

## GENOME EDITING FOR EARLY AND LATE FLOWERING IN PLANTS

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**Abstract** The use of genome editing to change the blooming period of plants has emerged as a valuable approach in contemporary agricultural research. This chapter delves into the complex processes that control early and late flowering in plants and how genome editing techniques such as CRISPR-Cas9 have altered the field. The chapter begins with an overview of the genetic pathways and regulatory networks that determine flowering time and then dives into the vital functions of key genes such as FLOWERING LOCUS T (FT), CONSTANS (CO), and FLOWERING LOCUS C (FLC). The chapter then delves into the many genome editing methods used to modify blooming time, focusing on augmentation and delay. Researchers have improved agricultural productivity, stress tolerance, and adaptation to changing climatic conditions by targeting regulatory genes. Case studies show effective genome editing applications in various plant species, indicating the possibility of crop development with personalized flowering time alterations. The ethical concerns and potential ecological implications of genome-edited plants with changed flowering times are also discussed, highlighting the significance of responsible research and environmental risk assessment. Furthermore, the chapter investigates the challenges and potential paths in the realm of genome editing for modifying flowering times in plants. This includes a comprehensive review of techniques to achieve more precise genetic modifications, strategies for reducing unintended alterations, and establishing regulatory guidelines.

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### Introduction

Genome editing is a new and powerful tool that allows scientists to modify the DNA of plants with great accuracy and precision. It is a revolutionary technique that has profound implications for plant genetics and biotechnology. Scientists have made specific plant mutations by exploiting the potential of genome editing, leading to breakthroughs in several aspects of plant growth and agricultural practices. One important area of study is regulating flowering time in plants, which is critical in influencing plant growth, reproduction, and total agricultural productivity. Genome editing technologies have transformed plant biology by allowing for precise modifications in plant DNA sequences (Puchta, 2017). These technologies have surpassed traditional breeding approaches by allowing researchers to make precise changes to specific genes, giving them unprecedented control over plant genomes (Jaganathan et al., 2018). The development of genome editing methods has greatly accelerated our understanding of gene function, genetic diversity, and the molecular mechanisms underlying many plant characteristics. Plant biology has undergone a revolution because of the

development of genome editing tools like CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9), which allow precise alterations to be made to a plant's DNA. Apart from CRISPR-Cas9, other genome editing techniques, such as TALENs (Transcription Activator-Like Effector Nucleases) and Zinc Finger Nucleases (ZFNs), have also been employed in plants (Bortesi and Fischer, 2015). With developments in genome editing, the alteration of flowering time genes has become an area of interest due to its enormous impact on plant development and crop yield (Ali et al., 2018). Flowering time management is vital in plant development because it directs how plants transition from vegetative to reproductive growth. The flowering time is critical in crop production, influencing pollination, seed production, and crop yield (Amasino, 2010). Furthermore, careful control over flowering time can help alleviate the negative impacts of environmental challenges like drought, heat, and cold, ensuring optimal plant growth and survival in difficult conditions (Qaim, 2020). Also, manipulating flowering time genes can be helpful in the

development of crops with customized flowering traits, such as altered day-length sensitivity or reduced vernalization requirements, thereby permitting crops to adapt to different geographical regions and changing climatic conditions (Thudi et al., 2021).

### **Understanding the Genetic Basis of Flowering Time**

#### **Key genes and pathways involved in flowering time regulation**

##### **Photoperiod pathway**

Plants possess an incredible ability to detect and react to modifications in the duration of daylight or photoperiod, an essential mechanism in controlling the timing of blooming (Jackson, 2009). The term photoperiod pertains to the length of lightness and darkness within 24 hours. Various plant types display unique reactions to particular photoperiods, leading to differences in the timing of their blooming. CONSTANS (CO) and FLOWERING LOCUS T (FT) are two key genes involved in photoperiod pathway (An et al., 2004). According to (An et al., 2004), CO is a central regulator of photoperiodic flowering in *Arabidopsis thaliana*. The photoperiod pathway heavily relies on the CONSTANS (CO) gene. It serves as a key controller that integrates light signals and promotes flowering when exposed to extended periods of daylight (Johansson and Staiger, 2015). CO is primarily expressed in leaves and functions in the phloem, the vascular tissue of plants that transports sugars and other signaling molecules (An et al., 2004). CO integrates information about day length and translates it into the systemic signals that induce flowering throughout the plant. This systemic signal is most likely transmitted through the phloem to the shoot apical meristem, the plant's growing tip, where flowering begins (Nakamichi et al., 2007). Studies have shown the interaction between CO and FT in regulating flowering. FT is expressed in leaves and functions as a flowering integrator. In other words, it coordinates distinctive signals to stimulate flowering (Nakamichi et al., 2007). FT is expressed in leaves and is activated by CO under long-day conditions. Further research has also demonstrated that histone modifications, especially trimethylation of histone H3 lysine 27, play a part in directing the expression of FT and other flower-related qualities (Turck et al., 2007). The CO gene acts as a crucial regulator, detecting changes in the length of daylight and sending a signal to the entire system to start the flowering process (An et al., 2004). As a downstream target of CO in flowering regulation, FT functions as a floral integrator, assimilating inputs from numerous pathways to facilitate the commencement of flowering (Yamaguchi et al., 2005). The CO and FT interactions are critical in starting the floral transition in *Arabidopsis* and regulating gene expression via histone changes (Turck et al., 2007).

##### **Vernalization pathway**

The process of vernalization is of utmost importance in controlling the timing of flower growth in plants and is activated when exposed to extended periods of low temperatures (Rouse et al., 2002). Multiple genes are involved in the vernalization pathway, such as FLC gene, VIN1 gene, VIN2 gene, and VIN3 gene. FLOWERING LOCUS C (FLC) functions as a key suppressor in flowering by hindering early flowering in adverse circumstances. FLC's high expression levels prevent the transition from vegetative growth to flowering without vernalization (Rouse et al., 2002). The mechanism of repression ensures that the plant remains in a non-reproductive state. The acquisition of the ability to bloom in plants after being exposed to long periods of low temperatures is known as vernalization (Alexandre and Hennig, 2008). The act of vernalization results in the suppression of the FLC gene, ultimately encouraging the initiation of the flowering process (Rouse et al., 2002). One of the genetic components, VIN3, plays a key role in establishing the plant's vernalization memory. VRN1 plays a role that comes after VIN3 and is crucial for the continuation of FLC suppression even after the cold phase's conclusion. VRN2, alternatively referred to as FLOWERING LOCUS D (FLD) or VERNALIZATION 2, is an additional pathway element that leads to vernalization. It functions collaboratively with VIN3 and VRN1 to facilitate the inhibition of FLC (Bastow et al., 2004). VRN2 plays a role in creating an inhibitory chromatin environment at the FLC locus via histone modifications. The mechanism of the FLC pathway is to inhibit the process of blooming utilizing the FLC gene, while the opposite effect is brought about by the vernalization process, which employs genes such as VIN3, VRN1, and VRN2 (Distelfeld et al., 2009). By using this pathway, plants can detect and react to low temperatures, which results in the suppression of FLC and stimulation of the blooming process (De Lucia and Dean, 2011). Overall, the pathway that regulates flowering is governed by the FLC gene and opposed by the process of vernalization, which involves the activation of genes such as VIN3, VRN1, and VRN2 (Alexandre and Hennig, 2008).

##### **Gibberellins Pathway**

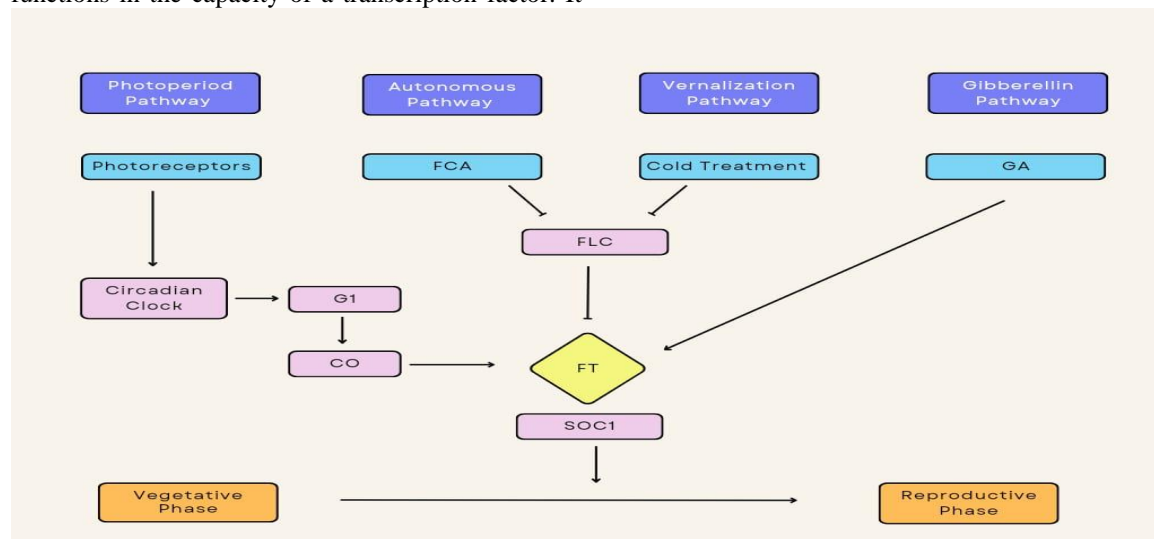
The gibberellic acid (GA) pathway is important in stimulating floral development and regulating several aspects of plant growth and development (Bao et al., 2020). Gibberellins, a kind of plant hormone, are synthesised and then degraded inside the cellular structure of plants (Sun, 2010). These molecules operate as signal transducers, interacting with certain receptors and proteins to elicit various physiological reactions. A crucial interaction between gibberellins and important proteins, notably the GID1 receptor and DELLA proteins, occurs inside the Gibberellic Acid pathway (Gomi and Matsuoka, 2003). The GID1 receptors, found in the cytoplasm of plant cells, have been identified as the particular receptors for gibberellins. Gibberellins can form a complex with

DELLA proteins after attaching to GID1 receptors ([Achar et al., 2007](#)).

### Autonomous Pathway

The autonomous pathway is a flowering regulatory pathway that operates independently of external environmental cues ([Wu et al., 2020](#)). The autonomous pathway is a regulatory mechanism that affects flowering without external environmental stimuli such as light or temperature. Endogenous stimuli internal to the plant control flowering induction ([Jung et al., 2014](#)). The action sequence includes a plethora of crucial elements and modulatory molecules that synchronise the chronological occurrence of blooming. The mutual engagement of two important proteins, FD (FLOWERING LOCUS D) and FT (FLOWERING LOCUS T), is a critical interaction in the self-governing pathway ([Castro Marín et al., 2011](#)). Foliar FT expression causes the mobilisation of a signalling molecule in plants, which travels to the shoot apical meristem, prompting the change from vegetative to floral growth ([Wigge et al., 2005](#)). The FD protein functions in the capacity of a transcription factor. It

interacts with the FT protein, promoting the transcriptional activity of floral meristem-identity genes and instigating the process of floral development ([Amasino and Michaels, 2010](#)). Furthermore, the exact control of flowering time within the autonomous pathway is governed by protein interactions and the integral participation of microRNAs (miRNAs) ([Hong and Jackson, 2015](#)). MicroRNAs (miRNAs) are small RNA molecules that can inhibit gene expression by selectively targeting certain messenger RNAs (mRNAs) and causing their destruction or translational repression. MiR156, miR172, and miR319 are important in the setting of blooming ([Jung et al., 2011](#)). The miR156 genetic sequence specifically targets genes encoding SPL proteins, which act as negative regulators of the essential blooming process. MiR172's molecular target includes AP2-like transcription factors, which are important regulators of floral organ identity ([Fan et al., 2018](#)). The regulation of transcription factors by miR172 significantly impacts the differentiation and characterization of floral structures.



**Figure 1 Metabolic pathways involved in reproductive and vegetative phases**

The miRNA locus miR319 has been shown to target TCP transcription factors, which have been linked to leaf development and flowering time control. MicroRNAs (miRNAs) and their target genes form a sophisticated regulatory network that precisely controls the commencement of blooming and ensures optimal floral maturity in response to intrinsic cues ([Yu et al., 2012](#)). Such regulatory mechanisms may be critical in sustaining plant reproductive fitness.

### Genome Editing Tools and Techniques

#### CRISPR-Cas9

CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated Cas9 endonuclease) has revolutionized biological research and agricultural enhancement owing to its specificity, simplicity, and adaptability ([Komor et al., 2016](#)). The CRISPR/Cas9 gene editing method requires three things: (1) nuclear Cas9 protein expression, (2)

creation of a guide RNA (gRNA) molecule with a complementary sequence to the target gene's first 20 nucleotides, and (3) the presence of a particular DNA sequence known as the NGG PAM site next to the 3' end of the target sequence ([Li et al., 2017](#)). When accompanied by a guide RNA (gRNA), the Cas9 protein causes a double-strand break (DSB) at the specified sequence ([Bortesi and Fischer, 2015](#)). Double-strand DNA break (DSB) restoration occurs by the inaccurate non-homologous end-joining (NHEJ) process, frequently resulting in small deletions or insertions at the intended position. The capacity to create knockout and loss-of-function mutants is useful in revealing gene functions ([Komor et al., 2016](#)).

#### TALEN (Transcription Activator-Like Effector Nucleases)

The Transcription Activator-Like Effector Nucleases (TALEN) technique is a molecular biology approach to editing the genome of living organisms, offering accurate modifications to the DNA structure (Malzahn et al., 2017). The cell's DNA repair mechanism changes, resulting in gene deletion, substitution, or insertion, in response to double-strand breaks. This results in double-stranded breaks (DSBs) at predetermined genetic locations within the genome. Double-strand breaks give rise to specific alterations within the DNA repair mechanism of the cell, including gene deletion, substitution, or insertion (Bogdanove and Voytas, 2011). The transcription activator-like effectors (TALEs) protein family may be traced back to bacterial sources, more especially pathogenic bacteria found in plant organisms, among which members of the *Xanthomonas* genus are particularly notable (Bogdanove and Voytas, 2011). TALE proteins work as transcriptional activators by building a complex with certain DNA sequences found within the host plant's genome, influencing gene expression. According to scientific writing traditions, the identification of DNA binding specificity in Transcription Activator-Like Effectors (TALEs) is dependent on the placement of amino acids inside their repeats, indicated as repeat variable di-residues (RVDs) (Christian et al., 2010).

The inclusion of the FokI endonuclease is a customary application in the TALEN strategy, wherein two TALEN units are affixed to adjoining segments of DNA, leading to the creation of double-stranded breaks (DSBs) (Malzahn et al., 2017). The identification of a specific DNA sequence by TALEN pairs can be accomplished by the TALE repeat subunit, which includes repeat variable residues (RVDs) that can connect with complementary base pairs (Mao et al., 2019). The genetic material, DNA, undergoes cleavage upon binding of transcription activator-like effector nucleases (TALENs) to its specific recognition motif. This initiates additional DNA repair mechanisms, namely NHEJ (Non-homologous end joining) and HDR (Homology Directed Repair), to manifest (Li et al., 2011). TALEN technology has been widely adopted and has demonstrated successful genome editing in various organisms, including plants, animals, and human cells.

#### **ZFNs (Zinc Finger Nucleases)**

Zinc Finger Nucleases (ZFNs) represent a specific category of purposely engineered DNA-binding proteins employed in the process of directed genome manipulation within plant biotechnology. Zinc finger nucleases (ZFNs) consist of two essential constituents, namely zinc finger proteins (ZFPs) and a DNA cleavage domain (Carroll, 2011). The ZFPs are designed to identify and interact with specific DNA sequences selectively, while the DNA cleavage domain catalyzes the creation of double-strand breaks (DSBs) at the designated target site (Weinthal et al., 2010). Zinc Finger Nucleases (ZFNs) represent a

specific category of purposely engineered DNA-binding proteins that are employed in the process of directed genome manipulation within the realm of plant biotechnology (Shukla et al., 2009). Zinc finger nucleases (ZFNs) consist of two essential constituents: zinc finger proteins (ZFPs) and a DNA cleavage domain. The ZFPs are designed to selectively identify and interact with specific DNA sequences, while the DNA cleavage domain catalyzes the creation of double-strand breaks (DSBs) at the designated target site (Petolino, 2015). Following the induction of double strand breaks (DSBs) by Zinc Finger Nucleases (ZFNs), the cellular mechanism responsible for DNA repair is activated. This ultimately leads to DNA repair through Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR) mechanisms. The extant corrective procedures can be utilized to implement desired alterations to the hereditary material, namely to instigate gene knockout, gene replacement, or gene insertion protocols (Urnov et al., 2010). The ZFN technology has been successfully employed for targeted gene editing in plant genomics, resulting in precise modifications within plant genes (Petolino, 2015). Multiple plant species have been the subject of their use for a range of purposes, including functional genomics, crop improvement, and biotechnology (Townsend et al., 2009).

#### **Base Editing**

Base editing is a powerful genome editing approach that allows for precise and concentrated changes to individual nucleotides in the DNA sequence while preventing double-stranded DNA breakage (Molla et al., 2021). The method includes purposefully altering one nucleotide base to another using a Cas protein with a limited catalyzed capacity and an enzyme that transforms the base (Komor et al., 2016). A approach called as targeted base-pair alteration is widely mentioned. This approach allows for correcting disease-causing mutations or introducing specific genetic alterations with few undesirable consequences and a high success rate. CRISPR-Cas9-mediated cytidine deaminase mechanisms, commonly known as CRISPR base editors (CBEs), are widely used as a base editing technique (Gaudelli et al., 2017). A single guide RNA (sgRNA) is used to precisely route the cytidine base editor (CBE) to a certain defined position to activate deaminase activity. The enzyme deaminase promotes a specific change within the sequence of DNA, which is then permanently altered by the cell's repair processes (Li et al., 2018).

#### **Prime Editing**

Prime editing represents an advanced genome editing method that enables precise DNA sequence alterations while circumventing the requirement for double-strand breaks (DSBs). The present study incorporates the CRISPR-Cas9 mechanism, a reverse transcriptase apparatus, and a self-designed primary editing guide RNA, commonly abbreviated as pegRNA (Anzalone et al., 2019). This method



facilitates precise and effective alterations of bases through swaps, deletions, and insertions. The prime editing process encompasses a series of distinct phases, including target site identification, DNA strand nicking, primer elongation, reverse transcription, and DNA ligation ([Gaudelli et al., 2017](#)). The Cas9 enzyme induces a single-stranded break in the DNA molecule after the guidance of the pegRNA molecule which directs the primary editing complex towards the predetermined location within the genome ([Lin et al., 2020](#)). Subsequently, the pegRNA serves as a template for the reverse transcriptase to synthesize a novel DNA strand incorporating the essential modifications ([Zhan et al., 2021](#)). Finally, the newly synthesized DNA strand is ligated to the other DNA strand, resulting in the modified genomic sequence ([Tang et al., 2017](#)).

#### **Designing and optimizing guide RNA sequences for target gene modification**

Formation and optimization of guide RNA (gRNA) sequences is critical in genome editing to ensure effective and focused target gene change. The gRNA sequence used can significantly impact the editing process's success and accuracy ([Wiles et al., 2015](#)). For this purpose, we must first choose the target site, carefully picked within the gene of interest to make the desired change. The target location is typically chosen within the gene's coding area or functional parts, such as promoters or regulatory regions. Several sequence characteristics impact gRNA design, including a protospacer adjacent motif (PAM), gRNA length, GC content, and secondary structure. The PAM is a short DNA sequence essential for Cas9 binding and cleavage ([Park et al., 2015](#)). The PAM sequence for the widely used *Streptococcus pyogenes* Cas9 is NGG (where N can be any nucleotide). gRNA length and GC content can impact stability and efficiency, with typical lengths ranging from 18 to 20 nucleotides and a 40-60% GC level.

#### **Off-target effects and strategies for minimizing unintended modifications**

##### **Off-Target Effects in Genome Editing**

Off-target effects are unintended modifications that occur in genomic regions other than the intended target site during genome editing. Off-target impacts must be reduced to ensure the editing process's precision and accuracy. Cas nuclease recognition and cleavage of DNA sequences with partial similarity to the target region might result in off-target effects. Off-target locations may have a few mismatches or partial complementarity with the guide RNA (gRNA), resulting in unintended DNA double-strand breaks and subsequent changes. Off-target effects can be troublesome due to selectivity and potentially unwanted genetic modifications.

##### **Strategies for Minimizing Off-Target Effects**

Several strategies and methods have been developed to mitigate off-target effects and improve genome editing specificity.

##### **High-Fidelity Cas Nucleases**

Engineered Cas nucleases with increased fidelity, such as high-fidelity Cas9 variants (e.g., eSpCas9, HypaCas9), have been designed to alleviate off-target effects while maintaining target site cleavage efficiency ([Kleinstiver et al., 2016](#)).

##### **gRNA Optimization**

Optimizing gRNA design can help reduce off-target effects. Strategies include choosing gRNA sequences with the fewest predicted off-target sites, optimizing gRNA length and composition, and considering secondary structure predictions ([Pattanayak et al., 2013](#)).

##### **Paired Nickases**

Using paired nickases, in which two Cas nucleases are targeted to adjacent regions on the target DNA rather than a single nuclease, can reduce off-target effects ([Cho et al., 2014](#)). Each nickase creates a single-strand break, while double-strand breaks are created only when both nickases are present, increasing specificity ([Bae et al., 2014](#)).

##### **Genome-Wide Specificity Analysis**

Experimental techniques such as GUIDE-seq and Digenome-seq may be employed to extensively identify potential off-target sites and assess the specificity of genome editing approaches ([Tsai et al., 2015](#)).

##### **Enhanced Specificity Variants**

Engineered Cas variants with greater specificity have been developed, such as the SpCas9-HF1 variation, which minimizes off-target effects while retaining on-target activity ([Kleinstiver et al., 2016](#)).

##### **Bioinformatics Tools**

Various bioinformatics tools, such as Cas-OFFinder, CRISPR, and COSMID, aid in predicting and minimizing off-target effects by identifying potential off-target sites and providing information for gRNA design optimization ([Listgarten et al., 2018](#)).

#### **Delivery methods for introducing genome editing components into plant cells**

Methods for introducing genome editing components into plant cells are critical for effective genome editing research. Here are some examples of widely utilized delivery methods:

##### **Agrobacterium-Mediated Transformation**

Agrobacterium-mediated transformation is a common approach for introducing genome editing components into plant cells ([Michielse et al., 2005](#)). *Agrobacterium tumefaciens*, a naturally occurring soil bacterium, has been engineered to contain the necessary genome editing components ([Opabode, 2006](#)). The *Agrobacterium* cells are then co-cultured with plant tissue or explants, allowing them to transmit the altered DNA into plant cells. This approach applies to a wide range of plant species and has the benefit of steady integration of the altered DNA into the plant genome ([Klee et al., 1987](#)).

##### **Biolistic Particle Delivery (Gene Gun)**

Biolistic particle delivery, commonly known as the gene gun method, includes the physical delivery of genome editing components into plant cells by high-

velocity microprojectiles ([Demirer and Landry, 2017](#)). A high-pressure helium gun propels tiny gold or tungsten particles coated with the appropriate DNA fragments, including genome editing components, into plant tissues ([Altpeter et al., 2005](#)). This approach is very helpful for getting DNA into plant cells that are difficult to change using other methods, such as cereal crops ([Ji et al., 2013](#)).

#### **Protoplast Transformation**

Protoplast transformation comprises separating plant cells that have had their cell walls removed (protoplasts), which are then transformed utilizing genome editing components. Plant tissue may be processed enzymatically to remove the cell walls, producing protoplasts ([Bates, 1999](#)). The genome editing components, such as plasmids or ribonucleoprotein complexes (RNPs), are transported into the protoplasts using techniques such as PEG-mediated transformation or electroporation. Protoplast transformation enables the efficient transport and temporary expression of editing components ([Wu and Hanzawa, 2018](#)).

#### **Viral vectors**

Viral vectors can introduce genome editing components into plant cells ([Oh et al., 2021](#)). Plant viruses, such as the Tobacco Rattle Virus (TRV), have been designed as vectors to transport the required DNA constructions. The viral vectors are subsequently injected into plant cells using mechanical inoculation or agroinfiltration ([Mallory et al., 2002](#)). The viral vectors transport the genome editing components, allowing for effective gene editing in infected plant cells ([Zaidi and Mansoor, 2017](#)). These are only a few examples of methods to introduce genome editing components into plant cells. The delivery method used is determined by parameters such as plant species, tissue type, and the availability of appropriate protocols for a certain system.

#### **Early Flowering in Plants**

##### **Role of early flowering in plant adaptation and productivity**

Early flowering is critical for plant adaptability and production because it allows plants to align their life cycle with optimal environmental conditions and maximize reproductive success ([Fornara and Coupland, 2009](#)). Flowering time is a complex feature controlled by several genetic, physiological, and environmental variables. Understanding the significance of early flowering and the resulting implications for plant adaptability has been the focus of substantial research in plant biology ([Amasino, 2010](#)). One of the primary benefits of early flowering is the ability of plants to protect themselves from adverse climatic circumstances such as drought, cold, or other environmental stresses ([Imaizumi and Kay, 2006](#)). By initiating flowering early, plants can complete their life cycle before the commencement of harsh circumstances, assuring the species' survival and reproductive success ([Putterill et al., 1995](#)). This

adaptive technique is especially useful in environments with seasonal changes or variable weather patterns ([Hepworth and Dean, 2015](#)).

For instance, plants that blossom early in the spring might avoid the drought and heat stress that often occur throughout the summer months in temperate countries ([Imaizumi and Kay, 2006](#)). By completing their life cycle before the advent of such harsh conditions, these plants enable the successful production of seeds or fruits, which are necessary for their proliferation and survival ([Fornara and Coupland, 2009](#)). Similarly, in frost-prone areas, early flowering allows plants to complete pollination and seed development before the arrival of frigid temperatures, increasing their chances of reproductive success ([Putterill et al., 1995](#)). Moreover, the ability of plants to flower early gives them a competitive advantage by allowing them to maximize the utilization of available resources. Plants that begin flowering early have a major advantage in acquiring and utilizing valuable resources such as light, nutrients, and water in habitats where these resources are rare ([Li and Dubcovsky, 2008](#)). They may get a greater portion of the available nutrients this way, resulting in improved growth and higher overall production compared to plants that bloom later in the season. Early-flowering plants have a better chance of capturing sunlight and efficiently converting it into energy via photosynthesis ([Putterill et al., 1995](#)). Light availability during the early stages of the growth season allows these plants to allocate more energy to critical components such as biomass accumulation, root development, and reproductive structure formation ([Amasino, 2010](#)). As a result, there is a productivity improvement ([Imaizumi and Kay, 2006](#)). Furthermore, by flowering early, plants can exploit nutrient-rich soil conditions that have not yet been depleted by competing vegetation, maximizing their availability of necessary nutrients for growth and reproduction ([Li and Dubcovsky, 2008](#)).

##### **Identification of genes associated with early flowering**

The regulation of flowering time is a complicated process mediated by a network of genes and signaling pathways. CONSTANS (CO), FLOWERING LOCUS T (FT), and SUPPRESSOR OF OVEREXPRESSION OF CO 1 (SOC1) are important regulators of the transition from vegetative to reproductive growth ([Raman et al., 2019](#)). Internal factors such as hormones regulate these genes, as do external environmental elements such as photoperiod and temperature. The CONSTANS gene, for example, works as a sensor for day length in plants, allowing them to assess the duration of the sunshine and determine the most favorable time to flower ([Putterill et al., 1995](#)). Longer days raise the levels of the CONSTANS protein, which stimulates the production of other flowering genes such as FT and SOC1, thus commencing the blooming process ([Michaels and Amasino, 1999](#)). On the other hand, shorter days

result in reduced amounts of CONSTANS, which causes flowering to be delayed. These genetic and molecular systems allow plants to assimilate internal and external cues to make informed decisions regarding flowering time (She et al., 2013). Numerous studies have been conducted to examine the molecular mechanisms influencing early flowering and to identify genetic variations associated with natural variation in flowering time (Putterill et al., 1995). In *Arabidopsis thaliana*, a model plant species, the flowering LOCUS C (FLC) gene has been discovered to regulate flowering time and allow populations to adapt to varied conditions (Kumar and Wigge, 2010). The FLC gene functions as a repressor, limiting the production of flowering-promoting genes. Variations in the FLC gene sequence can cause varying amounts of repression, leading to flowering time variations among *Arabidopsis* populations (Turck et al., 2008). Similar research has revealed significant genes and quantitative trait loci (QTLs) related to early flowering in crops such as rice and wheat, giving useful insights for crop development and breeding programs (Greenup et al., 2009). These discoveries contributed to the development of early-flowering cultivars that are better adapted to certain environments. Manipulation of crop flowering timing is crucial for agricultural output. Breeders can generate varieties that mature earlier by selecting for early-flowering attributes, allowing farmers to optimize their planting and harvesting schedules (Zhang et al., 2015). This is especially useful in areas with shorter growing seasons or areas prone to challenging seasonal fluctuations, since it ensures that crops achieve maturity before unfavorable circumstances set in. Furthermore, early-flowering crops can also increase production potential. Plants have more time for seed or fruit development when the reproductive phase is accelerated, resulting in bigger yields (Shavruk et al., 2017). This is especially beneficial for crops with indeterminate growth habits, such as tomatoes or soybeans, because a prolonged blooming period improves fruit set and overall output. To summarize, early flowering is an important adaptive strategy employed by plants to deal with environmental obstacles and maximize reproductive success. Understanding the genetic and molecular processes underpinning early flowering has offered useful insights into plant adaptation and has the potential to contribute to the development of climate-resilient crop types.

#### **Case studies highlighting successful genome editing for early flowering in various plant species**

The model plant *Arabidopsis thaliana* is commonly utilized in genetic studies. Researchers employed CRISPR-Cas9 genome editing to change the CONSTANS (CO) gene, which is important in the flowering pathway, in a research project (Papikian et al., 2019). They were able to cause early blooming in *Arabidopsis* plants by deleting the CO gene. Rice is a vital food crop, and early-flowering varieties can help

improve productivity and adapt to changing environmental circumstances (Nelson et al., 2010). Researchers have effectively modified the Grain Number, Plant Height, and Heading Date 7 (Ghd7) gene in rice by CRISPR-Cas9 (Li et al., 2021). As a result, plants bloomed earlier and produced more grain. The self-pruning 5G (SP5G) gene is a flowering time regulator in tomato plants. In a 2020 study, researchers used CRISPR-Cas9 to target this gene. The modified tomato plants displayed early blooming phenotypes, which might be useful for shortening harvest time and increasing crop output (Nekrasov et al., 2017). Wheat is one of the most essential staple crops in the world. In a 2019 study, researchers used CRISPR-Cas9 to target wheat's VRN1 (Vernalization 1) gene (Zhang et al., 2018). They induced early blooming in wheat plants by deleting this gene, which might produce novel wheat types with shorter growth periods and better adaptation to varied settings.

#### **Late Flowering in Plants**

##### **Significance of late flowering in plant physiology and stress tolerance**

Late blooming in plants is an important feature of plant adaptability and productivity, providing several benefits for growth and responsiveness to environmental challenges (Amasino, 2010). One notable advantage is that adverse circumstances can be avoided throughout the initial stages of the plant's life cycle (Hepworth and Dean, 2015). Late-flowering plants, for example, might delay their reproductive phase until more favorable conditions, such as the commencement of the rainy season, occur in places with prolonged dry seasons (Castiglioni et al., 2008). This postponement protects them from water stress and boosts their chances of effective seed production and progeny survival. Late-flowering plants benefit from greater resource availability as the growing season progresses. They can use resources that become available after the initial surge of plant development has subsided by flowering in early-flowering species. This delayed acquisition strategy can potentially boost plant growth, biomass accumulation, and overall production (Nowicka et al., 2018). Another advantage of late flowering is that it allows for more vegetative development. It enables plants to devote more resources to root development, stem enlargement, and leaf expansion (She et al., 2013). This longer vegetative period enhances soil nutrient and water absorption, stress tolerance, and overall plant performance. Late-flowering species frequently have bigger root systems, allowing them to explore deeper soil levels and obtain water reserves that early-flowering plants may not have (Castiglioni et al., 2008). Late blooming is also beneficial for pollination and seed dissemination. Plants ensure the presence of pollinators and raise the odds of effective pollination and seed production by blooming later (Riedinger et al., 2014). They may also exploit seasonal fluctuations in pollinator populations or attract specialized pollinators active at specified



times. Furthermore, delayed flowering enables seed dissemination to be synchronized with environmental cues such as wind patterns or animal movements, allowing for seed dispersal over greater distances and assisting in colonizing new ecosystems.

#### **Identification of genes involved in late flowering**

Late blooming is controlled by a complex combination of genetic, hormonal, and environmental variables. Key genes such as FLOWERING LOCUS C (FLC), GIGANTEA (GI), and SHORT VEGETATIVE PHASE (SVP) operate as flowering repressors and are essential for vegetative development ([Turck et al., 2008](#)). Environmental signals such as day duration and temperature also impact the expression and activity of these flowering period genes. The study of the molecular causes of late blooming has revealed significant insights on the genetic control of flowering time. Several regulatory mechanisms have been discovered in *Arabidopsis thaliana*, including the vernalization system, which stimulates blooming after extended cold exposure, and the autonomous pathway, which functions independently of environmental stimuli ([Hayama and Coupland, 2004](#)). These pathways interact and incorporate environmental information to fine-tune blooming time.

#### **Overview of genome editing strategies for manipulating late flowering genes**

Studying the molecular processes behind late flowering has implications for crop development and agricultural operations. Late blooming can benefit crops with determinate growth patterns, such as maize or soybeans, since it allows for longer vegetative development, leading to increased biomass production and better grain yields ([Manavalan et al., 2009](#)). Furthermore, delayed flowering might help crops avoid the detrimental impacts of pests or diseases that are common earlier in the growing season. Researchers have used genome editing tools like CRISPR/Cas9 to modify flowering time and promote late flowering in various plant species ([Bortesi and Fischer, 2015](#)). Scientists successfully targeted the FLOWERING LOCUS C (FLC) gene, a flowering repressor, in *Arabidopsis* using CRISPR/Cas9 to impair its function and induce late flowering ([Capovilla et al., 2017](#)). This accomplishment has provided vital insights into the genetic regulation of blooming timing and the possibility of comparable alterations in other plants ([Doudna and Charpentier, 2014](#)).

#### **Examples of successful genome editing for late flowering in different plant species**

In rice, a major staple crop, researchers have used genome editing to manipulate flowering time by targeting genes involved in photoperiodic regulation, such as Heading date 1 (Hd1) and Early heading date 1 (Ehd1) ([Gao et al., 2013](#)). They have effectively delayed flowering by lengthening the vegetative phase, improving biomass accumulation and higher grain yield potential, particularly in specific

environmental circumstances or cropping practices ([Matres et al., 2021](#)). In maize, genome editing methods have also been utilized to alter flowering time traits. Researchers effectively created a delayed flowering phenotype by targeting genes that govern the transition to flowering, such as ZmMADS1 or ZCN8 ([Yang et al., 2021](#)). This treatment prolongs the vegetative period, allowing for improved production potential under various environmental circumstances. Wheat has also been a target for genome editing to change the flowering timing. Researchers have concentrated on genes such as VERNALIZATION1 (VRN1) and VRN2, which are involved in the vernalization pathway and influence flowering time regulation ([Chen and Dubcovsky, 2012](#)). Flowering in wheat may be delayed by modifying these genes, allowing for improved management of wheat crops in diverse agroclimatic zones and improving grain yield ([Cortinovis et al., 2020](#)). These examples demonstrate a successful application of genome editing, specifically CRISPR/Cas9, to modify flowering time and promote late flowering in various plant species. By targeting key genes involved in flowering regulatory processes, researchers may precisely regulate flowering timing and generate a variety of plants with distinctive characteristics ([Mishra and Zhao, 2018](#)). This can improve crop adaptability, production, and agricultural management.

#### **Challenges and Future Perspectives**

##### **Regulatory aspects and public acceptance of genome-edited plants**

Properly managing the regulatory environment is a major barrier to the broad adoption of genome-edited plants. Genetically modified organisms (GMOs) and genome editing technologies are regulated differently in different nations and areas ([Sprink et al., 2016](#)). It is critical to create clear, evidence-based regulatory frameworks that recognize the particular characteristics of genome-edited plants and distinguish them from transgenic species. Furthermore, fostering public acceptability and participating in effective communication regarding the safety and benefits of genome-edited plants is critical to addressing any concerns and cultivating a supportive environment for their use ([Eş et al., 2019](#)).

##### **Potential risks and ethical considerations associated with genome editing**

Regardless of how specific genome editing is, a thorough assessment of the risks posed by off-target effects and unforeseen outcomes is essential. Comprehensive analysis and ongoing monitoring of genome-edited plants are necessary to uncover potential environmental or health consequences ([Araki and Ishii, 2015](#)). Furthermore, ethical problems around genome editing, such as equal access to technology, intellectual property rights, and social ramifications, must be thoroughly investigated to ensure the responsible and sustainable deployment of these technologies. Continuous research efforts are aimed at improving the precision and safety of



genome editing technology. Base editing and prime editing approaches have been developed to increase accuracy and reduce off-target impacts ([Voytas and Gao, 2014](#)). These techniques involve modified Cas enzymes or Cas9 variants that facilitate more precise genetic alterations, reducing the risk of unintended genomic changes. Furthermore, rigorous risk assessment procedures are being developed to identify possible dangers associated with genome-edited plants and ensure their safe use ([Mao et al., 2019](#)).

#### **Advancements in precision genome editing techniques**

The ongoing development of precise genome editing tools is critical for increasing the efficacy and diversity of controlling plant flowering time. CRISPR/Cas9 systems have revolutionized genome editing by allowing for the precise mutation of certain target genes ([Doudna and Charpentier, 2014](#)). These strategies open the door to more efficient and focused control of flowering-related genes. Currently, scientists are working hard to increase the specificity and efficiency of genome editing technologies. They are investigating improved versions of CRISPR/Cas systems with fewer off-target effects and other developing genome editing techniques ([Perez-Pinera et al., 2012](#)). These developments are expected to allow for more precise control over flowering timing and improve the effectiveness of producing desirable plant features.

#### **Integration of genome editing with other breeding methods for enhanced flowering control**

The combination of genome editing and traditional breeding strategies can have a synergistic effect on flowering time management. It is possible to accelerate the generation of better plant varieties with desirable flowering features by combining genomic selection, marker-assisted breeding, and genome editing ([Tsai et al., 2021](#)). The exact alterations made feasible by genome editing can be used to add or fine-tune flowering-related genes discovered by traditional breeding methods ([Scheben et al., 2017](#)). This integration improves breeding programs' efficiency and concentration. Plant breeders, geneticists, and biotechnologists must collaborate to successfully integrate genome editing with other breeding strategies. We can maximize the management of blooming time and generate plant types better adapted to changing climatic circumstances and specific agricultural requirements by combining the benefits of diverse strategies ([Thudi et al., 2021](#)).

#### **Conclusion**

##### **Summary of the current state of genome editing for early and late flowering in plants**

The recent developments in manipulating the genetic traits of plants to regulate when they flower hold immense potential for enhancing crop quality and ensuring long-term sustainability in agriculture ([Andrés and Coupland, 2012](#)). Researchers, using advanced gene-editing techniques

like CRISPR-Cas9, have successfully utilized gene-editing techniques to modify the timing of plant flowering by targeting specific genes associated with the blooming process, such as CONSTANS (CO), FLOWERING LOCUS T (FT), SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), FLOWERING LOCUS C (FLC), VERNALIZATION1 (VRN1), and VRN2, to manipulate flowering time and vernalization requirements in various plant species ([Wang, 2014](#)).

#### **Implications for crop improvement and sustainable agriculture**

The ability to precisely manipulate flowering time has substantial implications for crop improvement. Early flowering traits can be favorable in short-season crops because they allow plants to complete their life cycle and yield better in a shorter period ([Wang, 2014](#)). This is especially useful when dealing with issues such as shifting planting seasons and unexpected weather patterns caused by climate change ([Qaim, 2020](#)). Genome editing can increase crop output and resilience in such volatile situations by speeding flowering. Late blooming characteristics achieved through genome editing can be advantageous for crops with a long growing season. By making the growing period longer, these crops have more time to grow in size and produce grains, which leads to a higher overall potential for production ([Thudi et al., 2021](#)). Moreover, decreasing the time crops need to be exposed to cold temperatures allows them to grow in a wider geographical range ([Putterill et al., 2004](#)). Genome editing can help grow crops in areas with milder winters by reducing the need for an extended vernalization period, thus extending agricultural possibilities and strengthening food security ([Koorneef et al., 1998](#)).

#### **Future directions and research priorities in this field**

Looking ahead, various potential initiatives and research goals in genome editing for flowering time control might be recognized. Firstly, finding new blooming genes and understanding their functions in flowering regulation may broaden the repertoire of targets for genome editing. It will allow for more precise control of flowering timing and vernalization response. Furthermore, it is critical to decipher the complex gene regulatory networks involved in blooming time regulation and vernalization response. This understanding will give insights into the underlying mechanisms and aid in developing strategies for enhancing crop production and optimizing flowering timing ([Pramanik et al., 2021](#)). Additionally, efforts should be made to create more efficient and precise genome editing techniques. Improved transformation techniques or alternative gene-editing tools might broaden the applicability of genome editing technology to a larger variety of plant species, allowing for more efficient crop improvement efforts ([Li and Xia, 2020](#)). Furthermore, as genome editing advances, it is crucial to identify

and minimize any unintended consequences. To assess the precision and safety of genome editing techniques, create rigorous tools for detecting off-target effects, and ensure the ethical and sustainable use of these technologies in agriculture, ongoing research is highly desirable (Puchta and Fauser, 2013). Ethical problems, biosafety rules, and public acceptance must all be addressed in order to build trust and allow for the widespread application of genome editing in crop production (Bharat et al., 2020). In conclusion, the current state of genome editing for early and late flowering in plants offers immense potential for crop improvement and sustainable agriculture. Precise control over flowering time can enhance productivity, enable adaptation to changing environmental conditions, and optimize resource utilization (Yin et al., 2017). Future research should focus on expanding our understanding of flowering gene networks, improving delivery methods, and addressing biosafety concerns to fully unlock the transformative power of genome editing in flowering control for the benefit of agriculture and society (Li and Xia, 2020).

#### Declarations

#### Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

#### Ethics approval and consent to participate

Not applicable

#### Consent for publication

Not applicable

#### Funding

Not applicable

#### Conflict of Interest

The authors affirm that the research was conducted without any involvement of commercial or financial relationships that could be perceived as a possible conflict of interest.

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