Timing is everything: the genetics of flowering time in *Cannabis sativa*

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*Cannabis sativa* is well known for its production of psychoactive chemicals and medicinal products, but it also has huge potential to be a multipurpose crop. Cultivated for biofuel, building materials and textiles, *Cannabis* has a high carbon sequestration rate and is bound to be a key player in future sustainable agriculture. The distinct applications of *Cannabis* are directly or indirectly connected to flowering and require different flowering time phenotypes. As an annual short-day plant, *Cannabis* usually flowers in autumn when days get shorter after an initial vegetative growth period. However, differences in latitude, temperature and other environmental factors require the development of new *Cannabis* cultivars adapted to local climatic conditions. As such, a comprehensive understanding of the physiological and genetic basis of flowering time is crucial to integrating *Cannabis* into modern agriculture, benefiting global sustainability efforts.

**Why is Cannabis a promising high-value crop?**

*Cannabis sativa* (henceforth *Cannabis*) is probably most famous for the valuable secondary compounds, called cannabinoids, that are produced in the trichomes of the female flowers and which have a wide range of medical applications. In addition, *Cannabis* has potentially dozens of different uses: it can be used for biofuel, building material and textiles (Figure 1), making it a high-value multiuse crop.

The non-intoxicating cannabinoid cannabidiol (CBD) is gaining a lot of consideration for treating cancer, epilepsy, multiple sclerosis and anxiety. But *Cannabis* can also produce tetrahydrocannabinol (THC), an intoxicating cannabinoid used as medicine but also as a recreational drug. Legally *Cannabis* is divided into two categories based on THC content: hemp (low THC) and marijuana (high THC).

Innovation is booming in the *Cannabis* industry, with hemp now being used to produce bioplastics and microgreens for salads. But in contrast to other crop plants a huge knowledge gap exists for *Cannabis*, and our understanding of its genetics, physiology and development lags behind many other crops such as maize, rice or wheat.

**How do plants flower?**

Flowering time is hugely complex and depends on a multitude of different factors, such as day length, temperature and plant age. Comprehensive analyses of flowering time have been conducted in several model and crop plant species. The flowering time network in the model plant *Arabidopsis thaliana* (thale cress) is particularly well studied, and several pathways controlling flowering time have been identified, including the photoperiod, circadian clock, age, autonomous, temperature and gibberellin pathways.

To complicate matters, in plants, a range of flowering responses to light exists. Plants can be short-day species (require days with short periods of light to flower, e.g., *Cannabis*, rice), long-day species (require days with long periods of light to flower, e.g., *Arabidopsis*, wheat) or day-neutral (flower irrespective of the light conditions, e.g., cultivated tomato, strawberry; Figure 2). To react to changes in day length with the initiation of flowering, the plant needs to be able to detect these changes, therefore the photoperiod pathway is at the centre of many studies.

Light is perceived by photoreceptors, a group of proteins including the phytochromes and cryptochromes. Phytochromes can exist in active or inactive states. Inactive phytochromes are synthesized in darkness, and when light is perceived, phytochromes are activated and move to the nucleus. In the nucleus of the plant cell, the active phytochromes can interact with proteins called transcription factors that cause large-scale changes in gene expression. Beyond phytochromes, three genes, in particular, are central
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Players in the photoperiod pathway of Arabidopsis thaliana: GIGANTEA (GI), CONSTANS (CO) and FLOWERING LOCUS T (FT) (Figure 3).

In Arabidopsis under long days (as Arabidopsis is a long-day plant) the active form of the phytochrome PHYA stabilizes the transcription factor CO which activates FT. GI is a circadian clock-controlled gene that indirectly helps to promote FT expression by degrading repressors of CO. Interestingly, this signalling cascade does not begin in the shoot apical meristem (i.e., the ‘tip’ of the plant, from which eventually the flowers emerge), but in leaves. The activation of FT in leaves leads to the accumulation of its protein product, known as florigen. Florigen is a small mobile protein that travels through the phloem from the leaves to the shoot apical meristem, where it induces the vegetative to reproductive growth transition. Once transported to the apical meristem, florigen activates the gene SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1). SOC1 promotes flowering by activating LEAFY, a floral meristem identity gene.

Though much of the pioneering research on flowering time genes has been carried out in Arabidopsis, analyses have shown that the genes PHYA, GI, CO and FT are conserved in a wide array of species including tomato, barley, onion, wheat, grapevine and pea. Therefore, it is highly likely that these genes also play a central role in controlling flowering time in Cannabis. Nonetheless, it is worth considering that gene functions may not be similarly conserved as gene sequences across species.

Why is it important to understand flowering time in Cannabis?

From an evolutionary perspective ensuring appropriate flowering certifies the survival of the next generation. In an agricultural context, flowering time is directly related to yield, and the fine tuning of flowering is a major focus of crop improvement efforts. During crop domestication by humans, alterations in key flowering time genes were instrumental in adapting crops to different climatic conditions. The success and worldwide expansion of staple crops like wheat are partly attributed to variations in flowering time genes. During domestication and the artificial selection for specific flowering traits, such as altered flowering due to temperature insensitivity, cultivation was enabled at a wide range of latitudes and in different climates.

As early as 1912 it was known that the photoperiod influences Cannabis flowering time. As a short-day plant, Cannabis flowering time is particularly affected by day length. Cannabis remains vegetative under long days and flowering is accelerated after several consecutive short days. Therefore, to cultivate Cannabis at northern latitudes (summer day lengths can be over 17 hours in northern Europe), early flowering phenotypes would be favourable to ensure ample flower and seed production as well as to allow sufficient time for seed ripening. On the other hand, to grow Cannabis for fibre, for which early flowering is not desirable, at southern latitudes with short days, late flowering phenotypes are required.

Despite the photoperiod sensitivity, most individuals will eventually flower even under long-day conditions, which is why Cannabis is considered a facultative short-day plant. The optimal photoperiod required to induce flowering is cultivar-specific, varying between 9 and 14 hours of light. It is not yet known precisely how many consecutive short days are required to induce flowering.
Interestingly, ‘auto-flowering’ or photoperiod-insensitive Cannabis cultivars exist that flower independent of the day length. Such cultivars are popular in northern latitudes with long summer days. These auto-flowering cultivars appeal to farmers growing Cannabis for CBD as the plants are typically smaller in stature and mature much faster than photoperiod-sensitive cultivars.

Another important aspect of Cannabis biology is that the species is dioecious (a Greek word that translates to ‘two households’) meaning that separate male and female individuals exist (see Figure 4). Most flowering plants have bisexual flowers with male and female reproductive organs together in one flower, and dioecy like in Cannabis is found in only ~6% of flowering plant species. Dioecy makes the control of flowering in Cannabis even more interesting because if the males and females do not flower simultaneously pollination cannot occur, hence seed production might be impossible. However, there are scenarios where vastly different flowering times for male and female individuals under field conditions could be advantageous. If the crop is being grown for a medicinal harvest, as the cannabinooid-producing trichomes reside on female flowers, preventing pollination via contrasting male and female flowering times may be of interest.

Flowering denotes the shift from a vegetative to a reproductive state, and this process induces several physiological changes. Upon flowering, a change in carbon partitioning occurs as more resources are invested in the production of flowers and seeds and less in the development of stems, leaves and roots. Furthermore, at flowering stem lignification intensifies and so fibre quality decreases after flowering. This means the optimal time for fibre harvest coincides with flowering. Therefore, to integrate Cannabis into modern agriculture and support the production of sustainable resources, a comprehensive understanding of the genetic pathways controlling flowering time is required.

Figure 2. Photoperiod sensitivity in crops. In summer, long periods of light (e.g., 16 hours light per day) induce flowering in long-day plants such as wheat, but not in short-day plants such as Cannabis. When day lengths are short (like in early autumn), flowering in Cannabis is induced. A long-day plant like wheat, on the other hand, will not flower in early spring when day lengths are similarly short as in autumn. Some species such as cultivated tomato are day-neutral, meaning flowering occurs under both long and short-day conditions. Figure created with BioRender.com.
How does Cannabis flower?

Due to the illicit connotations of marijuana, Cannabis (including hemp) was banned for most of the 20th century. As a result, knowledge of this species has not progressed to a similar extent to that of wheat, rice or maize. However, in recent years, recognition of the medicinal application of CBD (produced in both hemp and marijuana) is warranting worldwide relaxation of previous legislation.

Though the photoperiod pathway is of major importance in controlling flowering time in Cannabis, other environmental signals are also relevant. Unlike some Arabidopsis and wheat varieties, vernalization (exposure to cold) is not a requirement for flowering in Cannabis. Nonetheless, temperature influences floral transition in Cannabis, with low temperatures increasing the length of the vegetative phase thus delaying flowering. Conversely, high temperatures accelerate the progression to flowering and floral development. The vegetative phase is temperature-dependent, and a certain number of degree days (a heat index that helps to estimate plant development) are required before the induction of flowering can occur.

In the model short-day plant rice, few genes have a large effect on flowering, whereas in maize, many loci contribute a small amount to the flowering time phenotype. These observations are known as the genetic architecture of the trait, and it remains to be seen what the equivalent architecture is for Cannabis. A recent genome-wide association study (investigates the relationship between a genotype and a trait) identified six regions of the Cannabis genome containing 28 candidate genes for flowering time. The candidate genes identified (the phytochromes, cryptochromes, FT, as well as SOC1) include those anticipated to be involved in Cannabis flowering given the extent of evolutionary conservation observed in other species.

Another recent study monitored various Cannabis cultivars over an entire growing season. The authors observed huge diversity of flowering times both within and between different cultivars and suggest that, as in rice, several major effect loci contribute to flowering time in Cannabis. They also hypothesize that in some Cannabis cultivars earlier flowering phenotypes are caused by single loci.

Efforts to identify the genes controlling flowering time in Cannabis are on-going, and this research will allow the development of more cultivars that could provide consistent flowering times for farmers and allow field cultivation of Cannabis over a wider geographic range. ■
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Further Reading


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