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### CRISPR Activation System for Flowering Regulation in Mung Bean

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**Abstract** This study demonstrates that CRISPR activation systems show great promise for mung bean flowering time control because they allow for yield improvement and adaptation. The research combines existing data about candidate flowering gene identification and functional analysis of VrFT1 and VrFT2 with CRISPRa applications for photoperiodic regulation in mung bean. The development of genome characterization and gene expression profiling techniques enables researchers to activate specific genes which should result in early or delayed flowering and better environmental tolerance. CRISPRa integration with advanced editing tools and multiplex gene activation methods shows potential for sustainable mung bean breeding and climate-resistant crop development but faces ongoing technical and regulatory hurdles.

Keywords CRISPRa; Mung bean; Flowering regulation; Gene activation; Crop improvement

#### 1 Introduction

The legume crop *Vigna radiata* or mung bean serves as a crucial plant species because it contains abundant protein and grows quickly while tolerating various agricultural environments. The crop maintains a vital position in food security and economic stability because it functions as the main food source and revenue generator for Asian smallholder farmers. The improvement of mung bean production stands as a vital necessity to fulfill nutritional requirements of expanding human numbers while maintaining environmentally friendly farming practices.

The process of flowering determines both the quantity and quality of crops as well as their ability to thrive in different environments. The timing of flower production determines when plants will reproduce during optimal environmental periods which affects seed production and crop yield. The control of flowering in mung bean and other crops enables better climate resistance and allows farming in different areas. The development of new crop improvement techniques depends heavily on the regulation of flower development.

Genome editing technology has evolved through CRISPR activation (CRISPRa) systems which enable scientists to manage gene expression with exactness while keeping DNA sequences intact. The CRISPRa system uses dCas9 which lacks catalytic activity to link with transcriptional activators for boosting the expression of natural genes that control flowering processes. The method provides a solution to traditional transgenic overexpression problems because it enables researchers to activate multiple genes at once through guide RNA design (Karlson et al., 2021; Ding et al., 2022). The dual functionality of CRISPRa systems enables them to study flowering time regulators in model plants and crops by performing both functional gene analysis and trait development (Lowder et al., 2017; Lee et al., 2019; Ding et al., 2022; Hodaei and Werbrouck, 2023).

This study aims to synthesize current knowledge on the application of CRISPRa systems for flowering regulation in mung bean. The research aims to demonstrate mung bean value in economic and agricultural practices and to explain the importance of flower control for breeding improvements and to assess CRISPRa technology capabilities for mung bean and other legume species.



#### 2 Flowering Regulation in Mung Bean: An Overview

#### 2.1 The genetic systems which control flowering in legume plants

Flowering in mung bean is governed by a complex genetic network, with key roles played by genes homologous to those in other legumes and Arabidopsis. The CONSTANS-LIKE (COL) gene *VrCOL1* functions as a photoperiod regulator through FT and TSF activation in short-day conditions (Zhang et al., 2022). The research of genome-wide studies revealed new candidate genes that include FERONIA receptor-like kinases which control FLC regulation and PhyA and PIF3 orthologs that participate in light signaling and flowering time regulation (Chiteri et al., 2024). The PEBP gene family in mung bean consists of FT, MFT, TFL and FT-like clades and VrFT1 induces flowering when days are short (Xue et al., 2024). The study of quantitative trait loci (QTL) has identified key genetic regions which determine flowering time and these regions show high heritability and genetic similarity between different legume species (Ye et al., 2021; Liu et al., 2022). The research demonstrates that *FT*, *CO*, *SOC1* and FLC-like genes function as key regulators for mung bean flower development.

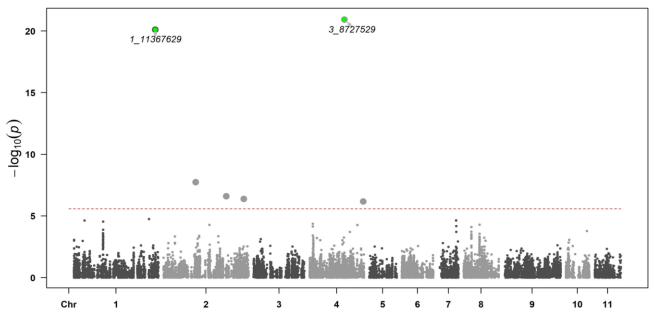


Figure 1 Manhattan plot showing significant SNPs associated with DTF. SNPs are labeled with the SNP name. SNPs in green are discussed further in the text. Trait-SNP associations were performed using FarmCPU in GAPIT. The horizontal dotted red line represents the Bonferroni correction as p = 0.05 (Adopted from Chiteri et al., 2024)

#### 2.2 Environmental cues: photoperiod sensitivity and circadian regulation

Mung bean plants show photoperiod sensitivity through their quantitative short-day plant behavior although their critical photoperiod and temperature responses vary between different genotypes. The process of flowering becomes faster when plants experience short-day conditions because *VrCOL1* and *VrFT1* genes become more active but flowering occurs later under long-day conditions (Zhang et al., 2022; Xue et al., 2024). The interaction between circadian regulation and photoperiodic pathways allows plants to adjust their flowering time according to environmental conditions such as temperature which enables them to thrive in different agro-ecological zones (Summerfield and Lawn, 1987; Imrie and Lawn, 1990; Ellis et al.,1994). The ability of plants to change their flowering response enables breeders to pick varieties that perform well in particular environmental conditions.

#### 2.3 Hormonal and epigenetic influences on flowering transition

Plant hormones together with epigenetic mechanisms play a role in controlling the flowering transition process in mung bean plants. The application of gibberellic acid (GA3) and kinetin and salicylic acid outside plants leads to major changes in flowering duration and plant development and yield production (Sharma et al., 2020; Chaudhary et al., 2024; Mitra and Kumar, 2024; Patidar et al., 2025). The study by Qiu et al. (2023) shows that GA3 and kinetin applications reduce flowering time and enhance plant development but DCPTA treatment leads to the best pod formation and yield results by modifying plant physiological and biochemical processes (Qiu et al., 2021;



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Gao et al., 2022). Research shows that epigenetic mechanisms which control gene presence/absence variation and expression levels contribute to the evolution of early flowering traits (Liu et al., 2022; Chiteri et al., 2024). The hormonal and epigenetic factors operate as additional control mechanisms which work together with genetic and environmental factors to regulate mung bean flowering.

#### 3 CRISPR Activation (CRISPRa) Systems: Concepts and Mechanisms

## 3.1 CRISPRa operates through dCas9 fusion with transcriptional activators to achieve its mechanism of action

The CRISPR activation system depends on dCas9 as its protein component because this enzyme variant maintains DNA targeting ability through single guide RNAs (sgRNAs) but lacks DNA cutting capability. The CRISPRa system enables gene expression enhancement through dCas9 connection to transcriptional activator domains which modify gene expression without modifying DNA sequences. The dCas9/sgRNA complex guides itself to promoter or enhancer regions to activate transcription by bringing transcriptional machinery through attached activator domains which results in elevated target gene expression (Karlson et al., 2021; Ding et al., 2022). Scientists use this method to precisely activate particular genes in plants and other biological systems.

## 3.2 CRISPR knockout and CRISPR interference (CRISPRi) systems are compared to the CRISPR-Cas13 system

The CRISPR knockout system employs active Cas9 to generate double-strand breaks which result in gene disruption through the error-prone DNA repair mechanism. The CRISPR interference system (CRISPRi) employs dCas9 linked to KRAB repressor domains to prevent gene transcription which results in gene silencing. CRISPRa functions differently from CRISPR because it works to increase gene expression rather than interrupting or blocking it. The regulatory outcome of CRISPRa and CRISPRi systems depends on the effector domain selection between activator and repressor because both systems use dCas9 for DNA binding (Lo and Qi, 2017; Karlson et al., 2021). The reversible nature of CRISPRa and CRISPRi systems allows them to function without creating lasting genetic alterations which makes them suitable for functional genomics and crop development applications.

#### 3.3 Common activation domains (VP64, VPR, SAM, SunTag) and their efficiency

Researchers have established multiple activation domains which function to boost CRISPRa performance.

The VP64 protein consists of four VP16 activation domains which function as a tetramer to activate genes at a moderate level (Park et al., 2017; Karlson et al., 2021).

The tripartite activator VPR unites VP64 with p65 and Rta to achieve higher activation levels than VP64 operates independently (Liu et al., 2023).

The SAM system (Synergistic Activation Mediator) enables gene activation through sgRNAs that include MS2 aptamers which bind p65 and HSF1 activators through MS2 coat proteins to achieve strong gene activation (Ding et al., 2022).

SunTag: Employs a repeating peptide array fused to dCas9, which recruits multiple copies of an antibody-activator fusion, amplifying transcriptional activation (Ding et al., 2022).

The study shows that VPR, SAM and SunTag systems generate superior results than dCas9-VP64 because they achieve 10 to over 1000-fold gene expression increases due to their precise targeting capabilities and optimized system architecture (Lo and Qi, 2017; Ding et al., 2022). The research of Liu et al. (2023) presents dCas9-VPRF as a new phase-separation protein fusion which enhances both activation efficiency and system design simplicity according to recent studies.

#### 4 Applications of CRISPRa in Plant Flowering Regulation

#### 4.1 Evidence from model plants (Arabidopsis, rice, tomato)

The CRISPR activation (CRISPRa) systems show promise for model plant applications to control flowering time by enabling the activation of vital floral regulators. The CRISPRa system in Arabidopsis used dCas9-VP64 and



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MS2-based systems to activate the FLOWERING LOCUS T (FT) gene which resulted in elevated FT expression and faster flowering (Lee et al., 2019). The research on rice and tomato demonstrates that scientists can use CRISPRa and other CRISPR-based tools to modify flowering genes for time-dependent crop development (Hodaei and Werbrouck, 2023). The research shows CRISPRa technology enables scientists to study gene function and control flowering characteristics in different plant species.

#### 4.2 Applications in legume crops (soybean, pea, related species)

CRISPRa pplications in legumes have mainly involved gene knockout techniques yet scientists now explore CRISPRa technology for activating flowering genes through transcriptional activation. Scientists used CRISPR/Cas9 technology to modify soybean genes *GmFT2a*, *GmPRR37* and *E1* for photoperiod sensitivity and flowering time regulation to improve regional adaptation and achieve maximum yields (Hodaei and Werbrouck, 2023). The knockout methods used in these studies indicate CRISPRa technology has the potential to activate specific genes in legumes through the same regulatory networks that control flowering genes. The research demonstrates that multiple flowering-related genes can be activated through multiplexed CRISPRa systems to manage intricate traits including flowering regulation (Lee et al., 2019; Selma et al., 2022).

#### 4.3 Mung bean flowering regulation has potential applications for translation

The demonstrated success of CRISPRa in model plants and the progress in legume genome editing provide a strong foundation for applying CRISPRa to mung bean. The CRISPRa system enables scientists to control flowering time by modifying FT, CO and SOC1 regulators which results in better crop yields and environmental resistance. CRISPRa systems show increased utility for mung bean breeding and functional genomics because they can be delivered by viruses to perform simultaneous multi-gene editing (Lee et al., 2019; Selma et al., 2022). The conserved flowering pathways of mung bean with other legumes make these discoveries directly applicable for speeding up mung bean improvement through specific gene activation methods.

#### 5 CRISPRa for Flowering Regulation in Mung Bean

#### 5.1 Candidate flowering genes for activation in mung bean

Genomic research has discovered multiple essential genes in mung bean which control the process of flowering. The Phosphatidylethanolamine-binding protein (PEBP) gene family contains FT (FLOWERING LOCUS T) clade members which function as key regulators for flower initiation. VrFT1, a member of this family, shows significantly higher expression under short-day conditions and is predicted to promote flowering, as demonstrated by overexpression studies in transgenic Arabidopsis (Xue et al., 2024). Research on *PEBP* genes shows that *VrMFT1* and *VrTFL2* and *VrTFL3* genes express based on photoperiod to control the timing of flowering transition (Xue et al., 2024). The researchers have determined which genes should receive CRISPRa activation treatment to achieve optimal control of mung bean flowering duration.

#### 5.2 Expected outcomes: early flowering, delayed flowering, adaptability

CRISPRa technology allows researchers to activate VrFT1 flowering-promoting genes through CRISPRa which leads to early flowering that benefits plant growth under short cultivation periods and harsh environmental conditions. The activation of TFL-like genes which act as flowering repressors would result in delayed flowering and longer vegetative growth periods for increased yields under particular environmental conditions. The modification of these genes through fine-tuning would lead to better mung bean performance in different agro-ecological zones and result in more stable yields and breeding programs for local cultivation (Xue et al., 2024).

#### 5.3 Technical challenges and regulatory considerations

Despite the promise of CRISPRa, several technical challenges remain. The delivery of CRISPRa components to mung bean cells faces two primary obstacles which are effective delivery techniques and stable expression of dCas9-activator fusions and precise targeting of endogenous gene promoters. The solution needs to include approaches for dealing with off-target effects and the inconsistent activation performance between different gene targets and tissue types. Different countries establish separate GMO regulations for CRISPRa because it functions



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as a tool that produces no permanent DNA changes. The successful implementation of CRISPRa-modified mung bean varieties depends on obtaining public approval and establishing precise regulatory frameworks for their use in agriculture.

#### 5 CRISPRa for Flowering Regulation in Mung Bean

#### 5.1 Candidate flowering genes for activation in mung bean

Research through genomic analysis has discovered multiple essential genes in mung bean which control the process of flowering. The Phosphatidylethanolamine-binding protein (PEBP) gene family contains the FT (FLOWERING LOCUS T) clade which functions as a key regulator for flower initiation. The family contains VrFT1 which shows increased expression under short-day conditions and studies with transgenic Arabidopsis overexpression demonstrate its role in promoting flowering. The study of *PEBP* genes progresses through VrMFT1 and VrTFL2 and VrTFL3 which show photoperiod-dependent expression to control flowering time changes (Xue et al., 2024). The researchers have determined which genes should receive CRISPRa activation to achieve the best results for mung bean flowering duration control.

#### 5.2 Expected outcomes: early flowering, delayed flowering, adaptability

The activation of VrFT1 flowering-promoting genes through CRISPRa technology will lead to early flowering which benefits plant adaptation to brief cultivation periods and harsh environmental conditions. The activation of TFL-like genes which function as flowering repressors presents a method to postpone flowering which could enhance plant growth and production in specific situations. The modification of these genes through fine-tuning would allow mung bean to grow in different agricultural areas and make breeding for local cultivation easier (Xue et al., 2024).

#### 5.3 Technical challenges and regulatory considerations

Despite the promise of CRISPRa, several technical challenges remain. CRISPRa technology encounters two major challenges which include delivering CRISPRa components into mung bean cells and sustaining dCas9-activator fusion expression stability and precise endogenous gene promoter targeting. The research requires solutions to handle off-target effects and variable activation efficiency that affects different gene targets and tissues. The classification of CRISPRa under GMO regulations varies across countries because it functions as a tool which makes no permanent changes to DNA sequences. CRISPRa-modified mung bean varieties need public approval together with specific regulatory frameworks to succeed in agricultural applications.

#### 6 Case Study: Activating VrFT1 Homolog in Mung Bean

#### 6.1 Experimental design: activating VrFT1 homolog in mung bean

The *VrFT1* gene, a member of the Phosphatidylethanolamine-binding protein (PEBP) family, has been identified as a key regulator of flowering in mung bean. Scientists studied *PEBP* gene function through genome-wide analysis of these genes and studied their expression patterns under various photoperiod conditions. The study of VrFT1 activation through CRISPRa in mung bean remains unreported but researchers used transgenic Arabidopsis to study VrFT1 function as a substitute for mung bean gene activation studies (Xue et al., 2024). The experimental design measured VrFT1 expression levels across different tissues and under short-day (SD) and long-day (LD) environmental conditions before researchers studied the model plant system for phenotypic effects.

#### 6.2 Observed results: changes in flowering time and yield traits

The research established that VrFT1 expression levels reached their peak under SD conditions which resulted in plants flowering at an earlier stage. Xue et al. (2024) proved VrFT1 functions as a flowering promoter through their study which showed that Arabidopsis plants with VrFT1 overexpression reached flowering stage before control plants did. The *VrFT1* and *PEBP* genes in mung bean showed photoperiod-dependent and tissue-specific expression patterns which indicates their function in flowering time regulation. The study does not include direct measurements of mung bean yield traits but suggests that VrFT1 overexpression enables researchers to control flowering time for better yield performance under different environmental conditions (Figure 2).



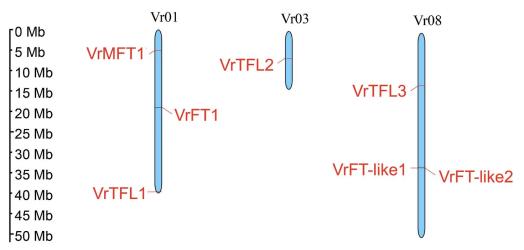


Figure 2 Distribution of *PEBP* genes on mung bean chromosomes. Chromosome size is indicated by its relative length. The scale on the left is shown in megabases (Mb) (Adopted from Xue et al., 2024)

#### 6.3 Lessons learned and implications for breeding programs

The research shows that VrFT1 functions as a potential target for managing flowering time in mung bean plants. The photoperiod-sensitive regulation of VrFT1 and its functional verification in a model plant system makes it an excellent candidate for CRISPRa-mediated activation. Breeding programs would benefit from VrFT1 activation because it would allow scientists to create mung bean varieties with specific flowering periods which would improve their performance across different agricultural environments. The study needs further investigation to test CRISPRa technology on mung bean plants and determine its impact on yield output and agricultural traits when used in real-world field settings (Xue et al., 2024).

#### 7 Future Directions for CRISPRa in Flowering Regulation of Mung Bean

#### 7.1 CRISPRa functions as a tool for base editing and prime editing through advanced editing technologies

CRISPR activation (CRISPRa) shows great potential for mung bean improvement through its combination with base editing and prime editing genome editing systems. The technology CRISPRa provides exact gene activation through reversible methods yet base and prime editing systems make it possible to perform permanent nucleotide modifications without creating double-strand breaks. Scientists can use these research methods to enhance flowering gene expression and introduce beneficial alleles which will speed up the development of mung bean varieties with better flowering and stress tolerance (Huppertz et al., 2023). The two breeding approaches need to be combined because modern mung bean breeding requires advanced methods for improving multiple traits.

#### 7.2 Multiplex gene activation for complex flowering networks

Flowering regulation in mung bean involves a network of genes, including FT, CO, SOC1, and TFL-like genes. The future applications of CRISPRa technology will concentrate on creating techniques to activate multiple genes simultaneously for controlling intricate flowering processes. This method enables scientists to study gene interactions while eliminating genetic redundancy which results in better control of flowering time and adaptation. The multiplex CRISPRa system which proved effective in other crops has the potential to control multiple flowering and stress-response genes in mung bean plants for improved breeding operations and multiple desirable trait stacking (Huppertz et al.,2023).

#### 7.3 Applications in climate-resilient and region-specific mung bean breeding

The growing severity of climate change requires researchers to develop mung bean varieties which show resistance to abiotic stress factors including drought and heat while being suitable for particular agricultural environments. CRISPRa enables the development of climate-resistant mung bean cultivars through its combination with genomic selection and molecular breeding (Huppertz et al., 2023). Breeders who activate specific genes which control early or late flowering and stress tolerance and yield stability can create new lines that adapt to various environments at a fast pace thus ensuring sustainable food production and food security.

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#### 8 Conclusion

Scientists use the CRISPR activation system to control native flowering gene expression with its programmable activation mechanism. CRISPRa enables scientists to activate specific flowering regulators including FT and related genes which results in new methods for crop adaptation and yield optimization and complex flowering network research in mung bean and additional crops.

CRISPRa technology provides researchers with multiple benefits because it enables exact gene expression management through reversible and multiplexable methods that protect cells from harm which makes it suitable for studying complex traits such as flowering. However, technical limitations remain. The delivery of CRISPRa components faces multiple obstacles which include inefficient delivery systems and target-specific activation variability and off-target effects and insufficient regulatory structures and public support. Additionally, most current applications and case studies are concentrated in model plants and major crops, with translational work in mung bean still in its early stages.

The future of mung bean development will benefit from CRISPRa integration with sophisticated editing tools and multiple gene activation methods to create varieties that match specific flowering schedules and show improved tolerance to various environmental conditions. The development of better delivery systems and clearer regulations will make CRISPRa a fundamental technology for sustainable mung bean breeding which will enhance food security and climate resilience by enabling fast and adaptable crop improvement.

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#### **Conflict of Interest Disclosure**

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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