PHOTOPERIODIC RESPONSES OF *CANNABIS SATIVA*:

DEVELOPMENTAL AND MOLECULAR ASPECTS

by

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ABSTRACT

The timing of reproductive development is influenced by photoperiod (daylength) in many plants, including *Cannabis sativa*. However, the developmental and molecular details and the variability of photoperiodic responses in *C. sativa* are not well understood.
I evaluated the photoperiod sensitivity of four high-CBD drug-type *Cannabis* varieties for four stages of reproductive development by comparing the timing of each stage between plants that received different periods of long-day (LD) exposure prior to the short-day (SD) treatment. In addition, I looked at the influence of photoperiod on the duration between different stages of development, and the effect of plant age on photoperiod sensitivity.

The timing of each stage, including the induction of solitary flowers and anthesis was accelerated in 'Eden' under SD conditions. 'Grape Indica' started developing inflorescences in 6 weeks under LD, but the process was also faster when SD treatment began earlier. The development of 'Auto Pivot', on the other hand, was slightly delayed under SD, but each stage occurred with a consistent number of leaves in all treatments. 'Russian Auto' had a shorter juvenile phase and showed accelerated inflorescence development under SD, but earlier stages were not accelerated even though SD development happened with fewer leaves at each stage. Also, older plants responded to SD slightly earlier, especially compared to plants directly started in SD. These results suggest there are important differences between *C. sativa* varieties in terms of the timing and the process of reproductive development, even within the day-neutral/auto-flowering varieties. I identified four *FLOWERING LOCUS T (FT)-LIKE* genes in the *Cannabis* genome and analyzed their diurnal expression patterns in 'Eden' and 'Auto Pivot' under LD and SD. *FTL2* showed a similar expression pattern to *FT* genes that are known to induce flowering in other SD species, and the expression in photoperiod-sensitive variety, Eden, was different between photoperiod treatments, while it was mostly unaffected in the day-neutral variety, Auto Pivot, suggesting *FTL2* could be a potential photoperiod-regulated floral promoter in *C. sativa*.

THE INFLUENCE OF PHOTOPERIOD ON REPRODUCTIVE DEVELOPMENT OF CANNABIS VARIETIES

Introduction

Plants show remarkable seasonal patterns in development, especially in the development of flowers. While these patterns are noticeable and knowledge of them is important for successful agriculture, the investigation of the environmental factors that influence flowering time began only about a hundred years ago. The works of Julien Tournois on hops and hemp are considered the earliest experiments on the relationship between flowering time and environmental factors. His choice of species allowed him to observe the effect of daylength (photoperiod) on flowering time. Later, Garner and Allard's experiments on tobacco, soybean, and other species have independently shown the importance of daylength for reproductive development (Garner and Allard 1920).

The timing of flowering is extremely important for the reproductive success of plants. Flowering too early or too late can result in failed pollination, seed development or dispersal (Gaudinier and Blackman 2020). Instead of responding to a single environmental factor, plants have evolved to integrate many sensory and internal states in order to timely flower. Based on extensive *Arabidopsis thaliana* studies, the mechanisms of endogenous and environmental signaling are distinguished as molecular pathways that show widespread crosstalk: the photoperiod, light-quality, vernalization, temperature, GA, age/sugar, and autonomous pathways (Hyun et al. 2017; Kim 2020; Simpson and Dean 2002; Yang et al. 2013; Yu et al. 2013).

Plant age and growth influence flowering time with endogenous sugar levels providing a cue for juvenile-to-adult transition (vegetative phase change) (Yang et al. 2013; Yu et al. 2013).

As juvenile plants do not respond to inductive environmental signals, this pathway acts as another mechanism to avoid premature flowering and ensure there is enough photosynthates available to support reproductive development. The autonomous pathway is thought to be independent of environmental factors and acts on flowering time by releasing floral repression via Flowering Locus C (Cheng et al. 2017). In contrast to the endogenous pathways just mentioned, plant hormone gibberellins (GA) are also known to promote or inhibit flowering depending on the species and the daylength conditions (Thomas and Vince-Prue 1996), and the GA pathway seems independent of the photoperiod- and vernalization-dependent pathways (Kim 2020).

Plant populations occupy different parts of the world, and they need to rely more on certain environmental signals than others to regulate flowering depending on their location. In the parts of the world where the temperatures drop below freezing in the winter, plants that grow in the fall need to make sure they do not develop flowers before the winter passes. For this reason, some plants show a vernalization requirement, meaning they do not flower under favorable conditions unless they have been exposed to cold for some time, so they are not misled by other signals. In addition to this particular effect of cold temperatures, warmer ambient temperatures tend to accelerate flowering (Samach and Wigge 2005).

Beyond the ratio of light wavelengths, photoperiod-sensitive plants respond to daylength by integrating their circadian rhythm with the timing of perceived light signals. Long-day plants (LDP) flower when the daylength is above a certain critical daylength unique to the plant, whereas short-day plants (SDP) flower when the daylength is below the critical daylength. These groups are further divided into obligate and facultative types. Flowering is delayed under non-inductive daylengths for facultative LDP/SDP, but obligate LDP/SDP do not flower at all under non-inductive daylengths. However, these four types do not capture the highly variable responses, and plants can be further classified into more groups, including long-short-day plants and short-long-day plants (requiring exposure to both daylengths in order), intermediate-day plants (flowering promoted in intermediate daylengths), amphiphotoperiodic plants (inhibited by intermediate daylengths). It is also possible to classify photoperiod sensitive plants into five groups based on their separate photoperiod requirements (or lack thereof) for the initiation and development of flowers (Kinet et al. 2018; Thomas and Vince-Prue 1996).

While each of these mechanisms is critical for adaptation to a particular (natural) environment, some of them are eliminated in cultivated plants as a result of domestication and breeding, especially photoperiod and vernalization requirements (Gaudinier and Blackman 2020). As much as the daylength provides information about the calendar date and the upcoming seasonal changes, it is also misleading in different geographical locations. For instance, short-day plants growing in higher latitudes experience longer daylengths and shorter seasons, which results in late or complete lack of flowering, and death before seeds can mature. Therefore, the photoperiod requirement needs to be relaxed or completely eliminated for successful flowering and seed development in both wild and agronomic environments, as observed in multiple legume species (Williams et al. 2022). Similarly, plants that lack vernalization requirement, such as spring-sown varieties of barley and einkorn wheat) can be utilized for spring planting in some environments (Gaudinier and Blackman 2020).

Cultivated cannabis is considered a facultative short-day plant (Lisson et al. 2000; Salentijn et al. 2019; Van der Werf et al. 1994), meaning that flowering can be accelerated when daylengths become shorter than the critical photoperiod. The range of critical photoperiod varies from 11 hours to 15.5 hours in cultivated cannabis varieties (Moher et al. 2021), though it has been suggested that some varieties might have a critical period of more than 20 hours (Heslop-Harrison and Heslop-Harrison 1969). It was also shown that photoperiod can influence not only the time of flowering but also the duration of inflorescence development in cannabis, suggesting the existence of multiple critical photoperiod thresholds for different stages of reproductive growth (Potter 2009). However, there are also natural populations of *Cannabis* that have adapted to northern latitudes and flower irrespective of daylength, thus considered day-neutral (McPartland 2018; Small 2016). Recently, the introgression of day-neutral cannabis plants into cultivated drug-type and seed-type lines allowed the development of day-neutral or fast-flowering lines (Crawford and Crawford 2019; Rodziewicz and Kayser 2020; Small 2018).

Uniform and timely flowering of cannabis plants is desirable in most agronomic settings. The photoperiod requirement implies the necessity of selection for a narrow range of critical daylength within the population for separate locations. In addition, the optimal timing of floral induction and further reproductive development in terms of yield and quality is somewhat different for varieties bred for different purposes. Generally, extending the vegetative phase as much as possible increases the yield of drug-type, seed-type, and fiber-type plants, except for some exceptions. In fiber-type cannabis, increasing the vegetative phase and reducing flowering events by exposing the plants to 24-hour photoperiod increased the fiber at the expense of quality (Van der Werf et al. 1994). Depending on the separation method, the optimal harvest time is either when the male plants start to flower, or 3-4 weeks after male flowering (beginning of seed maturity in females) (Keller et al. 2001). On the other hand, seed-type plants are constrained by the risk of frost and by seed shattering. As mentioned above, flowering should begin early enough to ensure seed maturation before the growing season ends. Additionally, since seeds (achenes, to be more accurate) shatter as they reach maturity, uniform seed development within the plant and the field is desirable. This uniformity could be improved by determinate growth, a trait potentially sensitive to photoperiod, or by modifying traits like plant architecture. Despite these details, combined harvest of seed and fibers from dual-purpose varieties is common. Still, longer vegetative phases can become undesirable if the plant architecture cannot support the dense inflorescences. Extending the vegetative phase can also come with the risk of major yield loss due to disease or insect damage. The use of day-neutral or early-flowering varieties becomes a necessity in higher latitudes. However, they can be utilized for denser planting due to their smaller sizes, or for multiple harvests per season where the weather permits (Stack et al. 2021).

The variation of responses among cannabis plants and varieties to different photoperiods and other environmental factors is not well-documented. While earlier studies on fiber- and seed-type plants show the photoperiod response to be facultative (flowering still occurs under long-days, although it is delayed), it is commonly noted (often in the context of drug-type varieties) that cannabis plants can be maintained vegetatively for years, as long as they are exposed to long-days, and used for clonal propagation (Clarke and Merlin 2016; Clarke and Watson 2007), which seems to be a common practice in the medical/recreational cannabis industry. There is also one recent study in which cuttings from a 1.5-year-old mother plant (of a drug-type variety) were taken to investigate the accumulation of somatic mutations (Adamek et al. 2022), documenting the existence of obligate short-day cannabis plants in the scientific literature. The obligate short-day response might be specific to certain high-THC drug-type varieties that are bred for and under indoor settings.

A recent study put forward an interesting argument regarding the short-day characterization of *Cannabis*. In this study involving drug-type cannabis plants, it was observed that single, solitary flowers were induced along the stem even under long-days, in plants that show no sign of inflorescence development (Spitzer-Rimon et al. 2019). While recognizing the effect of photoperiod on shoot-to-inflorescence transition, the authors concluded that *Cannabis* is day-neutral, as photoperiod-sensitivity is traditionally considered in relation to the (time of) induction of flowering. However, the study did not compare the timing of solitary flower induction between short-days and long-days, so their claim regarding the induction of solitary flowers remains to be tested. Regardless of this technical point about a biologically interesting trait, it is useful to consider the photoperiod-sensitivity of *Cannabis* in terms of inflorescence development, as solitary flowers on otherwise vegetative plants have no practical/agronomic importance. Furthermore, it is not known how common this trait is in different varieties. In a recent study on high-CBD varieties, it was observed that First Light™ cultivars do not produce axillary solitary flowers before inflorescence development begins, whereas 'Late Sue' showed axillary solitary flowers about three months before the inflorescence development (Stack et al. 2021).

Similarly, the flowering of day-neutral cannabis is another important area that needs to be explored. The effect of different environmental factors (temperature, water/nutrient availability, stress, etc.) on their flowering time, and how it varies between different day-neutral varieties is unknown. Understanding these factors would allow maintaining the optimal parameters in indoor cultivation, and choosing the right planting date, fertilization, and irrigation regimes in field settings, as well as breeding varieties tailored to different environments to optimize production or minimize agronomic inputs.

The following experiment (detailed in the next section) was designed to observe the effect of photoperiod on different stages of reproductive development, the relationship between vegetative growth and time to respond to short-days, and the effect of short-days on the development of day-neutral plants.

Materials and Methods

Cannabis varieties with varying photoperiod-sensitivity were identified. Seeds of two day-neutral (‘Auto Pivot’, ‘Russian Auto’), one early-flowering (‘Grape Indica’) and one late-flowering (‘Eden’) photoperiod-sensitive (as labeled by the distributor) drug-type high-CBD varieties were obtained from Hemp Seed Producers and grown in a growth room at the Plant Growth Center of Montana State University. The light intensity was between 430 and 590 µmol m^{-2} s^{-1} at the bench level (spectral quality of LED lights is shown in Figure 1.1), and the temperature ranged between 25-28 °C.

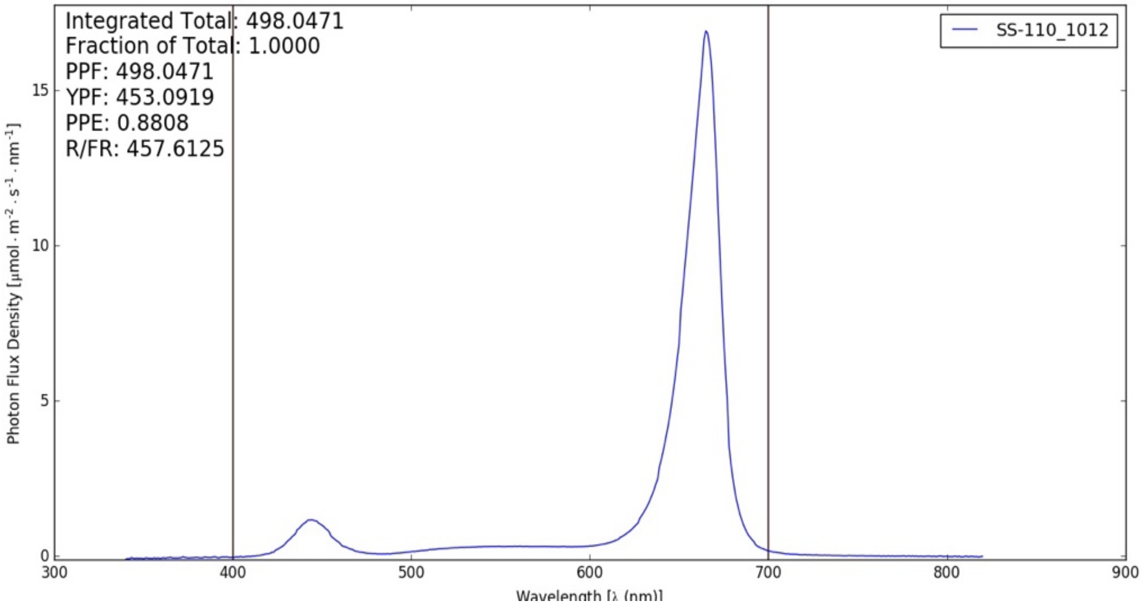


Figure 1.1 Relative spectral photon flux density distribution of the light-emitting diode (LED) fixture

The photoperiod was manipulated to create different lengths of exposure to long-day and short-day. The photoperiod was initially set to long-days (18:6 h light:dark) and changed to short-days (8:16 h light:dark) after six weeks (on day 42). Each variety was planted at two-week intervals to vary the number of days spent under long-days before the initiation of short-days.

The first treatment group was planted on day 0 and received 42 long-days, the second group 28 long-days, and the third group 14 long-days. The fourth treatment group was planted on day 42 and did not receive any long-days, starting directly under short-days.

Seeds were planted in 2.5 L plastic containers with Sunshine Mix #1 (3:1 peat:perlite) and fertigated as needed with Jack's Nutrients Tap 15-5-20 (100 ppm N). Containers were randomized to 58 positions and moved to the next position every three days to minimize the effect of variable light intensity. All male plants and flowered female plants were removed to create space for the next treatment group. The experiment was repeated in the same growth room.

Reproductive traits and plant growth were examined to determine photoperiod sensitivity. Plants were observed daily and scored for the first day of visible solitary flowers located at the leaf petiole base (Figure 1.2a, see (Spitzer-Rimon et al. 2019) for morphological details of *Cannabis* flowers), the first day of open flowers (Figure 1.2b), the day the shoot apex showed signs of shoot-to-inflorescence transition to inflorescence development (Figure 1.2c), and the day inflorescence formed with at least three open flowers (Figure 1.2d). When the first undifferentiated structures at the base of leaf petioles failed to develop further or developed into shoots (as observed in some plants of 'Grape Indica'), the first primordia that developed open flowers were scored as the first visible solitary flowers instead. The total number of nodes on the main stem was also recorded at each event.

Figure 1.2 Different stages of reproductive development of female *Cannabis* plants recorded in this study. Undifferentiated flowers at the leaf axil. (b) Open female flowers (several days after anthesis). (c) Raised shoot apex marking the shoot-to-inflorescence transition. (d) Early inflorescence development with several open flowers.

In order to determine the effect of treatments, ANOVA was used. Each trait was separately analyzed with ANOVA including variety, long-day treatment, replication, and their three-way interaction as predictor variables. For the models that did not meet the assumptions of linear regression, Box-Cox (days to solitary flowers, anthesis, shoot-to-inflorescence transition, and inflorescence development, and number of nodes at solitary flowers, anthesis, shoot-to-inflorescence transition, and inflorescence development) or logarithmic (days from first flower to anthesis, anthesis to shoot-to-inflorescence transition, shoot-to-inflorescence transition to inflorescence development) transformations were performed. The response variables that included negative values were adjusted to positive values prior to logarithmic transformation. Pairwise comparisons between long-day treatments and varieties were made using Tukey's HSD and statistical significance was determined at a = 0.05.

Results

Characterizing the photoperiod sensitivity of *Cannabis* involves some technical issues. There are multiple ways to measure photoperiod sensitivity. For example, it can be measured as the change in the mean number of days to flowering (chronological age) between different photoperiod treatments, or as the change in the mean number of leaves at flowering (developmental age) (Thomas and Vince-Prue 1996). Another issue is that different stages of reproductive development can be affected by photoperiod in different ways (Kinet et al. 2018; Thomas and Vince-Prue 1996), and in some cannabis varieties, the axillary solitary flowers develop without shoot-to-inflorescence transition (Spitzer-Rimon et al. 2019). To account for these issues, the influence of photoperiod on the cannabis varieties used in this experiment are measured and analyzed in different ways and for multiple stages.

Characterization of Photoperiod Sensitivity Based on two Stages of Inflorescence Development

The 'flowering' of *Cannabis* is usually understood as the development of inflorescences, as the development of solitary flowers at the leaf axils alone does not have much agronomic importance. In this section, the influence of photoperiod on the chronological age of flowering is analyzed for two stages of early inflorescence growth.

The first visual signs of shoot-to-inflorescence transition were followed by the formation of rapidly developing flowers, forming inflorescences. The effect of photoperiod on these developmental stages was initially measured by comparing the time it takes for a studied variety to reach that developmental stage when exposed to varied photoperiod lengths. Here, photoperiod-sensitivity is characterized based on the mean number of days it takes for a variety to reach two stages of inflorescence development (shoot-to-inflorescence transition and inflorescence development) in different photoperiod treatments. If development is significantly accelerated with earlier short-day (SD) exposure, that is taken as an indication of photoperiod-sensitive development.

‘Eden’ is described as photoperiod sensitive by the distributor, and I tested it for the traits transition and inflorescence. None of the Ed plants showed signs of shoot-to-inflorescence transition or inflorescence development during the first 6 weeks of LD exposure, and reproductive development began only after the initiation of short days. The number of days it took to reach the shoot-to-inflorescence transition stage is reduced from 42 days in plants that received 6 weeks of LD treatment prior to short days (SD), to 17 days in plants that started directly under short days, the 0-week LD treatment (Table 1.1, $p < 0.05$). Receiving SDs two weeks earlier accelerated the development by about 10 days ($p < 0.05$). The 0-week LD treatment reduced the number of days by only 5 days compared to plants that received the 2-week LD treatment ($p < 0.05$). The pattern was the same for inflorescence development: the mean number of days reduced from 46.5 days in 6-week LD treatment to 22 days in 0-week LD treatment ($p < 0.05$) with ~10-day reduction with each earlier SD exposure ($p < 0.05$), except the reduction from 2-week treatment to 0-week treatment was only 3 days ($p > 0.05$). Together, these results show that the variety ‘Eden’ (Ed) is photoperiod-sensitive for both traits.

‘Grape indica’ (GI) showed some similar photoperiod-sensitive responses to ‘Eden’. However, some GI plants in the 6-week LD treatment started shoot-to-inflorescence transition before SDs started. The reduction of time to shoot-to-inflorescence transition from 6-week to 4-week LD treatment was only 3 days (Table 1.1, $p > 0.05$) but decreased by 10 days from 4 week to 2-week LD treatment ($p < 0.05$). The mean number of days was further decreased by 4 days when started directly in SDs. The mean number of days to inflorescence development was similarly accelerated by 19 days with no LD treatment compared to 6 weeks of LD treatment ($p < 0.05$). Two weeks of LD exposure did not make a significant difference compared to 0-week plants. These results are consistent with what would be expected from a facultative-SD plant.

In contrast to Ed and GI, ‘Auto Pivot’ (AP) showed a very different pattern for number of days to shoot-to-inflorescence transition and inflorescence development. The mean number of days to shoot-to-inflorescence transition was around 17 days for all treatments with LDs (Figure 1.3a). The mean number of days to shoot-to-inflorescence transition increased to 21.5 days in the 0-week LD treatment relative to the 6-week LD treatment, although the difference between 4- and 0-week treatments was not significant. The development of inflorescences was also delayed by 6.5 days in the 0-week LD treatment, compared to treatments with LD exposure (Table 1.3b, $p < 0.05$). When measured in this way, AP showed a pattern that could be considered a weak long-day response.

‘Russian Auto’ (RA) was the fastest of all varieties tested for both measured traits. While shoot-to-inflorescence transition happened around 10 days and did not differ between treatments (Figure 1.3a), time to inflorescence development was accelerated by nearly 4 days when there was no LD exposure (Figure 1.3b, Table 1.1). This shows some varieties like RA can be considered both day-neutral and photoperiod-sensitive when analyzed for different stages.

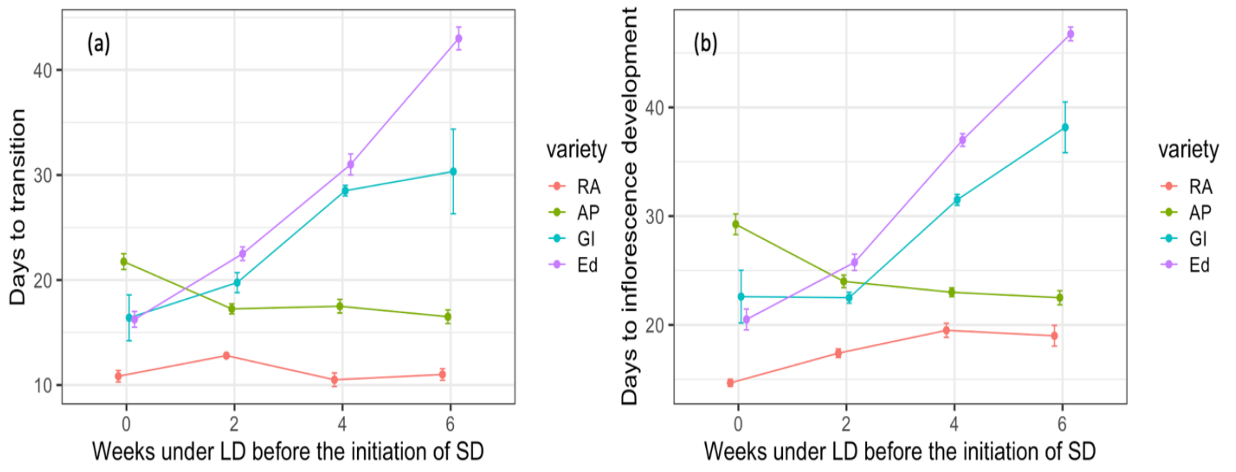


Figure 1.3 Mean number of days from germination to shoot-to-inflorescence transition (a) and inflorescence development (b) for each variety and long-day (LD) treatment. Error bars show means ± SE. Only the second replication is shown as a representative figure.

		Long-day treatment (number of weeks under LDs)			
Trait	Variety	0	2	4	6
Days to shoot-to-inflorescence transition	RA	10.5^{a}	12.2^{a}	10.3^{a}	10.6^{a}
	AP	21.5^{b}	16.7^{a}	17.6^{ab}	16.7^{a}
	GI	15.3^{c}	19.5^{b}	29.4^{a}	32.8^{a}
	Ed	17.0^{d}	21.7^{c}	32.1^{b}	42.3^{a}
Days to inflorescence development	RA	14.7^{b}	17.0^{ab}	17.4^{a}	18.9^{a}
	AP	27.9^{c}	23.4^{b}	22.3^{b}	21.3^{a}
	GI	20.7^{c}	22.4^{c}	32.5^{b}	39.9^{a}
	Ed	22.0^{c}	25.1^{c}	36.3^{b}	46.5^{a}

Table 1.1 Mean number of days to shoot-to-inflorescence transition and inflorescence development. Values that do not have the same superscript letter are significantly different ($p < 0.05$). Comparisons are shown only for each variety and trait (within the same row). Means are back-transformed after Box-Cox transformation.

Characterization of Photoperiod Sensitivity Based on Anthesis

Similar to shoot-to-inflorescence transition and inflorescence development, photoperiod-sensitivity of varieties was characterized based on the differences in the mean number of days to develop open flowers (anthesis) between different photoperiod treatments. All varieties in the 6-

week treatment reached anthesis before SDs began, although it took nearly twice as many days for GI and Ed compared to RA and AP (Figure 1.4).

For Ed and GI, anthesis occurred around 25-27 days from germination in 4 weeks and 6 weeks of LD treatments, before or slightly after SD treatment began. Anthesis occurred slightly earlier with 2 weeks of LD treatment, but the differences were not statistically significant. However, the time it takes to develop first open flowers was accelerated by ~10 days for both varieties in the 0-week LD treatment compared to 4-week and 6-week LD treatments (Table 1.2, $p < 0.05$).

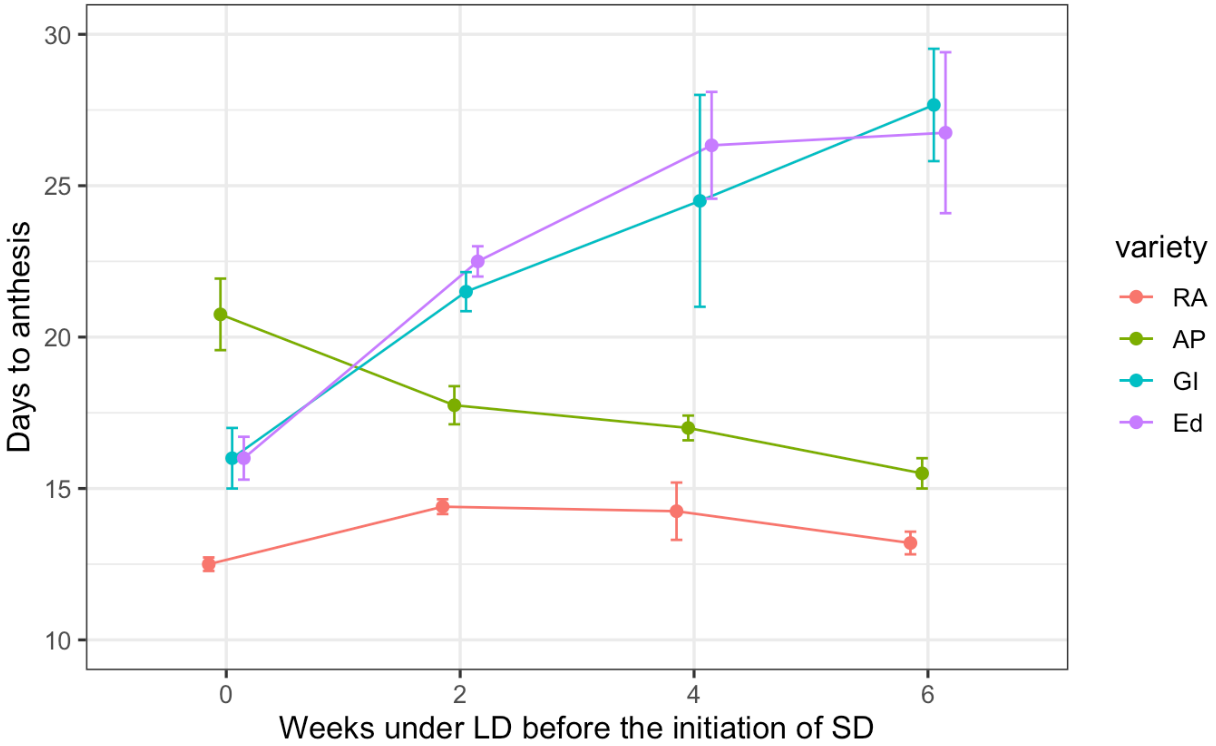


Figure 1.4 Mean number of days from germination to anthesis for each variety and long-day (LD) treatment. Error bars show means ± SE. Only the second replication is shown as a representative figure.

RA first showed open flowers nearly 13-14 days after germination, and there were no statistically significant differences between treatments, although there was a tendency for a small decrease in the number of days in the 0-week LD treatment relative to other treatments. AP, on the other hand, was delayed by 4.5 days in 0-week treatment compared to 6-week treatment (Table 1.2, $p < 0.05$), but the 0-week treatment was not significantly different from 2-week and 4-week treatments.

		Long-day treatment (number of weeks under LDs)			
Trait	Variety	0	2	4	6
Days to anthesis	RA	12.6^{a}	14.3^{a}	13.5^{a}	14.0^{a}
	AP	20.2^{b}	17.9^{ab}	17.2^{ab}	15.7^{a}
	GI	15.3^{b}	21.5^{a}	25.0^{a}	26.5^{a}
	Ed	16.7^{b}	22.2^{a}	27.0^{a}	27.4^{a}

Table 1.2 Mean number of days to anthesis. Values that do not have the same superscript letter are significantly different ($p < 0.05$). Comparisons are shown only for each variety (within the same row). Means are backtransformed after Box-Cox transformation.

The Influence of Photoperiod on Different Stages of Reproductive Development

Different stages of reproductive development can show distinct responses to photoperiod. For example, SD treatment can reduce the time to anthesis without affecting time to flower initiation in some sunflower cultivars (Marc and Palmer 1981). Similarly, in some genotypes of peas, flower initiation can be day-neutral while flower development and senescence are promoted by LD (Reid 1979). In SDP *Crysanthemum*, floral buds can be produced under LD but they stop developing if kept under LD (Schwabe 1951). The effect of photoperiod on termination of cannabis inflorescences was also observed previously (Potter 2009). Here, I tested whether the initiation of solitary flowers can also be considered photoperiod sensitive in *Cannabis*. I also evaluated whether the durations between different stages are affected by photoperiod.

In addition to the three stages of reproductive development above, four other stages were analyzed to evaluate photoperiod sensitivity. In all varieties, the first solitary flowers became visible within a few weeks in LDs (Figure 1.5a).

Ed and GI had shown photoperiod sensitive development for shoot-to-inflorescence transition and inflorescence development, whereas RA was unaffected, and AP was delayed under 0-week treatment for those traits. Results for solitary flower development are similar. For Ed, the appearance of solitary flowers along the stems occurred 5 days (Table 1.3, $p < 0.05$), 7 days ($p < 0.05$), and 2 days ($p > 0.05$) faster in 0-week treatment compared to 2-week, 4-week, and 6-week treatments, respectively. For GI, first flowers appeared 5.5 days earlier in 0-week treatment, compared to 6-week treatment ($p <0.05$). RA developed first flowers in 10-12 days in all treatments and was not affected by photoperiod. Similar to traits considered above, AP showed delayed flowering under 0-week LD treatment compared to 2- and 6-week treatments by ~4 days ($p < 0.05$), but the difference with 4-week treatment was not significant.

After considering photoperiod sensitivity in terms of different stages of reproductive development individually, the next question is whether the duration between each stage is influenced by photoperiod. The number of days from first flowers to anthesis was reduced from 7 days in 6-week LD treatment to 1 day in 0- and 2-week treatments for Ed (Figure 1.5b, Table 1.3, $p < 0.05$). For GI, there was a smaller reduction in mean number of days from 4-week to 0-week LD treatment ($p < 0.05$), although 0-week treatment was not significantly different from 6-week treatment. The gap between first appearance of flowers and anthesis was already small (2-3 days) for RA in 4- and 6-week treatments, but it further decreased to less than a day when grown in SDs ($p < 0.05$). AP did not show any significant difference between treatments.

The duration between anthesis and shoot-to-inflorescence transition was affected by photoperiod only for Ed, decreasing from 13 days in 6-week LD treatment to zero days in 0-week treatment (Figure 1.5c). GI also showed a decreasing trend from 6-week LD treatment (5 days) to 0-week treatment (0 day), but the difference was not significant. Shoot-to-inflorescence transition occurred about the same day as anthesis for AP in all treatments, whereas shoot-to-inflorescence transition occurred consistently earlier than anthesis in RA. The mean number of days from shoot-to-inflorescence transition to inflorescence development was not affected by photoperiod for AP, GI, and Ed, whereas it decreased from 8 days in 6-week treatment to 4 days in 0-week treatment for RA (Table 1.3, $p < 0.05$)

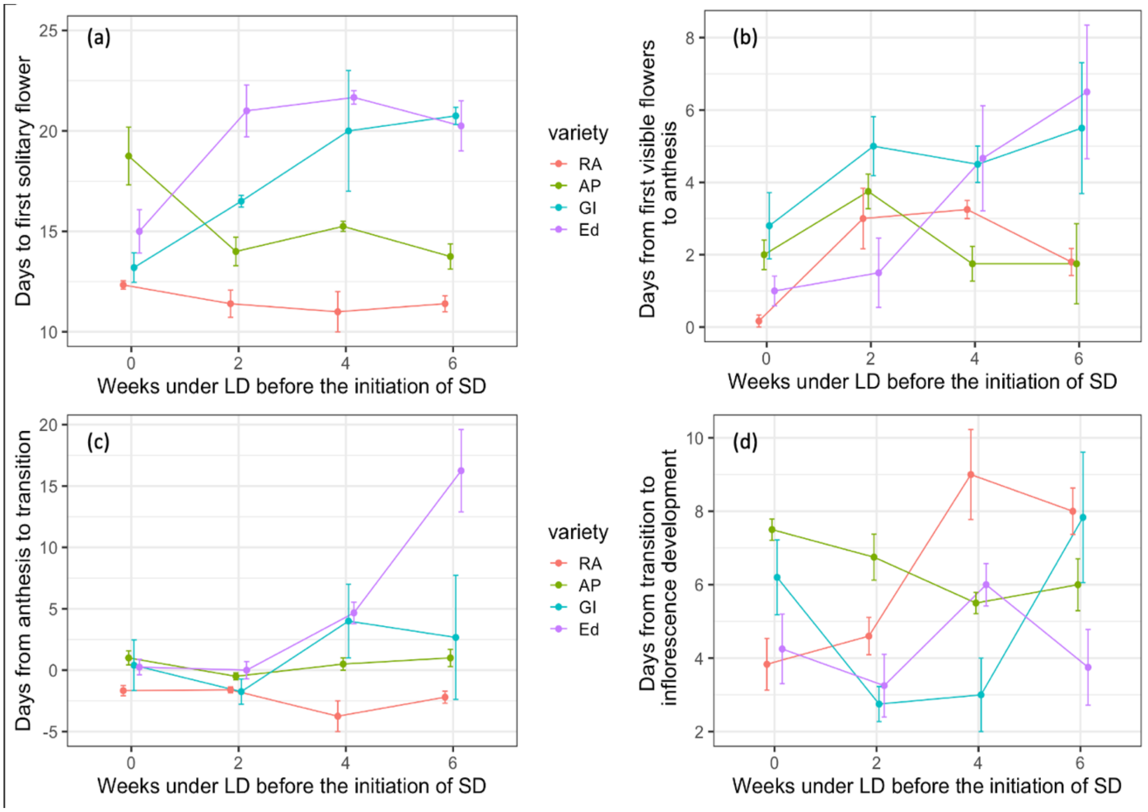


Figure 1.5 Mean number of days from (a) germination to first visible flower, (b) from first visible flower to anthesis, (c) from anthesis to shoot-to-inflorescence transition, and (d) shoot-to-inflorescence transition to inflorescence development for each variety and long-day (LD) treatment. Error bars show means ± SE. Only the second replication is shown as a representative figure.

		Long-day treatment (number of weeks under LDs)			
Trait	Variety	0	2	4	6
Days from germination to first solitary flower*	RA	12.1^{a}	11.6^{a}	10.4^{a}	11.5^{a}
	AP	18.1^{b}	14.1^{a}	15.3^{ab}	13.8^{a}
	GI	13.7^{b}	15.9^{ab}	15.8^{ab}	19.4^{a}
	Ed	15.3^{b}	20.2^{a}	22.8^{a}	18.7^{ab}
Days from first solitary flower to anthesis**	RA	0.4^{b}	1.9^{ab}	3.1^{a}	2.3^{a}
	AP	1.9^{a}	3.5^{a}	1.6^{a}	1.5^{a}
	GI	1.2^{b}	4.8^{ab}	7.4^{a}	3.2^{ab}
	Ed	1.0^{b}	1.3^{b}	4.1^{ab}	7.3^{a}
Days from anthesis to shoot-to-inflorescence transition**	RA	-2.2^{a}	-2.1^{a}	-3.5^{a}	-4.5^{a}
	AP	1.3^{a}	-1.3^{a}	0.3^{a}	0.9^{a}
	GI	-0.3^{a}	-1.9^{a}	3.8^{a}	5.3^{a}
	Ed	0.2^{b}	-0.6^{b}	5.0^{ab}	13.3^{a}
Days from transition to inflorescence development**	RA	4.0^{b}	4.7^{ab}	7.2^{ab}	8.0^{a}
	AP	6.3^{a}	6.6^{a}	4.7^{a}	4.4^{a}
	GI	5.3^{a}	2.7^{a}	3.0^{a}	4.9^{a}
	Ed	4.7^{a}	3.1^{a}	3.9^{a}	4.0^{a}

Table 1.3 Mean number of days from germination to first visible flower, from first flower to anthesis, from anthesis to shoot-to-inflorescence transition, and from shoot-to-inflorescence transition to inflorescence development. Values that do not have the same superscript letter are significantly different ($p < 0.05$). Comparisons are shown only for each variety and trait (within the same row). *Means are backtransformed from Box-Cox transformation. ** Means are backtransformed from logarithmic transformation.

Reproductive Development Rate
Measured as Developmental Age

Another possible way to compare the timing of developmental stages and photoperiod sensitivity is to compare the developmental age (usually measured in number of leaves) of the plants, as opposed to comparing chronological age (days from germination). Similar to evaluating photoperiod sensitivity by the reduction in number of days, it is measured in terms of reduction in number of nodes. There was a reduction in number of nodes with shorter LD treatments for all traits in Ed, GI, and RA, while they did not change between treatments in AP for any of the traits, despite being delayed in 0-week treatment for first solitary flower, anthesis, shoot-to-inflorescence transition, and inflorescence development in terms of chronological age (Figure

1.6). For AP, the first flowers appeared with about 5 nodes; anthesis and shoot-to-inflorescence transition happened at 6 node stage; and inflorescences developed with about 8.5 nodes in all treatments (Table 1.4).

For Ed, the number of nodes at inflorescence development decreased by nearly 3 nodes with each treatment with fewer LDs (Table 1.4, $p < 0.05$). The number of nodes at shoot-to-inflorescence transition similarly decreased from 15 in 6-week treatment to 5 in 0-week treatment, while the difference between 2-week and 4-week treatments were not significant. The number of nodes at first solitary flower and anthesis were both nearly halved in 0-week treatment compared to 6-week treatment.

For GI, the number of nodes at first solitary flowers decreased from 9 in 6-week treatment to 7 in 2-week treatment, and further reduced to 4 nodes in 0-week treatment. During anthesis, there were only 5 nodes on average in 0-week treatment, compared to 10-11 nodes in 2-, 4-, and 6-week treatments. The mean number of nodes at shoot-to-inflorescence transition decreased with less LD exposure as well, from 13 nodes in 4- and 6-week treatments, to 8.5 in 2-week treatment and to 5 nodes in 0-week treatment. Similarly, the mean number of nodes at inflorescence development was 15 and 17 (Table 1.4, $p > 0.05$) in 4 week and 6-week treatments, respectively, decreasing to 10 nodes in 2-week treatment ($p < 0.05$) and 8 nodes in 0-week treatment ($p < 0.05$).

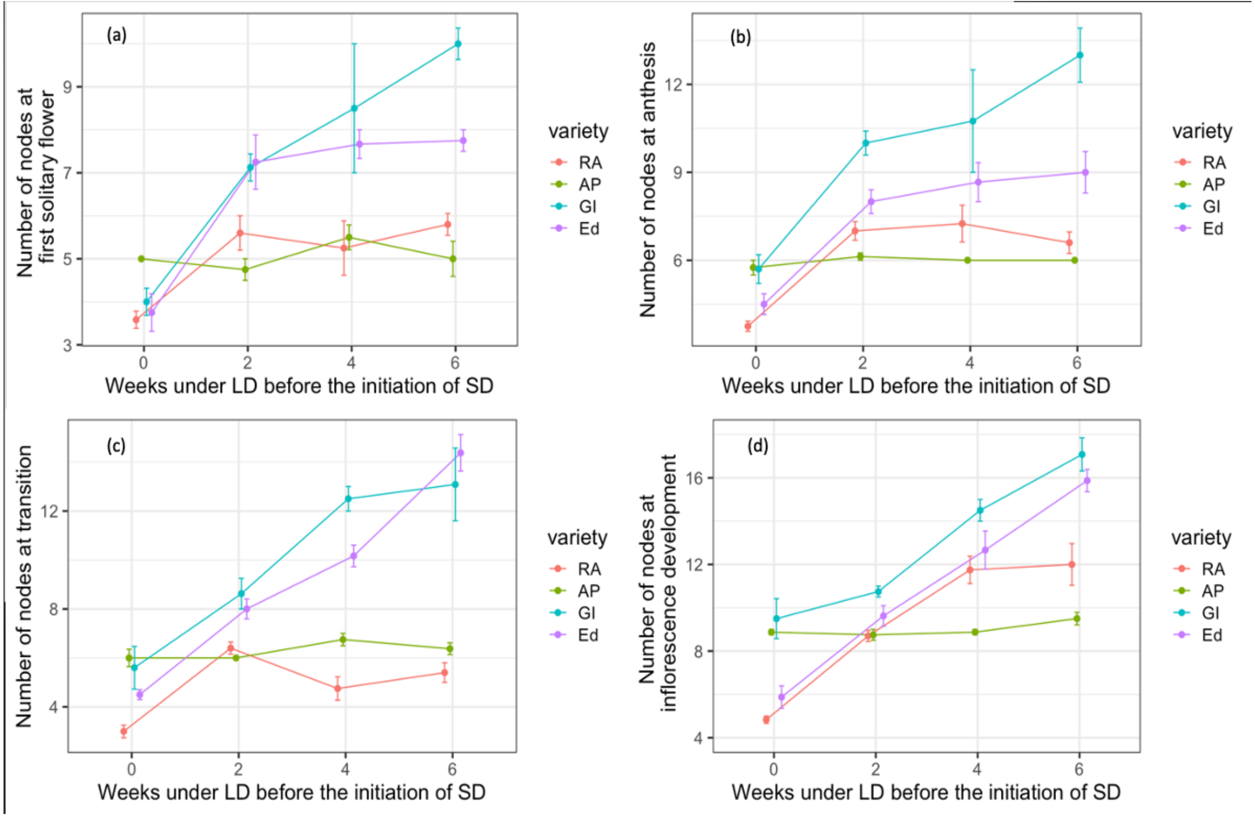


Figure 1.6 Mean number of nodes on the main stem at the time of (a) first visible flower, (b) anthesis, (c) shoot-to-inflorescence transition, and (d) inflorescence development for each variety and long-day (LD) treatment. Error bars show means ± SE. Only the second replication is shown as a representative figure.

Unlike chronological age-based evaluation of photoperiod sensitivity, the appearance of first flowers happened earlier in terms of developmental age for RA. The mean number of nodes decreased from 5-6 in 2-, 4-, and 6-week treatments, to 3.5 nodes in 0-week treatment (Table 1.4, $p < 0.05$). There was a similar reduction of number of nodes at anthesis between 0-week (four nodes) and 2-, 4-, and 6-week treatments (seven nodes), also being inconsistent with chronological age-based evaluation. The mean number of nodes at shoot-to-inflorescence transition decreased from 5 (2-week, 4-week, 6-week treatments) to less than 3 nodes in 0-week treatment ($p < 0.05$). The mean number of nodes at inflorescence development decreased from 11 in 6-week treatment to 8.5 in 2-week treatment, and further decreased to 5 nodes in 0-week treatment.

The Relationship Between Plant Age and Response to Short Days

Finally, the question whether the plant age affects how fast the plants respond to SDs for shoot-to-inflorescence transition and inflorescence development was investigated. If there is no relationship, plants of different ages should require the same number of days. If there is a relationship, older plants (those with longer LD treatment) were expected to respond sooner.

		Long-day treatment (number of weeks under LDs)			
Trait	Variety	0	2	4	6
Number of nodes at first solitary flower	RA	3.5^{b}	5.7^{a}	5.0^{a}	5.8^{a}
	AP	4.8^{a}	4.8^{a}	5.5^{a}	5.3^{a}
	GI	4.2^{c}	6.7^{b}	7.1^{ab}	9.1^{a}
	Ed	3.8^{b}	7.5^{a}	8.1^{a}	7.5^{a}
Number of nodes at anthesis	RA	3.7^{b}	7.0^{a}	6.7^{a}	7.0^{a}
	AP	5.5^{a}	6.2^{a}	6.0^{a}	6.0^{a}
	GI	5.3^{b}	10.0^{a}	10.9^{a}	11.4^{a}
	Ed	4.6^{b}	8.4^{a}	9.0^{a}	10.0^{a}
Number of nodes at shoot-to-inflorescence transition	RA	2.8^{b}	5.9^{a}	4.9^{a}	5.1^{a}
	AP	6.1^{a}	6.0^{a}	6.6^{a}	6.4^{a}
	GI	5.0^{c}	8.4^{b}	13.0^{a}	13.2^{a}
	Ed	4.7^{c}	8.2^{b}	10.7^{b}	14.6^{a}
Number of nodes at inflorescence development	RA	4.8^{c}	8.5^{b}	9.7^{ab}	10.7^{a}
	AP	8.6^{a}	8.5^{a}	8.3^{a}	8.6^{a}
	GI	8.1^{c}	10.4^{b}	14.9^{a}	17.1^{a}
	Ed	6.6^{d}	9.9^{c}	12.6^{b}	16.5^{a}

Table 1.4 Mean number of nodes on the main stem at the time of first solitary flower, anthesis, shoot-to-inflorescence transition, and inflorescence development. Values that do not have the same superscript letter are significantly different ($p < 0.05$). Comparisons are shown only for each variety and trait (within the same row). Means are backtransformed from Box-Cox transformation.

For Ed, the mean number of days to shoot-to-inflorescence transition after SDs were 17 in 0-week treatment, compared to 11.6 days (Figure 1.7a, $p < 0.05$), 8 days ($p < 0.05$), and 4 days ($p < 0.05$) in 2-week, 4-week, and 6-week treatments, respectively. Ed’s inflorescence development response was 7 days, 10 days, and 13.5 days later compared to 2-week ($p < 0.05$), 4-week

(Figure 1.7b, Table 1.5, $p < 0.05$), and 6-week ($p < 0.05$) treatments, respectively. For both traits, the differences between 2-week and 6-week treatments were significant, while 2-week and 4-week treatments were not significantly different.

GI plants in 0-week treatment started shoot-to-inflorescence transition about 6 days slower than 2-week-old plants, and 10 days slower than 4-week-old plants. The four-day difference between 2-week-old and 4-week-old plants were not statistically significant (Table 1.5). Plants in 6-week treatment started shoot-to-inflorescence transition before SD exposure (Figure 1.7a). The response to SDs for inflorescence development was 9 days slower compared to 2-week-old plants, and about 12.5 days slower compared to 4-week-old plants. The difference between 2- and 4-week-old plants was not statistically significant. Plants in the 6-week LD treatment developed inflorescences in less than 2 days after being exposed to SDs.

In 6- and 4-week treatments, both AP and RA started shoot-to-inflorescence transition (Figure 1.7a) and developing inflorescences (Figure 1.7b) before the initiation of SDs. Comparing 0-week and 2-week treatments, inflorescence development response was twice as slow in plants started in SDs for both varieties. shoot-to-inflorescence transition response was also slower in 0-week treatment (Figure 1.7a). However, since both varieties rapidly start developing inflorescences regardless of the daylength, these comparisons are not as informative for understanding age and SD response relationship as the comparisons made for Ed.

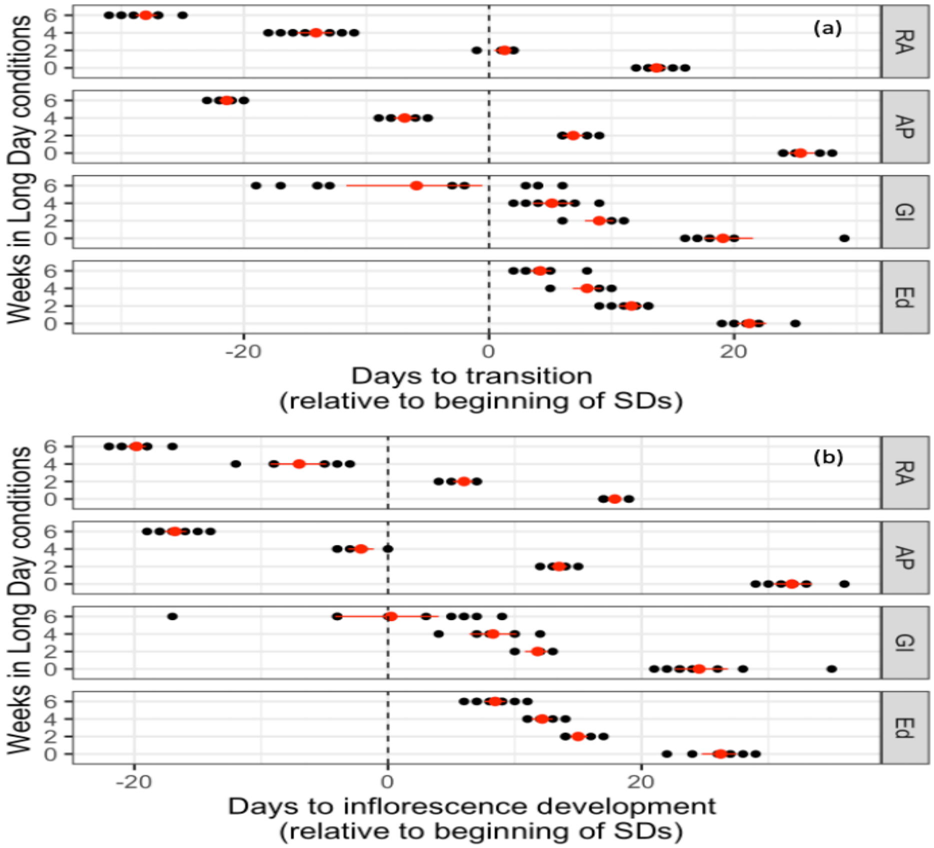


Figure 1.7 Mean number of days to shoot-to-inflorescence transition(a) and inflorescence development (b) after the beginning of short days (SD). Vertical dashed lines indicate the day photoperiod was set to short days. Datapoints left of the line show the plants that began shoot-to-inflorescence transition before short days started. Red bars show means and 95% confidence intervals based on bootstrapping. The confidence intervals are different from those observed in the statistical analysis. Only the second replication is shown as a representative figure.

		Long-day treatment (number of weeks under LDs)			
Trait	Variety	0	2	4	6
Days to shoot-to-inflorescence transition after SDs	RA	10.5^{d}	1.3^{c}	-14.1^{b}	-28.0^{a}
	AP	21.6^{d}	6.9^{c}	-6.9^{b}	-21.4^{a}
	GI	15.4^{c}	9.0^{b}	5.1^{b}	-4.6^{a}
	Ed	17.1^{c}	11.6^{b}	8.0^{ab}	4.2^{a}
Days to inflorescence development after SDs	RA	14.7^{d}	6.0^{c}	-6.8^{b}	-19.8^{a}
	AP	28.0^{d}	13.5^{c}	-2.1^{b}	-16.8^{a}
	GI	20.9^{c}	11.8^{b}	8.3^{b}	1.8^{a}
	Ed	22.1^{c}	15.0^{b}	12.2^{ab}	8.5^{a}

Table 1.5 Mean number of days to shoot-to-inflorescence transition and inflorescence development after the beginning of short days (SD). Values that do not have the same superscript letter are significantly different ($p < 0.05$). Comparisons are shown only for each variety and trait (within the same row).

Discussion

In this study, I aimed to characterize photoperiod sensitive development of *Cannabis*. Instead of trying to fit *Cannabis* into a photoperiodic category (e.g., SDP), I recognize and emphasize the variability of the process of reproductive development. In order to not get lost in the technical problems of defining and measuring photoperiod sensitivity, I have taken a pluralist position by evaluating photoperiod sensitivity from multiple perspectives. This approach showed that there are important developmental differences even within four high-CBD drug-type varieties. The major findings of this study are highlighted and discussed below.

‘Eden’ showed a clear photoperiod sensitive response for shoot-to-inflorescence transition and early inflorescence development. Both stages of reproductive growth were accelerated under short-days and happened with fewer nodes. Plants did not show any signs of shoot-to-inflorescence transition under long days for 6 weeks (the longest LD exposure in this study). However, it is not clear whether this variety has an obligate photoperiod requirement, as they may eventually develop inflorescences if they are kept under LDs for longer durations than tested in this study.

Spitzer-Rimon et al. (2019) had reported some drug-type varieties fully developed solitary flowers along the stem axis under LDs, without the formation of inflorescences. The development of Ed was similar to those observations. Interestingly, both the induction and opening of flowers were accelerated and occurred with fewer nodes when plants started directly in short-day treatment, suggesting a facultative SD response for these traits (as opposed to day-neutral development). Nevertheless, it is possible this acceleration is due to stress induced by the lack of LD exposure, independent of a photoperiod-sensitive response. Exposure to SDs also lowered the duration between the first appearance of solitary flowers and anthesis, as well as the duration between anthesis and shoot-to-inflorescence transition. This suggests photoperiod does not only affect the timing of initial reproductive development, but also the rate of development. An interesting hypothesis to test would be whether different stages of reproductive development show different critical daylength requirement as observed with the termination of new flower development (determinate vs indeterminate growth) in an earlier study (Potter 2009).

Similar to 'Eden', 'Grape Indica' showed photoperiod-sensitive flowering (both induction and anthesis) and inflorescence development (happening earlier and with fewer nodes under SDs). Unlike Ed, GI was able to develop flowers and start shoot-to-inflorescence transition within 6 weeks under LD treatment, showing a clear facultative SD response. While the timing of Ed and GI's shoot-to-inflorescence transition and inflorescence development were drastically different under LDs, they were similar under SDs, meaning the early-flowering phenotype of GI was specifically due to a relaxed long-day repression and not due to differences in the length of juvenile phase and general reduction of flowering time. Also, there was not enough evidence to suggest a photoperiod-sensitive reduction of the rate of reproductive development (duration between different stages) for GI, unlike what was observed with Ed.

For all reproductive traits measured, 'Auto Pivot' was surprisingly consistent among all treatments in terms of developmental age (measured as total number of nodes on the main stem), rather than chronological age (measured as number of days). The occurrences of all traits were slightly delayed when plants directly started in SDs, except for the duration between different traits. While it is possible to interpret this delay as a facultative LD response, it is better explained by a strict day-neutral response in terms of developmental age together with slowed growth rate due to receiving only 8 hours of light, making it slower to reach the required developmental age. This developmental age dependent day-neutral flowering was also observed in *Nicotiana tabacum* cv. Wisconsin 38, and it is interpreted as endogenous regulation of flowering (McDaniel and Hsu 1976), and it is likely based on sugar signaling (Yang et al. 2013; Yu et al. 2013). The plasticity of this trait in the presence of environmental stressors and limiting conditions (nutrition, light, soil space, etc.) in day-neutral cannabis is worth investigating.

Despite being labeled as a day-neutral variety by the distributor and showing very rapid reproductive development earlier than other varieties in all treatments, ‘Russian Auto’ showed some signs of photoperiod sensitivity. The number of days to shoot-to-inflorescence transition, first solitary flowers, and anthesis were not affected by different treatments, but the number of days to inflorescence development was. For all four traits, the total number of nodes were decreased when planted directly in SDs, similar to Ed and GI. The duration between the first solitary flower and anthesis, and between shoot-to-inflorescence transition and inflorescence development was also shorter under SDs. One potential explanation for these observations is that only the rate of floral development is sensitive to photoperiod in this variety. Therefore, this variety could be considered weakly photoperiod-sensitive for floral development and day-neutral for the rest of reproductive development.

Meanwhile, the observed reduction of total number of nodes for developmental traits that appear (chronologically) day-neutral could be explained by the endogenous signaling that somehow differs from that of AP. For instance, assuming the role of miR156 in sugar signaling (Yang et al. 2013; Yu et al. 2013) is conserved in *Cannabis*, the regulatory network in RA might be altered in a way to reduce or eliminate the expression of miR156 without the need of elevated sugar levels. This would explain the lack of developmental timing of solitary flower induction and shoot-to-inflorescence, and the manifestation of chronological age-based day-neutral development, as well as the extremely short juvenile phase observed in this variety.

In regular field settings, it is not possible to distinguish day-neutral plants from early-flowering photoperiod-sensitive plants: they both flower within a few weeks regardless of the photoperiod. Both can be grown in places where the growing season is short with long photoperiods. However, while both AP and RA can be practically considered day-neutral, their developmental differences described above may be crucial in stressful environments. For example, with the lack of juvenile phase and developmental timing of reproductive growth, RA may flower with very little vegetative growth and cause yield losses if the growth is stunted for any reason, whereas AP may be able to compensate by delaying the onset of reproductive development. For varieties that are intended to be grown as day-neutral plants, extended juvenile phase with strict developmental timing seems to be a better choice. For varieties intended to be photoperiod-sensitive, exploiting the variation in juvenility could potentially be a faster way to expand their cultivation to new locations. For instance, a variety with a relatively long critical daylength can be grown in lower latitudes without premature flowering if the juvenile phase can be extended easily. Furthermore, it may be possible to plant earlier than usual where the photoperiod is too short early in the season, but the environment is otherwise permissive for healthy growth.

One hypothesis regarding the relationship between the age of plants and the sensitivity of plants to inductive photoperiod was whether older plants can respond to SDs than younger plants. For both Ed and GI, plants started in SDs responded much slower than plants than received two or more weeks of LDs before SDs, indicating the existence of juvenile phase when plants do not respond to inductive photoperiod. For Ed, there was a clear trend of faster responses with older plants, but the differences between 2-week-old and 4-week-old plants, and between 4-week-old and 6-week-old plants were not statistically significant. However, there was a ~7-day delay in response to SDs between 2-week-old and 6-week-old plants. Whether this difference was due to juvenility or the age of the plants beyond the juvenile phase is not clear, as it was not possible to measure the length of the juvenile phase directly. Considering the earliest sign of reproductive development (first solitary flowers) was at about 15 days, we could infer that the length of the juvenile phase for Ed is a little short of 2 weeks in this particular controlled environment. While the responses of GI followed a similar trend, the difference between 6-week-old and 4-week-old plants were statistically significant. However, this difference was likely because of plants that were already committed to inflorescence development shortly before the initiation of SDs at sixth week. Since the differences were small (~3 days) and not statistically significant between 2-week and 4-week-old plants for both varieties, it could be said that the age of the plant (after the juvenile phase) is not agronomically relevant for the reproductive development of early-flowering, facultative photoperiod-sensitive plants. However, it is still possible that the age difference matters for obligate SD plants, or for facultative SD plants that can grow vegetatively much longer than 6 weeks.

DIURNAL EXPRESSION OF FT-LIKE GENES IN CANNABIS SATIVA L.

Introduction

What causes plants to flower or remain vegetative? During the 20th century, this question was initially addressed by investigating the environmental and internal factors that affect flowering time. Understanding these factors led to experiments that aimed to explain how plants sense and signal the information relevant for reproductive development from physiological and molecular perspectives. There were several critical findings that would result in a long-lasting debate, which would not be resolved until the 21st century.

The first important finding was that photoperiodic induction happens in leaves. Knott (1934) first demonstrated that in spinach, a long-day plant (LDP), exposing only the leaves to long days was sufficient to induce floral primordia at the shoot apex. A similar observation was later made with the short-day plant (SDP) *Xanthium strumarium* (Naylor 1941). In this study, keeping the plant in non-inductive (long day) conditions while exposing a single young leaf to short days (even for a single day) was enough to induce flowers. Furthermore, studying two SDPs, *Xanthium* and *Perilla*, Zeevaart (1958) found that leaves can be induced in short day conditions even when the leaves are detached from the plant. When grafted to plants kept in long days, induced leaves were able to trigger flowering in receptor plants. These experiments showed that daylength is perceived in the leaves independently.

More interestingly, grafting experiments demonstrated that induced leaves can successfully initiate flowering across species and photoperiod types. For example, flowering of the day neutral variety *Nicotiana tabacum* 'Trapezond' is accelerated when the shoots or leaves of short-day tobacco variety *Nicotiana tabacum* 'Maryland Mammoth' or LDP *Nicotiana sylvestris* are grafted on them while maintaining the photoperiods that are inductive for the donor plant (Lang et al. 1977). Similarly, the leaves of the day neutral soybean variety *Glycine max* 'Agate' were able to induce flowering in the short-day variety 'Biloxi' in LD conditions (Heinze et al. 1942). These findings together provided support to the idea that the flowering stimulus was a universal hormone induced in the leaves and transported to the shoot apex, leading to floral evocation. Even though this universal hormone concept (named 'florigen') was proposed in 1937 (Chailakhyan 1937), no one was able to isolate such a substance. This failure led to new hypotheses such as the "multifactorial control hypothesis" that suggested the transmissible signal is a combination of several chemicals, or the nutrient diversion hypothesis, which claimed the signal is a particular modification of source/sink relationship in the plant (Bernier et al. 1993). Another interesting hypothesis was that the floral stimulus could be electrical in nature, which was motivated by findings such as the presence of diurnal rhythms of membrane resting potentials and their alteration in induced plants, and the ability to promote or repress evocation under non-inductive and inductive conditions, respectively (Adamec et al. 1989; Macháčková and Krekule 1991; Macháčková et al. 1990; Wagner et al. 2006; Wagner et al. 1998). Despite promising findings, the role of bioelectric signaling in reproductive development of plants has not been studied systematically, and the area remains to be explored.

Flowering Locus T

After the decades-long search for the hypothetical florigen, the best candidate turned out to be a protein coding gene, *Flowering Locus T* (*FT*), which belongs to the phosphatidyl ethanolamine-binding proteins (PEBP) family. It was found that overexpression of *FT* leads to early flowering in *Arabidopsis* (Kobayashi et al. 1999), and *FT* expression in a single leaf is enough to initiate flowering (Corbesier et al. 2007). Also, both the mRNA and the protein move from leaves to the apex, and FT protein was detected across graft junctions (Corbesier et al. 2007; Huang et al. 2005; Jaeger and Wigge 2007). FT protein interacts with the bZIP transcription factor FD, and together they activate flower identity genes (Abe et al. 2005; Wigge et al. 2005). While it appears that the floral stimulus is mainly the FT protein, the role of *FT* mRNA in signaling is still debated (Jackson and Hong 2012; Yu et al. 2022). Finally, the FT signal is "universal" in the sense that heterologous expression of *FT* homologs can induce flowering in distant species (Harada et al. 2022; Heller et al. 2014; Patil et al. 2022; Zhou et al. 2018), so the extrapolation from limited intrafamily grafts regarding the florigen turned out to be true.

FT orthologues are known in many other species, and there are often multiple homologues, sometimes with diverged functions. For instance, in rice, both *Hd3a* and *RFT1* are floral activators (Komiya et al. 2008). In soybean, *GmFT2a/2b*, *GmFT3a/3b*, and *GmFT5a/5b* promote flowering, but *GmFT1a/1b*, *GmFT4*, and *GmFT6* delay flowering. The differences in their regulation and function allow plants to fine-tune their flowering time (Lee et al. 2021).

Phosphatidyl Ethanolamine-Binding Proteins (PEBP) Family

In the LDP *Arabidopsis thaliana*, *FT* belongs to a family of six genes. The *PEBP* family is generally involved in reproductive development with overlapping or antagonistic functions. According to phylogenetic analyses, the *PEBP* family consists of three subfamilies: *TFL1-LIKE*, *MFT-LIKE*, and *FT-LIKE* (Chardon and Damerval 2005). The genes of the family can also be involved in other developmental and physiological functions (Jin et al. 2021). For example, in addition to its floral promoting function, *FT* influences stomatal opening (Kinoshita et al. 2011).

More interestingly, it is expressed in fruits in response to temperature, which influences progeny dormancy (Chen et al. 2014).

Like FT, TERMINAL FLOWER 1 (TFL1) and BROTHER OF FT AND TFL1 (BFT) also interact with FD. By competing for binding to FD, TFL1 and BFT inhibit flowering (Hanano and Goto 2011; Ryu et al. 2014). Furthermore, the balance between FT and TFL1 influences the plant architecture (Moraes et al. 2019). While BFT functions redundantly with TFL1 (Yoo et al. 2010), it specifically represses flowering under salt stress (Ryu et al. 2014). ARABIDOPSIS THALIANA CENTRORADIALIS (ATC) is also an inhibitor, and it is induced by SDs (Huang et al. 2012).

TWIN SISTER OF FT (TSF), on the other hand, has a similar function to FT. They both induce flowering and show similar expression patterns. In the presence of FT, TSF seems to be mostly redundant, although it has a significant contribution to flowering in non-inductive (SD) conditions. Unlike FT, TSF is mainly expressed in the hypocotyl (Yamaguchi et al. 2005). Finally, MOTHER OF FT AND TFL1 (MFT) also promotes flowering, in addition to promoting seed germination (Xi et al. 2010).

Research in *A. thaliana* have provided a foundation for understanding flowering time control in plants, but studies have been absent for *Cannabis*, especially in terms of genetic research, due to decades-long criminalization of the plant. With the recent trend of legalization, *Cannabis* started receiving more attention. In one study involving 123 hemp accessions, six QTL were identified for flowering time traits. Candidate genes within these QTL include genes involved in light signaling and regulation of circadian rhythm such as *CRY1*, *PHYA*, *PHYE*, *UVR8*, *XCT*, and *SPA1*, as well as *miR156*, *VRN1*, *FLOWERING LOCUS D*, three *FT-like* genes, two

CONSTANS-like (*COL*) genes, *FLORICAULA/LEAFY*, and GA regulator *SPINDLY* (Petit et al. 2020). Another recent study characterized the expression of 13 *COL* genes in early and late flowering cannabis varieties under LD and SD. Under SD, diurnal peaks of *CsCOL4*, *CsCOL6*, *CsCOL7*, *CsCOL9*, *CsCOL11*, and *CsCOL12* expression showed differences between early- and late-flowering varieties. Amino acid differences were also found between early- and late-flowering varieties in *CsCOL3* and *CsCOL7* (authors failed to clone other *CsCOL* genes). Based on this study, authors proposed *CsCOL6/12* might play a similar role to *OsCOL16*, a negative regulator of rice under both LD and SD (Pan et al. 2021). Finally, in a study comparing morphological, developmental, and molecular diversity of wild and cultivated varieties in China, wild cannabis was found to flower within 50 days under LD, while cultivated varieties remained vegetative after 100 days. Based on nucleotide diversity analyses, the authors found evidence of positive selection for nine genes potentially related to flowering: *CET1*, *SUVR5*, *FY*, *RING1A*, *PAPS4*, *APRF1*, *PRR37*, *NFYC9*, and *RLT2*. Looking at the changes in gene expression over a period of 40 days under LD followed by 40 days of SD, higher expression of *FT-like* was observed in wild cannabis compared to cultivated cannabis under LD, and *FT-like* expression increased under LD for both types (the authors looked at only one of the *FT-like* genes in *Cannabis*, which corresponds to *FTL2* in this thesis). The expression of *SOC1* was also higher in wild cannabis under LD, but not under SD. On the other hand, the expression of *FLC-like*, *CET1*, *FY*, *PRR37*, and *NFYC9* did not change as much, and there was not a significant difference between wild and cultivated varieties (Chen et al. 2022). In my thesis research, I examined the differences in *FT* family gene expression by contrasting photoperiod sensitive and day-neutral varieties of cannabis.

Materials and Methods

One day-neutral ('Auto Pivot') and one late-flowering photoperiod-sensitive ('Eden') variety were planted in the same growth room described in the first chapter. The LD group received 2 weeks of long-days (18:6 h light:dark), and the SD group received 2 weeks of short-days (8:16 h light:dark) before the collection of leaves. Leaves were harvested from three plants for each variety and timepoint at seven timepoints (zeitgeber time (ZT) 0, 4, 8, 13, 18, 21, 24). Dim, green LED lights were placed under the bench and turned on temporarily to assist collecting leaves in the dark.

The youngest unfolded leaves of 14-day-old vegetative female plants were harvested and frozen in liquid nitrogen. Day 14 was chosen for sampling to allow the plants grow as much as possible before either of the varieties started shoot-to-inflorescence transition, informed by the previous experiments. Young leaves were chosen in order to be consistent with the reported methods in FT literature (Kong et al. 2010). At the time of leaf collection, plants had 6-8 unfolded leaves in LD conditions and 4 unfolded leaves in SD conditions. RNA was extracted using Qiagen RNeasy Plant Mini Kit following the manufacturer's protocol. To remove potential contaminating DNA, a DNaseI treatment was performed using TURBO DNase-free kit. cDNA was synthesized from extracted RNA using Invitrogen's SuperScript IV Reverse Transcriptase with oligo-dT to reverse transcribe mRNA.

qPCR was performed to measure relative gene expression. The BIORAD C1000 Thermal Cycler was used to quantify expression based on SYBR green fluorescence. Twenty microliter reactions were made using BIORAD iTaq Universal SYBR Mastermix, for each genotype, timepoint, biological replication with three technical repeats, following the manufacturer's protocol. Relative expression was calculated using delta-delta Ct with efficiency correction (Pfaffl 2004):

$$\text{Relative Expression} = \frac{(\mathrm{E_{FT}})^{\Delta \mathrm{CT_{FT}}(\mathrm{Control-Sample})}}{(\mathrm{E_{TUB}})^{\Delta \mathrm{CT_{TUB}}(\mathrm{Control-Sample})}}$$

$\mathrm{E_{FT}}$: Efficiency of each FT primer

$\mathrm{E_{TUB}}$: Efficiency of TUB primer

$\Delta\mathrm{CT_{FT}}$: Ct difference between reference sample and target sample for each FT gene

$\Delta\mathrm{CT_{TUB}}$: Ct difference between reference sample and target sample for TUB gene

In order to build the gene tree, amino acid sequences of selected PEBP proteins from *Arabidopsis thaliana*, soybean (*Glycine max*), and apple (*Malus domestica*) are aligned with the predicted PEBP proteins of *Cannabis sativa* using ClustalOmega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Simple Phylogeny tool (https://www.ebi.ac.uk/Tools/phylogeny/simple_phylogeny/) was used to build the tree, and the visualization was done by using ETE Toolkit (http://etetoolkit.org/treeview/).

For Arabidopsis, all reviewed PEBP sequences were obtained from InterPro (http://www.ebi.ac.uk/interpro/). Based on the literature on apple (Kotoda et al. 2010; Kotoda and Wada 2005), MdFT and MdFT2 were obtained from UniProt (https://www.uniprot.org/), and MdTFL from NCBI (ncbi.nlm.nih.gov). Soybean amino acid sequences were obtained from NCBI according to the genes reported in (Lee et al. 2021). *Cannabis* proteins with predicted PEBP domains were selected from Ensembl Plants (https://plants.ensembl.org/)

Cannabis genes that clustered with the FT/TSF subfamily were selected for expression analysis. Predicted cDNA sequences of the identified *FT-LIKE* genes were downloaded from Ensembl Plants, and primers were designed using Benchling Primer Design Tool (https://www.benchling.com/) The primers used for four *FT-like* genes and the reference gene Tubulin (Lee et al. 2021) are listed in Table 2.1.

Gene	Alternative name	Primer Sequence	Product Size
TU.01.1380 F	FT-LIKE 1	CACCTAGGGTTGAGATTGGTGG	89
TU.01.1380 R		AGGTTCGCTAGGGTTAGGAGC	
TU.09.1439 F	FT-LIKE 2	GGAACTACCGGGCCAAGCTT	123
TU.09.1439 R		CGCGAACACCGTTTGCCTT	
TU.10.2280 F	FT-LIKE 3	TTCTCAACCAACCTCGTGTTCA	81
TU.10.2280 R		GAGCATCCGGATCCACCATAAG	
TU.01.2530 F	FT-LIKE 4	AGGAGGTCAACAATGGTTCTGAG	124
TU.01.2530 R		CTAGGAGCATCAGGGTCAACCA	
TUB F		CTCGGCTGAGAAAGCATACC	102
TUB R		CCATGCCTAGGGTCACACTT	

Table 2.1 Primers used for amplifying cDNA of four FT-like genes and the reference gene (Tubulin)

Results

Phylogenetic Analysis

Twelve *PEBP* genes were found in the genome of *Cannabis sativa*. As revealed by the phylogenetic analysis, five of these proteins clustered with the CEN-like subfamily, three of them being an outgroup with respect to Arabidopsis, soybean, and apple proteins in the same subfamily (Figure 2.1). Three proteins are in the MFT-like clade and one of them is phylogenetically more distant to AtMFT and GmMFT compared to the other two, which reflect the phylogenetic relationship between soybean, *Arabidopsis* and *Cannabis*. Within the FT-like family, there are two main clades. CsFTL1 is in the first clade, clustered with AtFT, AtTSF, and GmFT5a and GmFT5b, which are all known to be floral inducers. The second clade has two main groups, reflecting the diversification of GmFT proteins. Unlike other FT proteins included in the analysis, GmFT1a, GmFT1b, GmFT4, and GmFT6 suppress flowering, and they form an outgroup with respect to GmFT2a, GmFT2b, GmFT3a, as well as apple and *Cannabis* FT proteins (MdFT1, MdFT2, CsFTL2, CsFTL3, and CsFTL4).

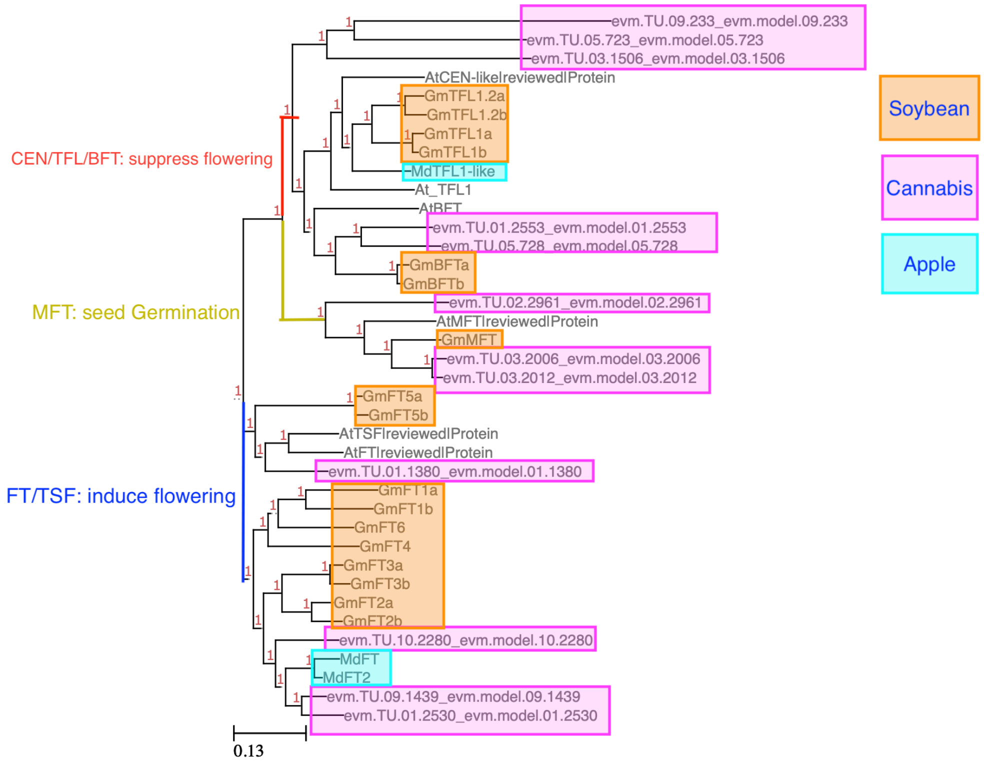


Figure 2.1 Phylogenetic tree of PEBP family from *Arabidopsis thaliana* (At), *Glycine max* (Gm), *Malus domestica* (Md), and *Cannabis sativa* (highlighted in pink). Branch colors indicate subfamilies within the PEBP family.

Gene Expression Analysis

For both the photoperiod-sensitive (PS) and day-neutral (DN) varieties, *FTL1* gene expression was consistently higher in SDs relative to LDs (Figure 2.2). Under SD conditions, PS plants had relatively stable expression, gradually increasing towards the end of the dark period (Figure 2.3a). Whereas for DN plants, the expression of *FTL1* started decreasing at dawn. The minimum expression was at ZT 13 (5h after dusk) and starts to increase again until dawn. Under LD conditions, DN plants showed generally higher gene expression compared to PS plants (Figure 2.3b). *FTL1* expression in DN plants increased for the first 4 hours after dawn (ZT 0) and stayed high until the next dawn (ZT 24). For PS plants, expression was generally stable until dusk; it was minimum at ZT 21 and increased again at dawn (ZT 24).

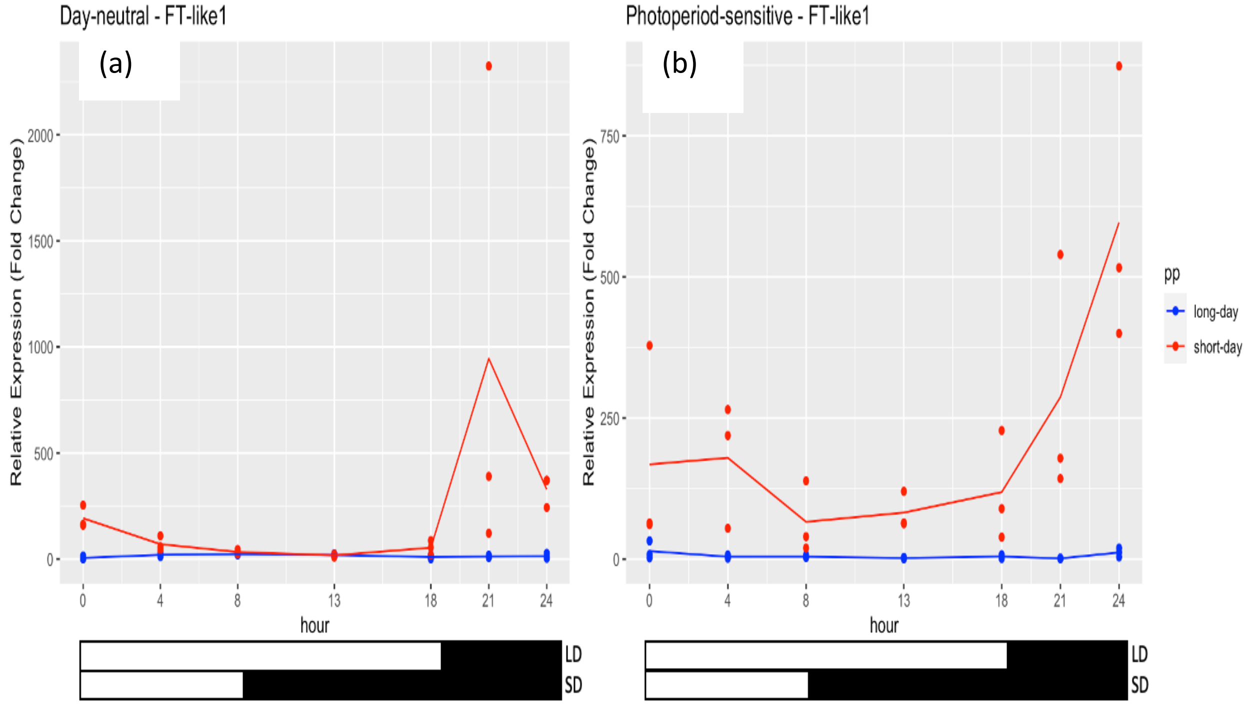


Figure 2.2 Diurnal expression of *FT-like1* under LD (blue) and SD (red) conditions in (a) day-neutral and (b) photoperiod-sensitive plants. White and black rectangles indicate subjective day and night times, respectively.

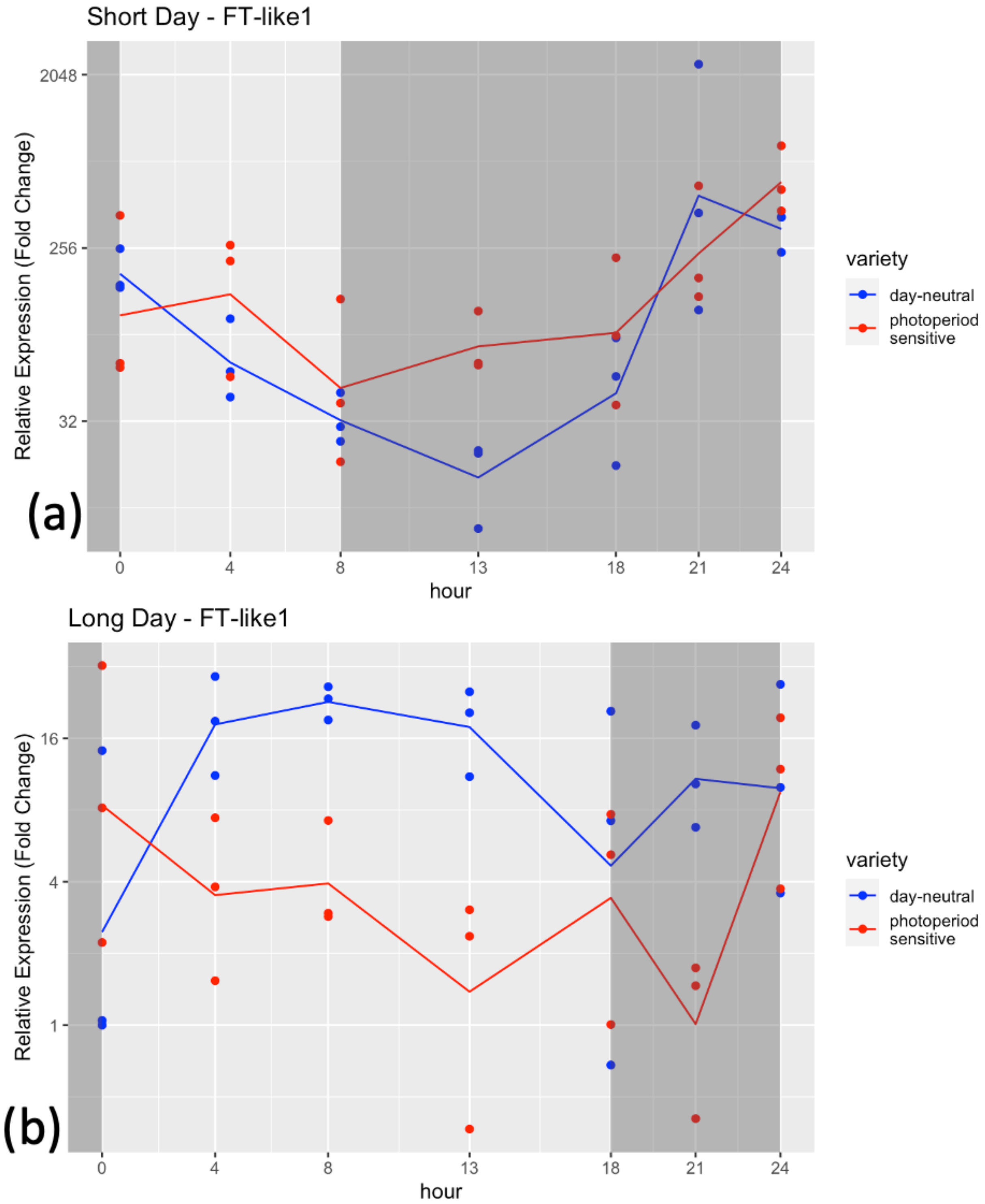


Figure 2.3 Diurnal expression of *FT-like1* in day-neutral (blue) and photoperiod-sensitive (red) plants under (a) short-day and (b) long day conditions. Shaded areas represent the dark period. Y-axis is in log scale in order to show the patterns of low gene expression.

FTL2 expression was higher for PS plants under SD compared to LD (Figure 2.4b). In SD treatment, expression decreased after dusk (ZT 8), and started increasing again after ZT 18, peaking at the next dawn (Figure 2.5a). Under LD, the expression level was very low and relatively stable, although there was a slight decrease after ZT 8, which increased back to the initial levels at dawn (ZT 24; Figure 2.5b). In DN plants, general expression levels of *FTL2* and its diurnal patterns were similar between LD and SD conditions, except there was an increase in expression at ZT 21 for the SD treatment but not for the LD treatment (Figure 2.4a). Unlike PS plants, expression started low at dawn and peaked during ZT 4 and ZT 8 in both treatments. The expression level of *FTL2* in PS plants was higher in SD compared to DN plants, but lower in LD.

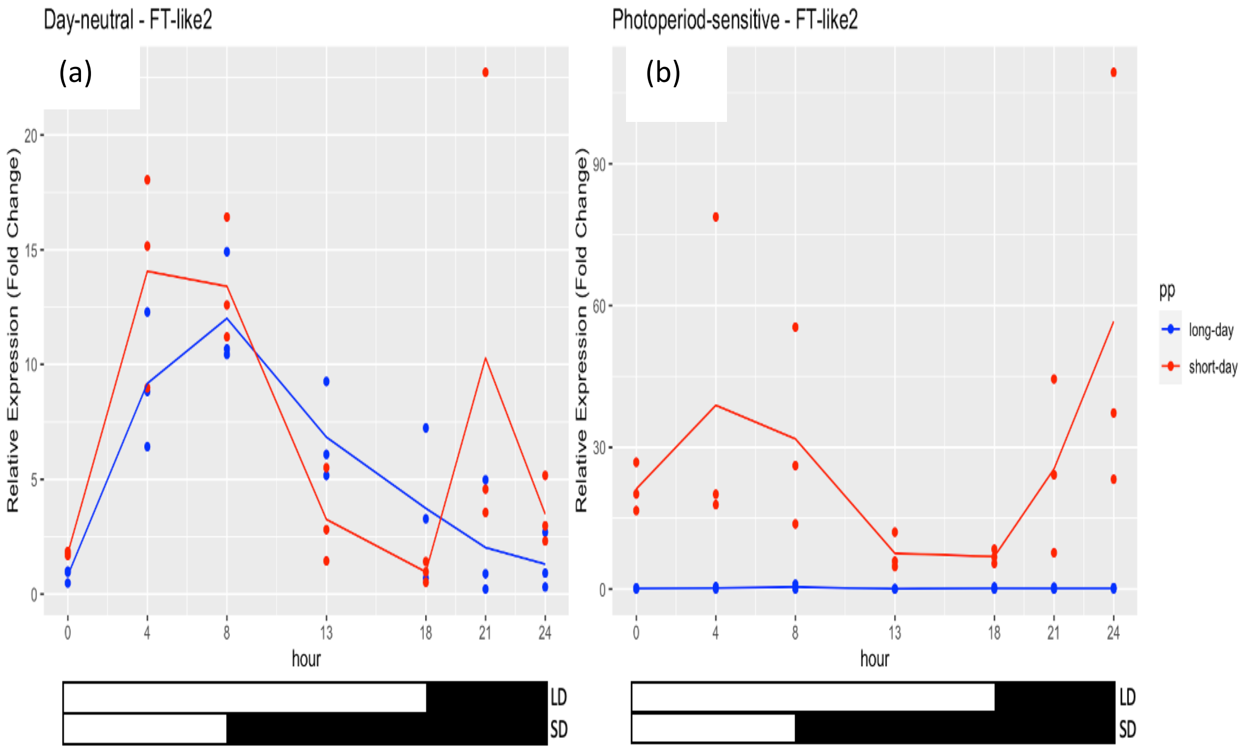


Figure 2.4 Diurnal expression of *FT-like2* under LD (blue) and SD (red) conditions in (a) day-neutral and (b) photoperiod-sensitive plants. White and black rectangles indicate subjective day and night times, respectively.

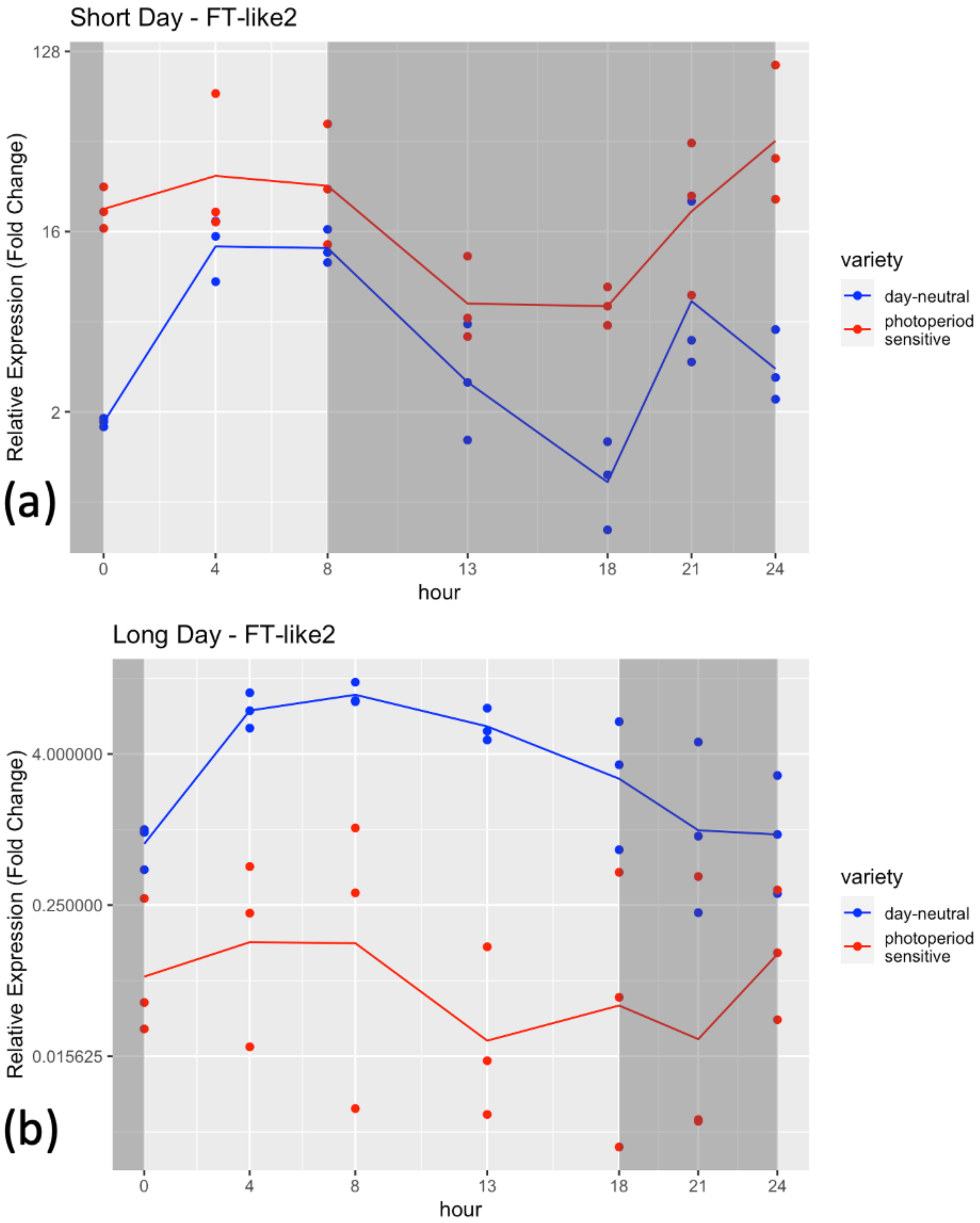
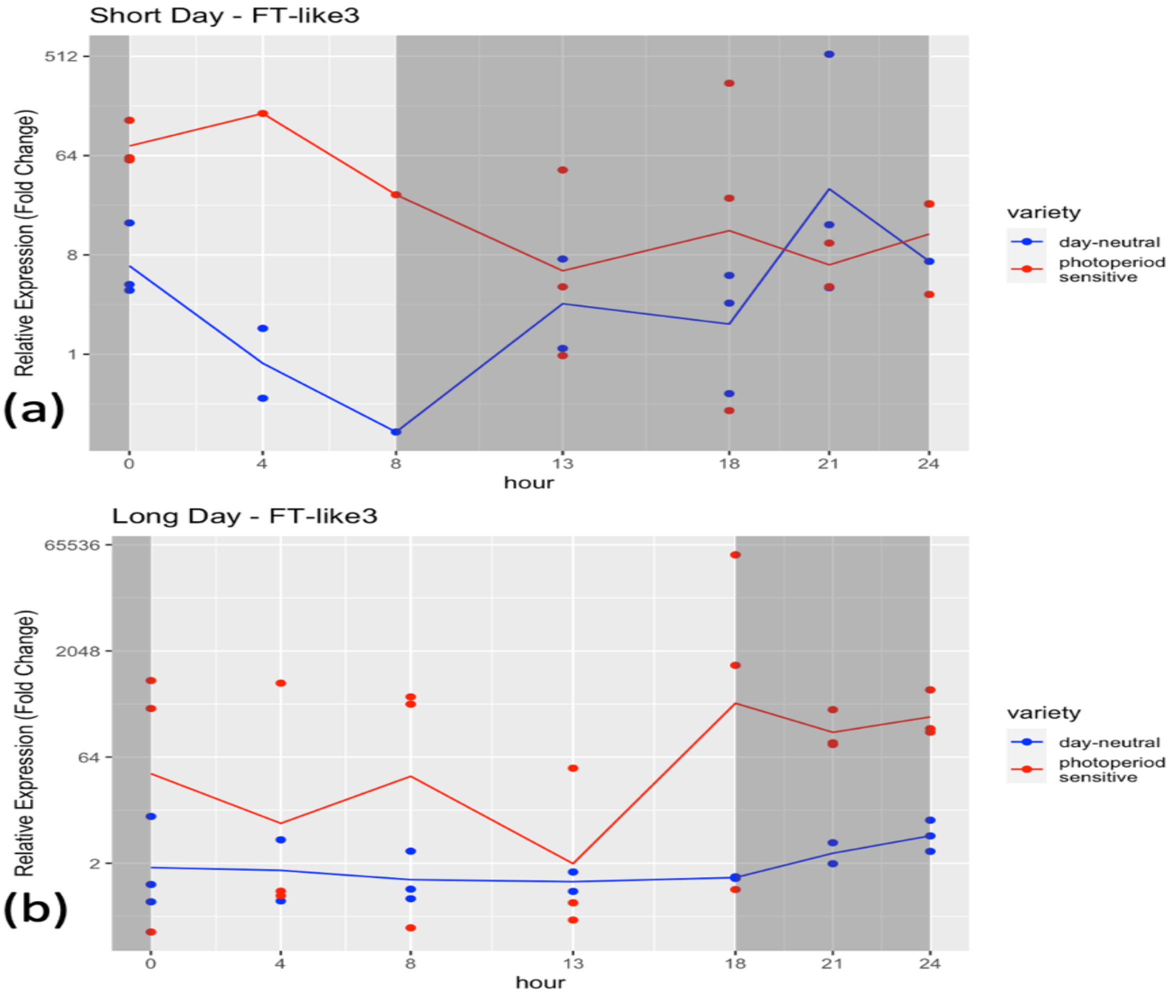


Figure 2.5 Diurnal expression of *FT-like2* in day-neutral (blue) and photoperiod-sensitive (red) plants under (a) short-day and (b) long day conditions. Shaded areas represent the dark period. Y-axis is in log scale in order to show the patterns of low gene expression.

The primer set used for *FTL3* was not reliable enough, and it was not possible to measure the expression level in fifteen samples at all. However, based on the limited data, the expression level of *FTL3* was generally higher in PS plants under both LD and SD treatments. Under LD, the expression in PS plants increased at dusk (ZT 18; Figure 2.6a), while it stayed at the same level under SD (Figure 2.6b). In DN plants, expression of *FTL3* was stable in LD for the most part, with a gradual increase after dusk (ZT 18). Under SD, expression level decreased from dawn to dusk (ZT 8) and reached the same level again at ZT 13.


Short Day - FT-like3

Long Day - FT-like3

Figure 2.6 Diurnal expression of *FT-like3* in day-neutral (blue) and photoperiod-sensitive (red) plants under (a) short-day and (b) long day conditions. Shaded areas represent the dark period. Y-axis is in log scale in order to show the patterns of low gene expression.

For both varieties, the expression of *FTL4* was higher under SD compared to LD (Figure 2.7). Under SD, expression in PS plants gradually increased and peaked at ZT 13, decreased at ZT 18, and peaked again at the next dawn, which resulted in higher expression compared to previous dawn (Figure 2.8a). The diurnal expression followed an opposite pattern in LD: expression level decreased until ZT 13, increased at ZT 18, and decreased again, starting the next day with lower expression compared to the previous dawn (Fig 2.8b). In DN plants, the expression level increased until ZT 8 in both treatments. However, expression gradually decreased under LD, returning to the initial level in the next dawn, while under SD expression remained higher until the next dawn.

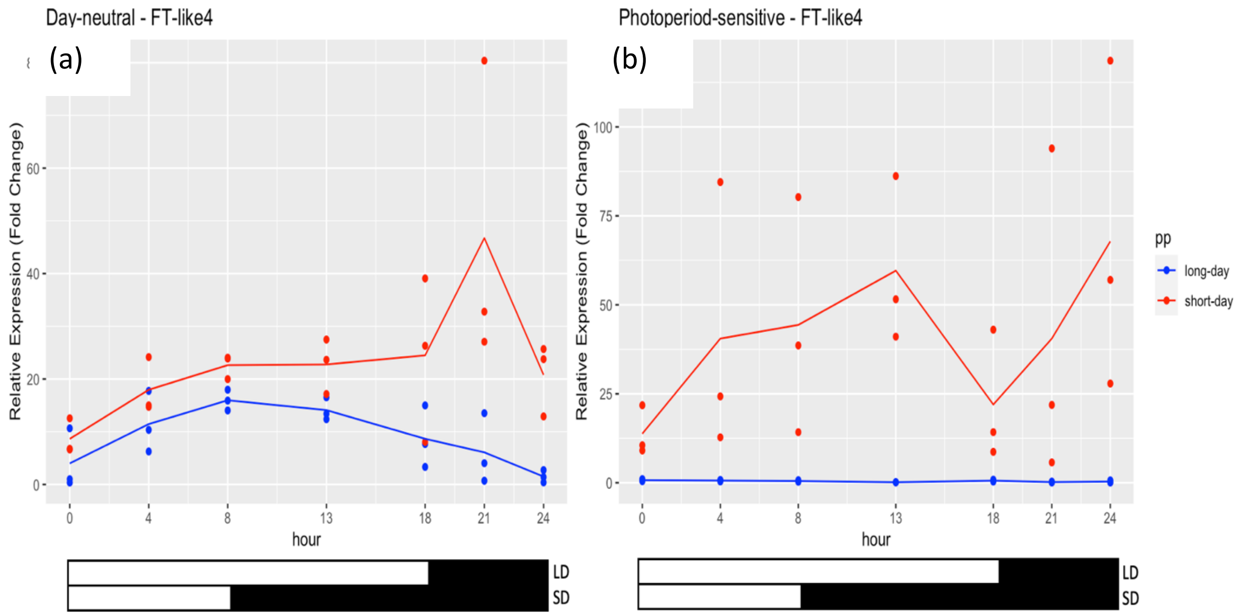


Figure 2.7 Diurnal expression of *FT-like4* under LD (blue) and SD (red) conditions in (a) day-neutral and (b) photoperiod-sensitive plants. White and black rectangles indicate subjective day and night times, respectively.

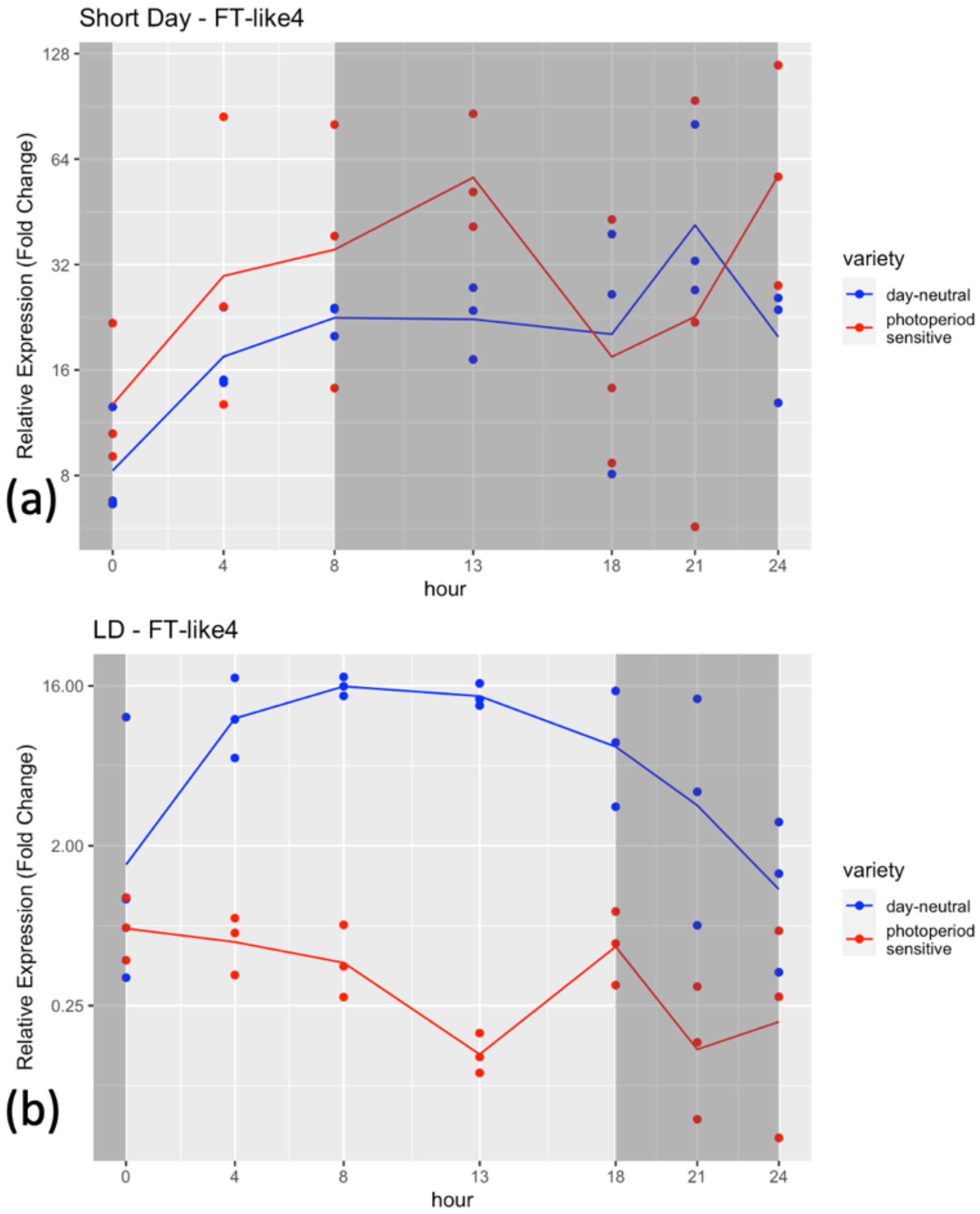


Figure 2.8 Diurnal expression of *FT-like4* in day-neutral (blue) and photoperiod-sensitive (red) plants under (a) short-day and (b) long day conditions. Shaded areas represent the dark period. Y-axis is in log scale in order to show the patterns of low gene expression.

Discussion

The discovery of *FT* and its functions in *Arabidopsis thaliana* was a critical development in understanding the nature of floral stimulus in plants, and it paved the way for further research into molecular mechanisms of flowering time regulation. Fortunately, *FT* homologs and associated genes found in model species *Arabidopsis thaliana* (LDP) and *Oryza sativa* (SDP) are well-conserved, and they often play similar roles in distant species (see (Turck et al. 2008) for a review), which enables a starting point for studying reproductive development in novel species. However, as studies on different species discover further details, major differences in regulatory mechanisms become apparent, even within plants that are classified in the same photoperiod-sensitivity groups (Hayama et al. 2007). Considering nuances in photoperiod-sensitive development (discussed in Chapter 1), it is expected that more mechanisms will be discovered to explain those nuances. Despite the benefits of model plants, it is important to study previously ignored species, for both practical (e.g., crop improvement) and theoretical (understanding the various processes plants employ) reasons. Being an emerging crop showing a variety of flowering behaviors, *Cannabis sativa* deserves more attention from researchers than it is receiving today. In this study, I attempted to identify potential *FT* homologs in *Cannabis* and characterize their diurnal expression patterns in DN and PS plants under LD and SD conditions.

According to the phylogenetic analysis and gene expression experiment, there are four *FT-like* genes in *Cannabis*, showing different expression patterns in different varieties and conditions. Currently, it is unknown what functions these genes have. While gene expression patterns cannot provide any straightforward evidence for their functions, it is possible to hypothesize potential functions with some assumptions. Given our knowledge about other flowering plants, we can assume at least one of the *FT-like* genes is a floral inducer and regulated by the photoperiod pathway. Based on the expression profiles observed in other species, the expression of a photoperiod-regulated floral inducer is expected to be expressed at higher levels in inductive photoperiod compared to non-inductive photoperiod in PS plants (Corbesier et al. 2007; Izawa et al. 2002), while the expression level is expected to be high in DN plants under both conditions (Guo et al. 2015). Additionally, gene expression likely oscillates in a circadian manner, potentially following a pattern similar to another SDP.

Of the four genes analyzed, *FTL2* appears to be the best candidate for a photoperiod-regulated floral inducer given these assumptions. In DN plants, the expression level and pattern were unaffected by photoperiod treatments, whereas PS plants showed increased expression level in SD treatment. The pattern appeared to follow a ~24h cycle, although this needs to be confirmed by measuring the expression over the course of multiple days. Also, similar to SDP genes *Pharbitis nil FT1* and *FT2* (Hayama et al. 2007), maize *ZCN8* (Meng et al. 2011), and *Chenopodium rubrum FTL1* (Cháb et al. 2008), expression level of *CsFTL2* in PS plants increased at night and peaked at dawn under SD conditions, which is different than some other SDP, such as soybean (Lee et al. 2021) and rice (Kojima et al. 2002). A recent study has also found that the expression level of this gene is higher in early-flowering wild cannabis plants than late-flowering cultivated cannabis plants under LD, and increased only in SD conditions for the late flowering cultivars, consistent with my findings (Chen et al. 2021). For future studies modelling the regulatory mechanisms, the differences in peak times between DN and PS plants might be significant to understand regulatory differences between the two.

Similar to *FTL2, FTL1* expression was increased under SD for PS plants, and expression increased during night, peaking at dawn. But unlike *FTL2*, the expression of *FTL1* was increased in SD for DN plants as well, with different patterns between LD and SD. Under LD, the pattern was similar to *FTL2* (increasing during the day), but under SD, it increased during the night like in PS plants. There could be several explanations as to why the expression of DN plants is affected by photoperiod. The gene could be responsive to photoperiod with some reduction in LD-dependent suppression. This could mean it functions redundantly with *FTL2* in promoting flowering under LD in DN plants, while potentially facilitating LD flowering in facultative-SD plants. On the other hand, photoperiod treatment may have caused alterations in other mechanisms regulating *FTL1* expression, such as changes in photosynthesis (Oh et al. 2009), metabolism (Ponnu et al. 2011; Wahl et al. 2013), and hormone levels and gradients (Alabadí and Blázquez 2008; Blázquez et al. 2002; Campos-Rivero et al. 2017). It is also possible that slight increase in temperature during light periods have resulted in some circadian entrainment based on temperature cycles (Gil and Park 2019; James et al. 2012).

The expression of *FTL4* was also affected by photoperiod treatment in DN plants, with slightly higher expression level under SD. Interestingly, the ongoing light period after the peak at ZT 8 under LD seemingly decreased the expression gradually until dawn, while expression remained high during the same period under SD. The same explanations for the change in expression *FTL1* in DN plants could apply here. It is interesting that the expression patterns of PS plants followed opposite patterns between LD and SD treatments. This also could hint at processes that lead to suppression of *FTL4* expression at around ZT 13 under LD. However, variation within biological replicates ambiguated the reality of observed trends.

Unlike other *FTL* genes, there was not a clear difference in the general expression level of *FTL3* between treatments. Also, expression level was higher in PS plants in both treatments. Due to high variability, missing data points, and unreliable measurements, it is not possible to interpret this data.

The above-mentioned hypotheses about the roles of *FTL* genes in *Cannabis* assume these genes have some role in shoot-to-inflorescence transition. While it is possible that they are all involved in the timing of flowering, future studies may show that is not the case. They could be more involved in different stages or other aspects of reproductive development or in certain physiological processes. In fact, flowering might be occurring without the involvement of the *FTL* genes (Štorchová et al. 2019). Still, it is conceivable that a species with multiple *FTL* genes is utilizing all of them to be able to flower timely depending on different cues by having various levels of sensitivity to upstream pathways or different levels of efficiency in inducing flowering.

Hopefully, data presented here will motivate further research to understand the reproductive development of *Cannabis* and be useful for future studies investigating the functions of *FTL* and other *PEBP* genes, as well as their regulation. Our understanding of the roles of these genes in Cannabis can be improved in the future by utilizing genetic transformation/modification techniques.

REFERENCES CITED

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T. 2005. Fd, a bzip protein mediating signals from the floral pathway integrator ft at the shoot apex. Science. 309(5737):1052-1056.

Adamec L, Macháčková I, Krekule J, Nováková M. 1989. Electric current inhibits flowering in the short-day plant chenopodium rubrum l. Journal of plant physiology. 134(1):43-46.

Adamek K, Jones AMP, Torkamaneh D. 2022. Accumulation of somatic mutations leads to genetic mosaicism in cannabis. The Plant Genome. 15(1):e20169.

Alabadí D, Blázquez MA. 2008. Molecular interactions between light and hormone signaling to control plant growth. Plant Molecular Biology. 69(4):409.

Bernier G, Havelange A, Houssa C, Petitjean A, Lejeune P. 1993. Physiological signals that induce flowering. The Plant Cell. 5(10):1147-1155.

Blázquez MA, Trénor M, Weigel D. 2002. Independent control of gibberellin biosynthesis and flowering time by the circadian clock in arabidopsis. Plant Physiology. 130(4):1770-1775.

Campos-Rivero G, Osorio-Montalvo P, Sánchez-Borges R, Us-Camas R, Duarte-Aké F, De-la-Peña C. 2017. Plant hormone signaling in flowering: An epigenetic point of view. Journal of plant physiology. 214:16-27.

Cháb D, Kolář J, Olson MS, Štorchová H. 2008. Two flowering locus t (ft) homologs in chenopodium rubrum differ in expression patterns. Planta. 228(6):929-940.

Chailakhyan MK. 1937. Hormonal theory of plant development. Hormonal theory of plant development.

Chardon F, Damerval C. 2005. Phylogenomic analysis of the pebp gene family in cereals. Journal of molecular evolution. 61(5):579-590.

Chen M, MacGregor DR, Dave A, Florance H, Moore K, Paszkiewicz K, Smirnoff N, Graham IA, Penfield S. 2014. Maternal temperature history activates flowering locus t in fruits to control progeny dormancy according to time of year. Proceedings of the National Academy of Sciences. 111(52):18787-18792.

Chen X, Guo H-Y, Zhang Q-Y, Wang L, Guo R, Zhan Y-X, Lv P, Xu Y-P, Guo M-B, Zhang Y. 2021. Whole-genome resequencing of wild and cultivated cannabis reveals the genetic structure and adaptive selection of important traits.

Chen X, Guo H-Y, Zhang Q-Y, Wang L, Guo R, Zhan Y-X, Lv P, Xu Y-P, Guo M-B, Zhang Y et al. 2022. Whole-genome resequencing of wild and cultivated cannabis reveals the genetic structure and adaptive selection of important traits. BMC Plant Biology. 22(1):371.

Cheng J-Z, Zhou Y-P, Lv T-X, Xie C-P, Tian C-E. 2017. Research progress on the autonomous flowering time pathway in arabidopsis. Physiology and Molecular Biology of Plants. 23(3):477-485.

Clarke RC, Merlin MD. 2016. Cannabis domestication, breeding history, present-day genetic diversity, and future prospects. Critical reviews in plant sciences. 35(5-6):293-327.

Clarke RC, Watson DP. 2007. Cannabis and natural cannabis medicines. In: ElSohly MA, editor. Marijuana and the cannabinoids. Totowa, NJ: Humana Press. p. 1-15.

Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C. 2007. Ft protein movement contributes to long-distance signaling in floral induction of arabidopsis. science. 316(5827):1030-1033.

Crawford SS, Crawford EB, inventors; Jack Hempicine LLC, assignee. 2019. Cross-hybridization of distinct homozygous cannabis plants to produce consistent early flowering seeds.

Garner WW, Allard HA. 1920. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Monthly Weather Review. 48(7):415-415.

Gaudinier A, Blackman BK. 2020. Evolutionary processes from the perspective of flowering time diversity. New Phytologist. 225(5):1883-1898.

Gil K-E, Park C-M. 2019. Thermal adaptation and plasticity of the plant circadian clock. New Phytologist. 221(3):1215-1229.

Guo D, Li C, Dong R, Li X, Xiao X, Huang X. 2015. Molecular cloning and functional analysis of the flowering locus t (ft) homolog ghft1 from gossypium hirsutum. Journal of Integrative Plant Biology. 57(6):522-533.

Hanano S, Goto K. 2011. Arabidopsis terminal flower1 is involved in the regulation of flowering time and inflorescence development through transcriptional repression The Plant Cell. 23(9):3172-3184.

Harada A, Tsuji N, Fujimoto N, Matsuo M, Saito M, Kanzawa N. 2022. Heterologous expression of flowering locus t promotes flowering but does not affect diurnal movement in the legume lotus japonicus. Plant Biotechnology. 39(2):155-163.

Hayama R, Agashe B, Luley E, King R, Coupland G. 2007. A circadian rhythm set by dusk determines the expression of ft homologs and the short-day photoperiodic flowering response in pharbitis. The Plant Cell. 19(10):2988-3000.

Heinze PH, Parker MW, Borthwick HA. 1942. Floral initiation in biloxi soybean as influenced by grafting. Botanical Gazette. 103(3):518-530.

Heller WP, Ying Z, Davenport TL, Keith LM, Matsumoto TK. 2014. Identification of members of the dimocarpus longan flowering locus t gene family with divergent functions in flowering. Tropical Plant Biology. 7(1):19-29.

Heslop-Harrison J, Heslop-Harrison Y. 1969. Cannabis sativa l. In: Evans LT, editor. The induction of flowering some case histories. Cornell University Press. p. 205-226.

Huang N-C, Jane W-N, Chen J, Yu T-S. 2012. Arabidopsis thaliana centroradialis homologue (atc) acts systemically to inhibit floral initiation in arabidopsis. The Plant Journal. 72(2):175-184.

Huang T, Böhlenius H, Eriksson S, Parcy F, Nilsson O. 2005. The mrna of the arabidopsis gene ft moves from leaf to shoot apex and induces flowering. Science. 309(5741):1694-1696.

Hyun Y, Richter R, Coupland G. 2017. Competence to flower: Age-controlled sensitivity to environmental cues. Plant physiology. 173(1):36-46.

Izawa T, Oikawa T, Sugiyama N, Tanisaka T, Yano M, Shimamoto K. 2002. Phytochrome mediates the external light signal to repress ft orthologs in photoperiodic flowering of rice. Genes & development. 16(15):2006-2020.

Jackson S, Hong Y. 2012. Systemic movement of ft mrna and a possible role in floral induction. Frontiers in Plant Science. 3.

Jaeger KE, Wigge PA. 2007. Ft protein acts as a long-range signal in arabidopsis. Current biology. 17(12):1050-1054.

James AB, Syed NH, Brown JWS, Nimmo HG. 2012. Thermoplasticity in the plant circadian clock. Plant Signaling & Behavior. 7(10):1219-1223.

Jin S, Nasim Z, Susila H, Ahn JH. 2021. Evolution and functional diversification of flowering locus t/terminal flower 1 family genes in plants. Seminars in Cell & Developmental Biology. 109:20-30.

Keller A, Leupin M, Mediavilla V, Wintermantel E. 2001. Influence of the growth stage of industrial hemp on chemical and physical properties of the fibres. Industrial crops and products. 13(1):35-48.

Kim D-H. 2020. Current understanding of flowering pathways in plants: Focusing on the vernalization pathway in arabidopsis and several vegetable crop plants. Horticulture, Environment, and Biotechnology. 61(2):209-227.

Kinet J-M, Sachs RM, Bernier G. 2018. The physiology of flowering: Volume iii the development of flowers. CRC Press.

Kinoshita T, Ono N, Hayashi Y, Morimoto S, Nakamura S, Soda M, Kato Y, Ohnishi M, Nakano T, Inoue S-i. 2011. Flowering locus t regulates stomatal opening. Current Biology. 21(14):1232-1238.

Knott JE. 1934. Effect of a localized photo-period on spinach. Proceedings American Society Hort Science. 31:152-154.

Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T. 1999. A pair of related genes with antagonistic roles in mediating flowering signals. Science. 286(5446):1960-1962.

Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M. 2002. Hd3a, a rice ortholog of the arabidopsis ft gene, promotes transition to flowering downstream of hd1 under short-day conditions. Plant and Cell Physiology. 43(10):1096-1105.

Komiya R, Ikegami A, Tamaki S, Yokoi S, Shimamoto K. 2008. Hd3a and rft1 are essential for flowering in rice. Development. 135(4):767-774.

Kong F, Liu B, Xia Z, Sato S, Kim BM, Watanabe S, Yamada T, Tabata S, Kanazawa A, Harada K et al. 2010. Two coordinately regulated homologs of flowering locus t are involved in the control of photoperiodic flowering in soybean. Plant Physiology. 154(3):1220-1231.

Kotoda N, Hayashi H, Suzuki M, Igarashi M, Hatsuyama Y, Kidou S-i, Igasaki T, Nishiguchi M, Yano K, Shimizu T et al. 2010. Molecular characterization of flowering locus t-like genes of apple (malus × domestica borkh.). Plant and Cell Physiology. 51(4):561-575.

Kotoda N, Wada M. 2005. Mdtfl1, a tfl1-like gene of apple, retards the transition from the vegetative to reproductive phase in transgenic arabidopsis. Plant Science. 168(1):95-104.

Lang A, Chailakhyan MK, Frolova IA. 1977. Promotion and inhibition of flower formation in a dayneutral plant in grafts with a short-day plant and a long-day plant. Proceedings of the National Academy of Sciences. 74(6):2412-2416.

Lee SH, Choi CW, Park KM, Jung W-H, Chun HJ, Baek D, Cho HM, Jin BJ, Park MS, No DH et al. 2021. Diversification in functions and expressions of soybean flowering locus t genes fine-tunes seasonal flowering. Frontiers in Plant Science. 12.

Lisson SN, Mendham NJ, Carberry PS. 2000. Development of a hemp (cannabis sativa l.) simulation model 2.The flowering response of two hemp cultivars to photoperiod. Australian Journal of Experimental Agriculture. 40(3):413-417.

Macháčková I, Krekule J. 1991. The interaction of direct electric current with endogenous rhythms of flowering in chenopodium rubrum. Journal of plant physiology. 138(4):365-369.

Macháčková I, Pospíšková M, Krekule J. 1990. Further studies on the inhibitory action of direct electric current on flowering in the short-day plant chenopodium rubrum l. Journal of plant physiology. 136(3):381-384.

Marc J, Palmer JH. 1981. Photoperiodic sensitivity of inflorescence initiation and development in sunflower. Field Crops Research. 4:155-164.

McDaniel CN, Hsu FC. 1976. Position-dependent development of tobacco meristems. Nature. 259(5544):564-566.

McPartland JM. 2018. Cannabis systematics at the levels of family, genus, and species. Cannabis and cannabinoid research. 3(1):203-212.

Meng X, Muszynski MG, Danilevskaya ON. 2011. The ft-like zcn8 gene functions as a floral activator and is involved in photoperiod sensitivity in maize. The Plant Cell. 23(3):942-960.

Moher M, Jones M, Zheng Y. 2021. Photoperiodic response of in vitro cannabis sativa plants. HortScience horts. 56(1):108-113.

Moraes TS, Dornelas MC, Martinelli AP. 2019. Ft/tfl1: Calibrating plant architecture. Frontiers in Plant Science. 10.

Naylor AW. 1941. Effect of nutrition and age upon rate of development of terminal staminate inflorescences of xanthium pennsylvanicum. Botanical Gazette. 103(2):342-353.

Oh W, Cheon IH, Kim KS, Runkle ES. 2009. Photosynthetic daily light integral influences flowering time and crop characteristics of cyclamen persicum. HortScience horts. 44(2):341-344.

Pan G, Li Z, Yin M, Huang S, Tao J, Chen A, Li J, Tang H, Chang L, Deng Y et al. 2021. Genome-wide identification, expression, and sequence analysis of constans-like gene family in cannabis reveals a potential role in plant flowering time regulation. BMC Plant Biology. 21(1):142.

Patil HB, Chaurasia AK, Kumar S, Krishna B, Subramaniam VR, Sane AP, Sane PV. 2022. Synchronized flowering in pomegranate, following pruning, is associated with expression of the flowering locus t homolog, pgft1. Physiologia Plantarum. 174(1):e13620.

Petit J, Salentijn EMJ, Paulo M-J, Denneboom C, Trindade LM. 2020. Genetic architecture of flowering time and sex determination in hemp (cannabis sativa l.): A genome-wide association study. Frontiers in Plant Science. 11.

Pfaffl MW. 2004. Quantification strategies in real-time pcr. AZ of quantitative PCR. 1:89-113.

Ponnu J, Wahl V, Schmid M. 2011. Trehalose-6-phosphate: Connecting plant metabolism and development. Frontiers in Plant Science. 2.

Potter D. 2009. The propagation, characterisation and optimisation of cannabis sativa l as a phytopharmaceutical. [London]: King's College London.

Reid JB. 1979. Red-far red reversibility of flower development and apical senescence in pisum. Zeitschrift für Pflanzenphysiologie. 93(4):297-301.

Rodziewicz P, Kayser O. 2020. Cannabis sativa l. –cannabis. In: Novak J, Blüthner W-D, editors. Medicinal, aromatic and stimulant plants. Cham: Springer International Publishing. p. 233-264.

Ryu JY, Lee H-J, Seo PJ, Jung J-H, Ahn JH, Park C-M. 2014. The arabidopsis floral repressor bft delays flowering by competing with ft for fd binding under high salinity. Molecular plant. 7(2):377-387.

Salentijn EM, Petit J, Trindade LM. 2019. The complex interactions between flowering behavior and fiber quality in hemp. Frontiers in plant science. 10:614.

Samach A, Wigge PA. 2005. Ambient temperature perception in plants. Current opinion in plant biology. 8(5):483-486.

Schwabe WW. 1951. Factors controlling flowering in the chrysanthemum: Ii. Day-length effects on the further development of inflorescence buds and their experimental reversal and modification. Journal of Experimental Botany. 2(5):223-237.

Simpson GG, Dean C. 2002. Arabidopsis, the rosetta stone of flowering time? Science. 296(5566):285-289.

Small E. 2016. Cannabis: A complete guide. CRC Press.

Small E. 2018. Dwarf germplasm: The key to giant cannabis hempseed and cannabinoid crops. Genetic Resources and Crop Evolution. 65(4):1071-1107.

Spitzer-Rimon B, Duchin S, Bernstein N, Kamenetsky R. 2019. Architecture and florogenesis in female cannabis sativa plants. Frontiers in Plant Science. 10.

Stack GM, Toth JA, Carlson CH, Cala AR, Marrero-González MI, Wilk RL, Gentner DR, Crawford JL, Philippe G, Rose JK. 2021. Season-long characterization of high-cannabinoid hemp (cannabis sativa l.) reveals variation in cannabinoid accumulation, flowering time, and disease resistance. GCB Bioenergy. 13(4):546-561.

Štorchová H, Hubáčková H, Abeyawardana OA, Walterová J, Vondráková Z, Eliášová K, Mandák B. 2019. Chenopodium ficifolium flowers under long days without upregulation of flowering locus t (ft) homologs. Planta. 250(6):2111-2125.

Thomas B, Vince-Prue D. 1996. Photoperiodism in plants. Elsevier.

Turck F, Fornara F, Coupland G. 2008. Regulation and identity of florigen: Flowering locus t moves center stage. Annual review of plant biology. 59:573-594.

Van der Werf H, Haasken H, Wijlhuizen M. 1994. The effect of daylength on yield and quality of fibre hemp (cannabis sativa l.). European Journal of Agronomy. 3(2):117-123.

Wagner E, Lehner L, Normann J, Veit J, Albrechtová J. 2006. Hydro-electrochemical integration of the higher plant — basis for electrogenic flower induction. In: Baluška F, Mancuso S, Volkmann D, editors. Communication in plants: Neuronal aspects of plant life. Berlin, Heidelberg: Springer Berlin Heidelberg. p. 369-389.

Wagner E, Normann J, Albrechtová JT, Walczysko P, Bonzon M, Greppin H. 1998. Electrochemical-hydraulic signalling in photoperiodic control of flowering: Is "florigen" a frequency-coded electric signal? Flowering Newsletter.62-74.

Wahl V, Ponnu J, Schlereth A, Arrivault S, Langenecker T, Franke A, Feil R, Lunn JE, Stitt M, Schmid M. 2013. Regulation of flowering by trehalose-6-phosphate signaling in arabidopsis thaliana. Science. 339(6120):704-707.

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D. 2005. Integration of spatial and temporal information during floral induction in arabidopsis. Science. 309(5737):1056-1059.

Williams O, Vander Schoor JK, Butler JB, Ridge S, Sussmilch FC, Hecht VFG, Weller JL. 2022. The genetic architecture of flowering time changes in pea from wild to crop. Journal of Experimental Botany.

Xi W, Liu C, Hou X, Yu H. 2010. Mother of ft and tfl1 regulates seed germination through a negative feedback loop modulating aba signaling in arabidopsis. The Plant Cell. 22(6):1733-1748.

Yamaguchi A, Kobayashi Y, Goto K, Abe M, Araki T. 2005. Twin sister of ft (tsf) acts as a floral pathway integrator redundantly with ft. Plant and Cell Physiology. 46(8):1175-1189.

Yang L, Xu M, Koo Y, He J, Poethig RS. 2013. Sugar promotes vegetative phase change in arabidopsis thaliana by repressing the expression of mir156a and mir156c. elife. 2:e00260.

Yoo SJ, Chung KS, Jung SH, Yoo SY, Lee JS, Ahn JH. 2010. Brother of ft and tfl1 (bft) has tfl1-like activity and functions redundantly with tfl1 in inflorescence meristem development in arabidopsis. The Plant Journal. 63(2):241-253.

Yu S, Cao L, Zhou C-M, Zhang T-Q, Lian H, Sun Y, Wu J, Huang J, Wang G, Wang J-W. 2013. Sugar is an endogenous cue for juvenile-to-adult phase transition in plants. elife. 2:e00269.

Yu Z, Chen W, Wang Y, Zhang P, Shi N, Hong Y. 2022. Mobile flowering locus t rna – biological relevance and biotechnological potential. Frontiers in Plant Science. 12.

Zeevaart JAD. 1958. Flower formation as studied by grafting. Veenman.

Zhou S, Jiang L, Guan S, Gao Y, Gao Q, Wang G, Duan K. 2018. Expression profiles of five ft-like genes and functional analysis of phft-1 in a phalaenopsis hybrid. Electronic Journal of Biotechnology. 31:75-83.