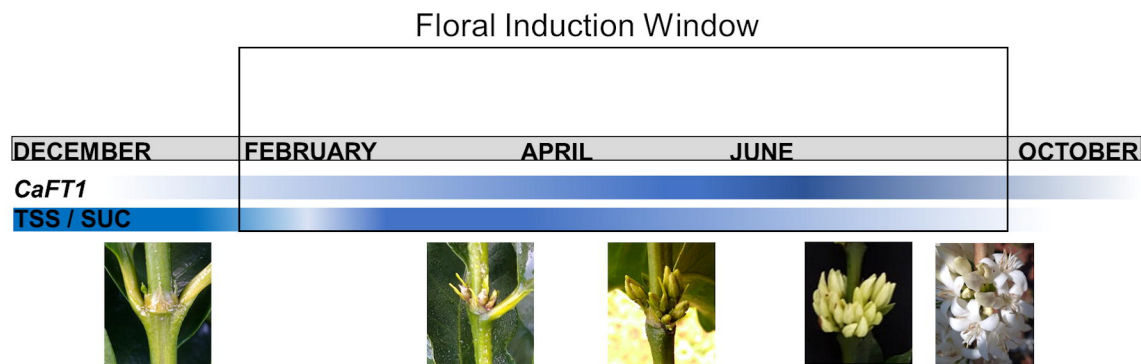


C



D



A**B****C**