Journal of Pure and Applied Agriculture (2022) 7(2): 73-82
ISSN (Print) 2617-8672, ISSN (Online) 2617-8680
http://jpaa.aiou.edu.pk/

Bloom or not to bloom: Understanding the MiR-156 based regulation of flowering genes in rice *(Oryza sativa)*

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Abstract

Flowering at the proper time is critical for effective sexual reproduction and the subsequent development of seeds and fruits in plants. This entails synchronising flowering with the right season and the plant's developmental history. The cruciform weed Arabidopsis has shown discrete but connected pathways for sensing the primary seasonal cues of day length and low temperature, as well as other local external and internal signals, using genetic and molecular analyses. A common group of genes integrates the balance of signals from these pathways to decide when blooming commences. Billions of people around the world directly or indirectly depend on rice *(Oryza sativa*) for their daily nutrition needs. To fulfill the demands of the growing population, scientists are looking for ways to improve the yield by tweaking the flowering genes and controlling the expression of the miR156 gene. The circuitry of the miR156 and its effect on flowering is complex and involves various pathways; some of those pathways are yet to be discovered. The most critical interactions in the SPL-miR156 gene result in the plant's shift from vegetative to reproductive phase. This involves several environmental, morphological, and internal factors coherently effect. Future research will look into the importance of these interactions and more yields will follow by manipulating the genetic interactions. This paper is an attempt to understand the complex nature of the SPL-miR156 gene and how it regulates the flowering process. © 2022 Department of Agricultural Sciences, AIOU

Keywords: *Arabidopsis thaliana*, Flowering genes, miR156, *Oryza sativa*, QTLs (Quantitative Trait Loci), SPL (SQUAMOSA-promoter binding protein-like)

Abbreviations: Cu = Copper; Fe = Iron; Mn = Manganese; Zn = Zinc; Ca = Calcium; Mg = Magnesium; P = Phosphorus; MiRNA = MicroRNA; RISC = Complex RNA-induced silencing complex; FLC = FLOWERING LOCUS C; SD = Short day; LD = Long day; Hd3a = Heading date 3a; RFT1 = RICE FLOWERING LOCUS T1; Hd1 = Heading date 1; OsABF1 = *Oryza sativa* ABA-responsive element binding factor 1; QTLs = Quantitative Trait Loci; PPS = Peter Pan Syndrome; Ehd1 = Early heading date 1; PIF = Phytochrome interacting factors; MRU = miRNA responsive Unit; UTR = Untranslated region; SAM = Shoot apical meristem; FUL = FRUITFUL; AP1 = APETALA1; LFY = LEAFY; SPL = SQUAMOSA-promoter binding protein-like

To cite this article: Ahmad, A., Khan, H. A., Nadeem, M., & Kazmi, A. (2022). Bloom or not to bloom: Understanding the MiR-156 based regulation of flowering genes in rice *(Oryza sativa*). *Journal of Pure and Applied Agriculture, 7*(2), 73-82.

Introduction

In most countries, rice (*Oryza sativa* L.) is an essential element of most people's diets. Due to its importance either directly as human food or indirectly as animal feed, it is regarded as one of the world's most vital nutritious staple food crops among cereals (Verma & Srivastav, 2020). Rice is a carbohydrate-rich meal with a little amount of protein and fat, as well as vitamin B complexes including niacin, riboflavin, and thiamine. Amylose and amylopectin, a kind of starch, make up the majority of rice carbohydrates (Makky et al., 2021). Rice contains traces of copper (Cu^{2+}), iron (Fe^{3+}), manganese (Mn^{+}), and zinc (Zn^{+}), as well as minerals such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and phosphorus (P) (Ravoson et al., 2019). Bioactive substances are additional nutritious components that are generally found in tiny amounts in different sections of rice (bran, germ fraction, and endosperm) (Ghanghas et al., 2020). Cellulose, hemicellulose, pectin, arabinoxylan, lignin, -glucan, polyphenolics, -oryzanol, -sitosterol, vitamins B9 and E isoforms, and important amino acids are some of the bioactive chemicals found in rice (Calderan-Rodrigues et al., 2019).

The genetics of flowering in plants and the development of sexual structures is a complex process that takes place in phases. Of all the important features that develop over time flowering is an important phenomenon and must be controlled efficiently to coordinate the flowering season to ensure pollination and reproductive success (Kumar et al., 2021). The development is controlled by many enzymes and an orchestra of miRNA and other non-coding nucleotide structures. To date, scientists have reported discovering a total of 700 miRNA through direct experiments and bioinformatics analysis (Li et al., 2010; Shi, 2022). However, the process that goes into the regulation of bolting and flowering through miRNA circuitry is largely unexplored. This is mainly because of the short life span of the miRNA and the lack of sophisticated techniques that go into the identification of small sequences of miRNA (Amasino & Michaels, 2010). The research is particularly important in the case of the rice plant as it is a staple food of billions of people around the world. Through regulation, understanding, and development of techniques and interventions to control the flowering in rice means more production and food for millions (Sanan-Mishra et al., 2009; Vega Rodríguez et al., 2022). One of the most significant agronomic variables for rice seed yield is the flowering period. It is primarily regulated by genes linked to photoperiod sensitivity, especially in short-day plants like rice. Rice breeders and academics have been interested in explaining the genetic basis of blooming time since the early twentieth century since it is crucial for regional adaptation and yield optimization. Despite the fact that blooming time is a complicated feature governed by several quantitative trait loci (QTLs), traditional genetic investigations have revealed that many related genes are passed down through the generations according to Mendelian principles. On the basis of genome-wide mapping and gene cloning, decoding the rice genome sequence ushered in a new era in understanding the genetic regulation of flowering time. The first flowering time QTL to be identified using spontaneous variation in rice was heading date 1 (Hd1) (Fujino et al., 2022). Other QTLs, even those with minimal impacts on flowering time, have been cloned thanks to the recent collection of knowledge on the rice genome. With this knowledge, we were able to rediscover several of the blooming genes that were previously found using standard Mendelian genetics. Hd1 is one of the genes that has been identified so far and has been assigned to a specific photoperiod pathway.

Mature miRNA is formed as a result of the cleavage of pre-miRNA and is carried out through the RISC complex (MacFarlane & R Murphy, 2010). Formed as a result of the transcription of the noncoding regions of the plant genome, these small structures usually 19-24 nucleotides long play an important role in mRNA pre- and post-transcriptional modification hence gene expression. Literature and studies of the past decade have elaborated on the importance of miRNA in terms of development, hormonal control, organ polarity, and development of specific organs (Ali et al., 2020; Chen et al., 2021). The genetics and timing of flowering is a complex process and is not controlled by only one factor but is multigene and multifaceted. There are various factors other than genetics that play a vital role in the onset of the flowering process such as the light intensity and quality, hormones like the auxin and gibberellin balance (Rabinowitch, 2018), temperature as in vernalization that occurs in presence of the vernalin and the age of the plant (Nie et al., 2015). A critical balance is maintained among all these factors to bring a coordinated response. However, these microRNAs are the most important factor that can be controlled and tweaked to delay or induce flowering.

Factors affecting flowering in rice

The flowering period is directly linked to crop production and quality and is primarily controlled by environmental variables (Kazan & Lyons, 2016). Rice grain yield is greatly reduced by early or late-blooming due to weak fertility or insufficient vegetative growth. The flowering period must be carefully managed by combining internal and external signals to enhance reproductive success and grain yield. The highly consistent seasonal signal through which plants divine imminent modifications in environmental circumstances is photoperiod, or the length of the light period among the different environmental cues (Guadagno et al., 2018). As a result, the pathway that senses and responds to photoperiod is a blooming time regulator in plants. Plants have an independent mechanism to trigger blooming in the absence of external stimuli, in addition to the photoperiodic system. The components of the autonomous route regulate the expression of the floral repressor FLC (FLOWERING LOCUS C) by transcription, epigenetic regulation, and RNA processing (Eom et al., 2018). Once the rice was domesticated, it spread from Asia's tropical and subtropical areas to temperate regions at different altitudes. The development of photoperiod-insensitive characteristics allowed rice to adapt to these latitudes (Goretti et al., 2017). Several environmental stressors influence the expression of genes involved in the photoperiodic flowering pathway, which helps to regulate blooming timing. The framework for generating superior varieties of rice with the features necessary to respond to specific conditions will be laid by identifying essential components influencing photoperiod responsiveness and researching their responses to other climatic stresses.

Moisture stress slows blooming in most rice cultivars, but it can potentially speed up flowering in others (Sridevi & Chellamuthu, 2015). Differences in genetic backgrounds in genes involved in flowering-time regulation are likely to be the cause of this paradoxical occurrence among cultivars. Drought suppresses the expression of the rice florigen genes Hd3a and RFT1 as well as Ehd1 in both SD and LD conditions (Zhang et al., 2016). Furthermore, Hd1 cycling amplitude is decreased, but GI cycling amplitude is unaffected. During drought conditions, Hd3a and RFT1 expression levels in hd1 and gi mutants are similar to those of the WT, although blooming occurs later in the mutants (Cho et al., 2017). These findings show that GI and Hd1 are not engaged in the drought response in Arabidopsis and that the regulatory mechanisms in Arabidopsis and rice are different (Todaka et al., 2015). Under both SD and LD circumstances, a bZIP TF called *Oryza sativa* ABA-responsive element binding factor 1 (OsABF1) slows the floral transition by inhibiting Ehd1 (Hossain et al., 2010). While overexpression of Ehd1 prevents the blooming delay induced by OsABF1 overexpression, RNAi silencing of Ehd1 reduces the time shift produced by OsABF1 (Zhang et al., 2016). This suggests that Ehd1 is a downstream effector of OsABF1. Drought-induced OsABF1 induction and downregulation, as well as its nearest homolog, OsbZIP40, delays blooming. Alternatively, an undiscovered repressor might be important in delaying floral induction in rice during dry periods. Even at modest NaCl concentrations (20–50 mM), salt causes rice to bloom later (Srivastava et al., 2016). Salt-sensitive species experience a longer delay. However, the chemical mechanism that causes this reaction in rice is mainly unclear.

Management of Flowering duration in Rice

To better understand the molecular machinery driving the floral alteration in rice, many QTLs (Quantitative Trait Loci) for heading dates are recently identified (Xu et al., 2016). RICE FLOWERING LOCUS T1 (RFT1) and Heading date 3a (Hd3a), both Arabidopsis FT orthologs, encrypt rice florigens. These regulators were discovered to be important in controlling flowering time in this research (Jing Zhao et al., 2015). Comparable to the flowering time of Arabidopsis, the proteins of these genes are generated in the leaves and then transferred to the shoot apex to trigger blooming in rice (Cerise et al., 2021). Hd3a promotes rice blooming under short-day situations, while RFT1 is involved in blooming under long-day circumstances (Fig. 1). The Arabidopsis CO-FT module-like Hd1-Hd3a route and the rice-specified Ghd7-Ehd1-Hd3a-RFT1 route both control RFT1 and Hd3a expression (Shim & Jang, 2020). Heading date 1 (Hd1) was the first flowering time gene to be discovered in rice, due to map-based cloning (Hori et al., 2016). Hd1 is an Arabidopsis CO ortholog that encodes a zinc finger protein. In rice, Hd1 is essential for day-time-definite blooming stimulation; much in Arabidopsis CO (Lee & An, 2007). Hd1 is a bifunctional protein that regulates the expression of the rice florigen gene Hd3a by either repressing or activating it. The circadian clock and light signaling govern the Hd3a expression is regulated by Hd1 in a Hd1-dependent manner. (Song et al., 2010). Under induced short-day circumstances, Hd1 is mostly regulated at night, but it is also highly translated from night until dawn under non-inductive long-day conditions (Itoh & Izawa, 2013). Hd1's diurnal expression is controlled by OsGI, an Arabidopsis GI homolog. In the dark conditions, Hd1 up-regulates the expression of Hd3a. In the occurrence of light, Hd1 is changed to a repressor of Hd3a production from a trigger, a procedure reconciled by phytochrome. In the mutant with phytochrome deficiency photoperiod sensitivity 5 (se5), expression of Hd3a is regulated by Hd1, independent of light duration (Andrés et al., 2009). Thus, Daily expression samples mediated by the light-dependent developmental translation of Hd1, and the circadian clock help in the activation of Hd3a regulation under short-day situations. The repressive act of Hd1 is boosted by Hd6's kinase action, although Hd6 emerges to have an indirect effect on Hd1's repressive activity on Hd3a translation under long-day circumstances (Shim & Jang, 2020). Rice has homologs of Arabidopsis CDFs, GI, and FKF1 which control CO expression in Arabidopsis based on day duration. Despite the fact that OsFKF1 interacts with OsDOF12, and OsGI, it is OsFKF1 that regulates rice-specific blooming genes rather than Hd1 (Han et al., 2015). OsDOF12 might be to account for the divergent outcomes of OsFKF1-dependent flowering pathway modulation in rice and Arabidopsis. By controlling the translation of Hd3a only, OsDOF12 aids photoperiodic blooming. Rice Hd1 has a different posttranslational regulation than Arabidopsis CO. CO is vigorously deteriorated in the dark in Arabidopsis by the COP1-SPA complex, which inhibits FT expression. Rice likewise has a low Hd1 protein content at night. In Hd1 over-expressing plants, however, Hd1 protein was identified in equal quantities during the day and night, showing that Hd1 protein appears to be constant in rice in the absence of light. As a result, the activities of COP1 and SPA in rice may differ. An ortholog in rice of Arabidopsis COP1 is considered as Peter Pan Syndrome (PPS) (Tanaka et al., 2011). The shift from the vegetative to the reproductive stage is aided by PPS1, like COP1, although it is not regulated by the Hd1-Hd3a/RFT1 passageway. In accumulation to PPS1, HAF1, a C3HC4 RING domain-including E3 ligase, affects the protein of Hd1 gene stabilization in rice (Yang et al., 2015). HAF1 interacts physically with Hd1, and haf1 mutants have high amounts of Hd1. As a result, HAF1 may play a role in the ubiquitination of Hd1 in preparation for degradation by the 26S proteasome (Zhu et al., 2018). A molecular system that comprises rice-specific B-type response regulators like Ghd7, and Early heading date 1 (Ehd1) also controls rice blooming and other several characteristics (heading date, plant height, and Grain number) (Zhou et al., 2021). Under both short-day and long-day circumstances, Ehd1 stimulates flowering by enhanced regulation of RFT1 and Hd3a regulation. Ehd1 expression in rice is regulated by several upstream regulators. Under both short-day and long-day circumstances, Ehd2, Ehd3, and Ehd4 affirmatively controlled Ehd1 regulations (Gao et al., 2013). Ghd7, on the other hand, suppresses Ehd1 expression. In response to the lengthening of the day, Ghd7 expression rises progressively. Phytochrome is engaged in the day-duration-reliant accretion of Ghd7 transcripts (T. Zheng et al., 2019). Under long-day circumstances, Hd5/LH8/Ghd8/DTH8 is a transcription mediator of CCAAT-box-binding that slows blooming in rice by downregulating Hd3a expression (Wang et al., 2019). Importantly, DTH8 up-regulates Hd3a regulation during short-day circumstances. It was discovered that the genetic consequences of DTH8 on rice blooming time regulation are influenced by the genetic conditions. The physical interaction of DTH8 with Hd1 might explain its bi-functionality. H3K27 trimethylation at the Hd3a gene is increased by the DTH8-Hd1 complex, which inhibits Hd3a production (Du et al., 2017). As a result, the development of the DTH8-Hd1 complex is critical for Hd3a transcriptional suppression by Hd1 during the long days (Zong et al., 2021). DTH8 also inhibits Ehd1, an upstream regulator of Hd3a, in an indirect manner. DTH8 is thought to bind to the Ghd7 promoter and stimulate its expression, according to recent research. In the presence of Hd1, this binding is boosted. As a result, the Hd1-DTH8 complex may inhibit Hd3a expression both directly and indirectly through Ghd7 (Hori et al., 2013).

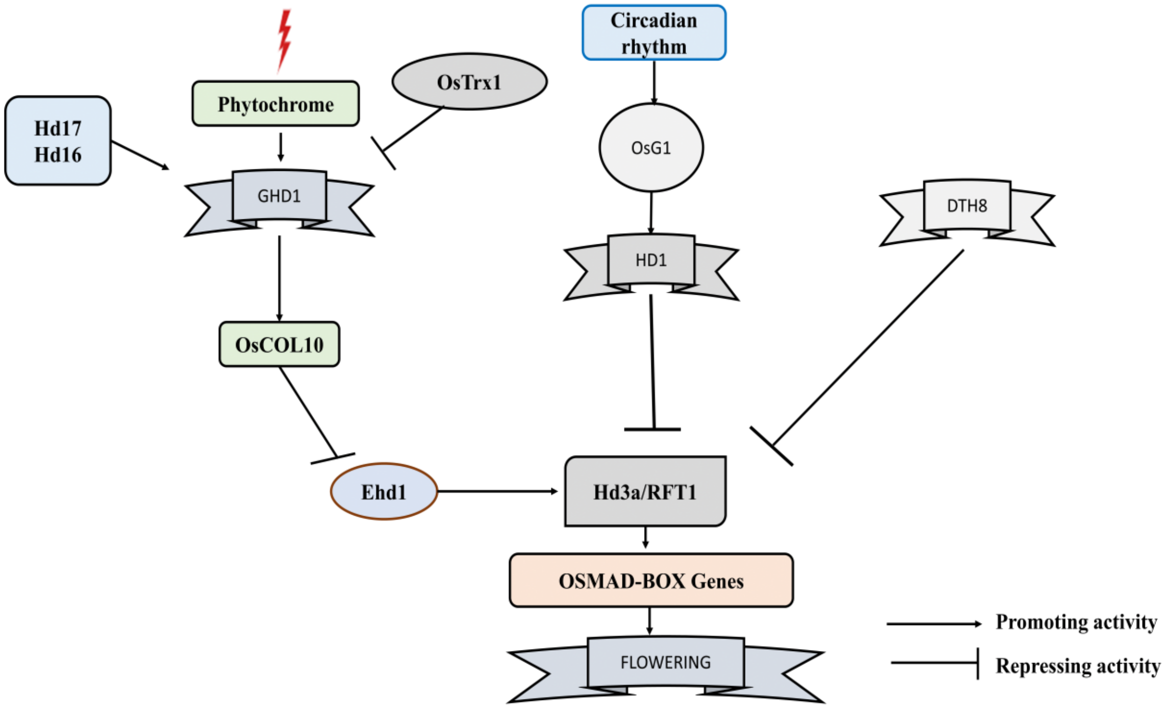

Fig. 1 A partial map of the genes involved in the flowering onset, various factors collectively regulate the flowering based on the external and internal factors

Regulation of flowering time by miR-156

The vegetative stage and the reproductive stage are the two major stages of blooming. Although there is just one flowering phase in the reproductive stage, the vegetative stage is split into two sub-phases: juvenile and adult (Bahuguna et al., 2017). The shoot formation transition between juvenile, adult, and reproductive phases and the phase shift phenomena are important contributors to heteroblasty. Although the floral induction process and reproductive development phenomena are well understood, the molecules involved in the transition from juvenile to adult phase (change in the vegetative phase) have just recently been discovered (Ahsan et al., 2019; Kinoshita & Richter, 2020). In Arabidopsis and maize, the microRNA (miRNA) miR156 is one of the essential RNAs (D'Ario et al., 2017). Expression of miR156 is high early in the development of a shoot, but it drops substantially during the juvenile-to-adult transition. This miR156 expression prolongs the expression of the juvenile phase; yet, when miR156 activity decreases, the transition to the vegetative phase is expedited, making miR156 an important factor in this transition (Guo et al., 2017; Manuela & Xu, 2020). MiR156 supports juvenile development by suppressing members of transcription variables SPLs. The transcription factors AP1, miR172, LFY, AGL42, SOC1, and FUL are the major targets of the suppression (Teotia & Tang, 2015). While there is a lot of miR156 in the juvenile period, the levels of miR156-targeted SPL genes are low. As plants develop the quantity of miR156 declines and cause the SPL level of miR156-targeted to rise (Wang, 2014).Two processes, regulated by SPL9 and SPL3, stimulate flowering in the shoot apex and leaves (Andrés et al., 2014). MADS-box genes such as LFY, SOC1, AP1, and FUL are activated at the shoot apex through SPL3 and SPL9 mechanisms, resulting in flowering in the shoot apex. In both long-day and short-day regimes, inducing SPL3 or SPL9 expression under a promoter-specific to the shoot apex improves early-blooming characteristics (Golembeski et al., 2014).

MiR156 and heading time in plants

The flowering time of the plants or heading time is the most crucial time for the plant. Studies on *Arabidopsis thaliana* have produced a considerable insight into the circuitry that controls this flowering time. MiR156 is one of the most crucial factors that regulate flowering or bolting. Unlike other plants where miR156 diminished with age in rice, it gradually increases from young to old leaves and brings about the phase change process that controls the flowering process (Xie et al., 2012).

MiR-156 is a short non-coding RNA of 19-24 nucleotides long and is found both in plants and animals. It is one of the most conserved RNA and modifies the expression of genes through post Transcriptional modification of the mRNA or by modification of the translational assembly. The main target of miRNA156 is the SQUAMOSA PROMOTER BINDING LIKEs (SPLs) (Yu et al., 2012). The overproduction of miRNA 156 is involved in senescence and delayed flowering in all experimental plants. The expression decreases with age in most plants and that is due to the higher amount of circulatory sugar phytochromes and age-related factors. Similarly, the SPL factors also play an antagonistic role and promote flowering when overexpressed in a plant at a late stage of development (Wang et al., 2009). These are a group of promoters that enhance flowering in the plant by careful molecular crosstalk that involves many molecules all working to balance the flowering time with the day length and temperature (Fig. 2).

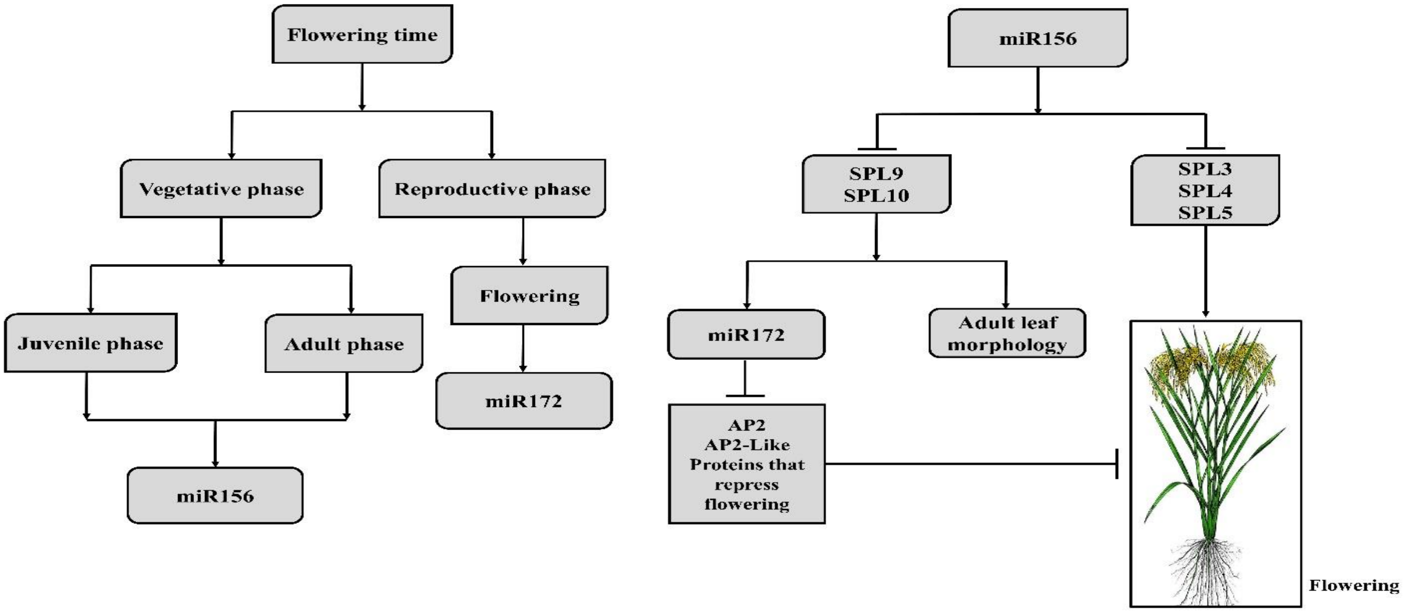

Fig. 2 The detailed diagram of the factors that are inhibited by the miR156 and how the regulation cases a transition from the vegetative to reproductive phase in rice. The role of miR156 and miR172 is shown with the result of flower

Phytochromes and miR156 expression

As mentioned earlier, the regulatory mechanism of any phenotype expression is not simple and requires multiple factors to initiate and stop the process. This is done to maintain an expression of the phenotype or character even when one pathway fails or reaches its limitations. Similarly, the shade avoidance mechanism which is originally developed by the plant to seek more light and grow at a rapid rate in presence of low light intensity and quality plays a vital role in miR156 expression. The phytochromes are a family of proteins expressed in plants under red or far-red exposure (Stief et al., 2014). Overexpression or accumulation of the phytochromes affects the expression of the miRNA in two ways i.e., it directly binds with the promoters of the miRNA genes thus hindering the transcription process, secondly by promoting the SPL proteins that antagonize the effect of miRNA and induce flowering (Xie et al., 2017). This two-way suppression brings a rapid flowering onset even when the flower is artificially kept in a reduced light period.

Similarly, other studies also suggest the role of different phytochromes like PHYA, PHYB, and PHYC genes as a promoter of phytohormones like gibberellin and auxin that promote apical growth and thus flowering. A simultaneous mutation in all the PHY genes induces early flowering that suggests the role of these genes in regulating the morphogenesis and also suppression of miR156 genes. The phytochrome interacting factors (PIF) are directly involved in the downregulation of the miR156 gene box by allowing the PHYA, PHYB, and PHYC proteins to occupy the G-box motif in the promoter of miR156 genes. With the advanced studies and literature review, it is obvious that four factors regulate the flowering and other transitional changes in plants from vegetative to reproductive state. The PHY-PIF-miR156-SPL interaction regulates flowering in Arabidopsis (Yu et al., 2015). However, there is a need for further investigation to know if any such completely developed system is found in rice as well. This would help us develop crop varieties that yield better and develop faster.

Epigenetics and DNA modification

There is a consensus among scientists that the methylation of DNA, histone proteins, and small non-coding RNA plays an important role in the expression of particular genes. It all happens in response to certain internal or external factors. As mentioned above light, sugar, and exposure to shade play a vital role in the plant conversion to reproductive generation from vegetative (Jinlei Shi et al., 2015). Unlike Arabidopsis onto which most of the flowering-related work has been done rice is a short-day plant (SD) so there is no influence of temperature in its flowering phase transition but there are other genes such as HD3a (Heading date 3a) gene and RFT1 (flowering locus T1) genes. Both of these genes are activated in rice plants exposed to SD. In shorter days (SD) length, both of these genes are produced in higher amounts which in turn activate the *"florogin"* protein that travels to the apical shoots and induces the flowering (Sun et al., 2012). There are two ways the HD3a and RFT1 genes are activated i.e., either via *early heading date 1* (*EHD1)* or through *Heading date 1 (Hd1)* genes. Where the *EHd1* genes activate the B-type activators and that in turn upregulates Hd3a and *RFT1* genes. However, the genes once activated start the flowering process (Wang et al., 2013). However, the process is far more complex and needs more attention to uncover more of the factor that regulates the crucial timing. Ehd1 gene expression is regulated via three different pathways all converging at the endpoint of upregulating the *Hd3a* and *RTF1* gene expression. The *Oryza sativa OsId1* gene pathway regulates the *Ehd1*gene that eventually affects *Hd3a* and *RTF1* genes. In this pathway, there is also *Edh4* that encodes zinc finger-like transcription factors which directly act on SD and LD plants. This makes this pathway unique as it can influence the flowering time in both types of plants. The remaining two factors are light length-dependent and involve many genes all eventually influencing *Hd3a* and *RFT1* genes (Shafiq et al., 2014).

MiR156-SPL toolbox for plant architect improvement

A plant is always battling with many biotic and abiotic factors and produces a variety of enzymes and proteins to maintain steady growth and fight off the problems (Long et al., 2018). Members of the SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (SPL) family of transcription factors, were first discovered in Antirrhinum majus, have emerged as critical regulators of a wide range of biological processes in plants, including leaf development, tillering/branching, plastochron, panicle/tassel architecture, fruit ripening, fertility, and stress response. MicroRNA156s target the transcripts of a subset of SPLs for cleavage and/or translational suppression (miR156s). Endogenous developmental signals and a variety of exogenous stressors both influence the levels of miR156s. Increasing evidence suggests that the regulatory circuit focused on the miR156/SPL module is largely conserved across phylogenetically diverse plant species and plays a key role in plant fitness, biomass, and yield regulation. We can now use the miR156/SPL module as a plethora of tools to genetically manipulate crops for optimal parameters in growth and development, and ultimately to maximize yield through intelligent crop design, thanks to growing knowledge and a mechanistic understanding of their roles and regulatory relationships (He et al., 2022; Lai et al., 2022; Xing et al., 2022).

The miR156 and SPL are two of the main factors that help plants tolerate the changing abiotic stress such as temperature, light intensity, quality, and water and salt problems. There are virtually enough SPL factors discovered by many studies however they are divided into four groups and the confirmed number is 16. All these SPL proteins show a diverse function and regulation of plant development aspects from morphological changes in the long term, yield, and tolerance to the biological and abiotic stress (Cui et al., 2014). The MiRNA and SPL are associated with each other as the miRNA 156 regulates and binds with many SPL protein genes and downregulates their function during early development. This, however, reduces with age and the plant produces more SPL proteins once mature and miR156 expression subsides once the leaf development is high and the circulating sugar is enough to suppress its expression. Also, the SAS mechanism and related proteins suppress the expression of miR156. Out of 16 SPL found in rice 11 are targeted and regulated by miR156. The miR156 attaches to the 3UTR of the SPL gene also called a miRNA responsive Unit (MRU) and reduces its transcription process (Wang et al., 2009). There are many potential uses of exploring and tweaking this toolbox to produce higher quality and elite crops especially rice but the lack of complete control over the expression and its other unexplored functions are bottlenecks that are to be solved.

Age and miR156/SPL pathway

Till now the literature has established that the plant has internal clocks that regulate the physical and hormonal changes. These changes are highly sophisticated and result in the changes in all the plants of a particular species undergoing a morphogenic change at the same time. This is ensured to bloom and flower simultaneously. This mass synchronization is based on the balance of expression of miR156 and SPL genes (Zheng et al., 2019). The miR156 genes are highly expressive when the level of glucose is low as a result of fewer leaves and photosynthesis is not established yet. Treatment of the plant at the early stage of its development with sugars results in a rapid decrease in miR156 gene expression. This decrease occurs due to the interference in the transcriptional and post-transcriptional levels of the miR156 gene. However, with the development and identification of shoot apical meristem (SAM) the expression of miR156 starts to decrease which in turn causes a surge in SPL gene expression. SAM works in two ways: it serves as a reservoir for the cells during the elongation and leaves the production phase also called the vegetative phase while the same cells under the influence of internal and external stimuli start the reproductive phase (Wang, 2014). Similarly, a miR172 molecule promotes the flowering and is produced in response to the external stimuli and decrease of miR156.

Recently, plant scientists turned their attention toward the miR156-SPL pathway and its associated genetic circuits. Although there is a strong association of miR156 overexpression with the exogenous pathways, the scientists are looking at the role of light and the SPL-targeted MADS-box genes. These genes including *AP1*, *LFY*, *FUL*, and *SOC1* are regulated by the level of SPL3 and SPL9 genes which are in turn regulated by the level of miR156 (Wang et al., 2009). This complex integrated and intricate system of gene regulation brings about the growth of the plant and also its shift to the reproductive phase from the vegetative phase.

Role of SD preferential activators in Ehd1 genes regulation

Of the total three Ehd1 genes, Ehd2 and Ehd3 genes are light-induced and are activated under specific day length while the third Ehd4 is light-independent and works with the OsId1 gene in the upregulation of Ehd1 genes. While the *Ehd3* gene works with the OsMAD51 gene box that regulates the Ehd1 gene upstream and induces florogin production through Hd3a and RFT1 gene pathway. The remaining Ehd2 gene is LD preferential gene and works with the *GHD7* gene that encodes the CCT protein in rice and *OsLFLs* proteins. Together all these proteins encode the B3 type factors that again converge at the Hd3a /RFT1 gene pathway (Tsuji et al., 2011). The mentioned three pathways are much conserved in all rice varieties but the Edh4 and Edh2 pathways are found both in Arabidopsis and rice alike although both of these plants bloom in different light conditions.

There is also another gene called OsELF3 that has two homologs in rice, the *ELF3-1* and ELF3-2. These genes are also engaged in the expression of Ehd1 genes through LD and SD pathways (Junming Zhao et al., 2012). Both *ELF3-1 and ELF3-2* are involved in the circadian rhythms of the plant through their influence over the *OsPRR* gene box *(OsPRR1, OsPRR37, OsPRR73, and OsPRR95)*. Similarly, *ELF3-1* also regulates the effect of the *GHD7* gene that was involved in the LD-preferential pathways in the flowering phase transition in long-day plants (Junming Zhao et al., 2012). A mutation in ELF3 homologs showed a late flowering or heading time. An orthologue of the same gene was discovered in Arabidopsis Thaliana, which displayed similar alterations as the rice plant, confirming its critical role as a flowering phase regulator in plants.

SPL4 and flowering

For a young plant, the most important thing is to develop structures that help it perform well in a challenging environment such as shade, resource deficit, and root formation to compensate for the absorption of nutrients and water. At this stage, the flowering is of the least importance (Gou et al., 2019). The shade and higher number of cell divisions, an indication of the vegetative growth phase, induce the miR156 and miR172 production in a higher amount. Both of these microRNAs, as described earlier, are instrumental in the suppression of the transition to the flowering or reproductive phase. One of the most important genes for both of these microRNA targets is the osSPL4 gene which induces flowering (Teotia & Tang, 2015).

With age the apical tissues especially the phloem cells start producing the osSPL4 enhancing factors that travel to the nodes and start the flowering process. The phloem under the higher amount of sugar starts the production of such SPL4 enhancing factors. Recent development has revealed a direct role of SPL4 in the activation of the *FRUITFUL (FUL)*, APETALA1 (*AP1*), and *LEAFY* (*LFY*) all of these have a direct role in the flowering initiation. These genes can only be activated in the absence of miR156 or miR172. Because the flowering initiation process is a complex mechanism there is no consensus on what factor predominantly leads to the flowering as there are multiple factors that induce miR156 suppression and osSPL4 expression (Preston & Hileman, 2010).

Conclusion

Rice is a major crop in the world with more than half of the earth's population directly or indirectly dependent on it. The last decade has been a crucial year for the redevelopment of the strategies to identify and develop plant varieties that flower early and yield more than the natural plant varieties. This has been possible through the investigation and uncovering of the gene circuits that regulate the flowering time. Rice is a short-day (SD) plant but most of the research for the identification of the factors and genes that influence the plant's flowering time has been done on the Long Day plants like Arabidopsis. This is the reason a detailed pathway of LD is available as compared to the SD plants that the scientists believe have the same number of genes but currently, very few are known. This review paper has identified the genes and factors that involve heading time in rice plants. The major influencers of heading time in rice plants are the miRNAs that downregulate the heading time genes and delay flowering which is in turn regulated via external and internal factors. Other genes that actively participate in flowering time are *Ehd1, Ehd2, Ehd3, Ehd4, OsId1, OsMADBox 51* and *50, GHD7, CCT* proteins, and *OsLFL* and *ELF3* variants. However, the complex pathway is still far from being complete and needs more attention and the development of more sophisticated techniques to uncover more hidden gene regulation and communication pathways. This would help develop the varieties that are easy to cultivate under artificially induced day period length and temperature.

Acknowledgment: The authors are thankful to the Department of Genetics, State Key Laboratory of Hybrid Rice, College of Life Science, Wuhan University, Wuhan, PR China, and the team of Big Bio for their support.

Conflict of interest: The authors declare no conflicts of interest.

References

Ahsan, M. U., Hayward, A., Irihimovitch, V., Fletcher, S., Tanurdzic, M., Pocock, A., & Mitter, N. (2019).

Juvenility and vegetative phase transition in tropical/subtropical tree crops. *Frontiers in Plant Science*, *10*, 729.

Ali, S., Khan, N., & Xie, L. (2020). Molecular and hormonal regulation of leaf morphogenesis in Arabidopsis. *International Journal of Molecular Sciences*, *21*(14), 5132.

Amasino, R. M., & Michaels, S. D. (2010). The timing of flowering. *Plant Physiology*, *154*(2), 516-520.

Andrés, F., Galbraith, D. W., Talón, M., & Domingo, C. (2009). Analysis of PHOTOPERIOD SENSITIVITY5 sheds light on the role of phytochromes in photoperiodic flowering in rice. *Plant Physiology*, *151*(2), 681-690.

Andrés, F., Porri, A., Torti, S., Mateos, J., Romera-Branchat, M., García-Martínez, J. L., & Coupland, G. (2014). SHORT VEGETATIVE PHASE reduces gibberellin biosynthesis at the Arabidopsis shoot apex to regulate the floral transition. *Proceedings of the National Academy of Sciences, 111*(26), E2760-E2769.

Bahuguna, R. N., Solis, C. A., Shi, W., & Jagadish, K. S. (2017). Post-flowering night respiration and altered sink activity account for high night temperature-induced grain yield and quality loss in rice (*Oryza sativa* L.). *Physiologia Plantarum*, *159*(1), 59-73.

Calderan-Rodrigues, M. J., Guimarães Fonseca, J., de Moraes, F. E., Vaz Setem, L., Carmanhanis Begossi, A., & Labate, C. A. (2019). Plant cell wall proteomics: A focus on monocot species, Brachypodium distachyon, Saccharum spp. and Oryza sativa. *International Journal of Molecular Sciences*, *20*(8), 1975.

Cerise, M., Giaume, F., Galli, M., Khahani, B., Lucas, J., Podico, F., & Brambilla, V. (2021). *OsFD4* promotes the rice floral transition via florigen activation complex formation in the shoot apical meristem. *New Phytologist, 229*(1), 429-443.

Chen, Z., Li, Y., Li, P., Huang, X., Chen, M., Wu, J., & Li, Y. (2021). MircroRNA profiles of early rice inflorescence revealed a specific miRNA5506 regulating development of floral organs and female megagametophyte in rice. *International Journal of Molecular Sciences, 22*(12), 6610.

Cho, L. H., Yoon, J., & An, G. (2017). The control of flowering time by environmental factors. *The Plant Journal*, *90*(4), 708-719.

Cui, L. G., Shan, J. X., Shi, M., Gao, J. P., & Lin, H. X. (2014). The miR156-SPL 9-DFR pathway coordinates the relationship between development and abiotic stress tolerance in plants. *The Plant Journal, 80*(6), 1108-1117.

D'Ario, M., Griffiths-Jones, S., & Kim, M. (2017). Small RNAs: big impact on plant development. *Trends in Plant Science, 22*(12), 1056-1068.

Du, A., Tian, W., Wei, M., Yan, W., He, H., Zhou, D., & Ouyang, X. (2017). The *DTH8-Hd1* module mediates day-length-dependent regulation of rice flowering. *Molecular Plant, 10*(7), 948-961.

Eom, H., Park, S. J., Kim, M. K., Kim, H., Kang, H., & Lee, I. (2018). *TAF15b*, involved in the autonomous pathway for flowering, represses transcription of FLOWERING LOCUS C. *The Plant Journal*, *93*(1), 79-91.

Fujino, K., Kawahara, Y., & Shirasawa, K. (2022). Genetic diversity among the varieties exhibiting early heading date in rice. *Euphytica, 218*(2), 1-14.

Gao, H., Zheng, X.-M., Fei, G., Chen, J., Jin, M., Ren, Y., & Zhou, F. (2013). Ehd4 encodes a novel and Oryza-genus-specific regulator of photoperiodic flowering in rice. *PLoS Genetics*, *9*(2), e1003281.

Ghanghas, N., MT, M., Sharma, S., & Prabhakar, P. K. (2020). Classification, composition, extraction, functional modification and application of rice (Oryza sativa) seed protein: A comprehensive review. *Food Reviews International*, *38*, 354-383.

Golembeski, G. S., Kinmonth-Schultz, H. A., Song, Y. H., & Imaizumi, T. (2014). Photoperiodic flowering regulation in Arabidopsis thaliana. *Advances in Botanical Research, 72*, 1-28.

Goretti, D., Martignago, D., Landini, M., Brambilla, V., Gómez-Ariza, J., Gnesutta, N., & Terauchi, R. (2017). Transcriptional and post-transcriptional mechanisms limit heading date 1 (Hd1) function to adapt rice to high latitudes. *PLoS Genetics*, *13*(1), e1006530.

Gou, J., Tang, C., Chen, N., Wang, H., Debnath, S., Sun, L., & Allen, R. D. (2019). SPL 7 and SPL 8 represent a novel flowering regulation mechanism in switchgrass. *New Phytologist*, *222*(3), 1610-1623.

Guadagno, C. R., Ewers, B. E., & Weinig, C. (2018). Circadian rhythms and redox state in plants: Till stress do us part. *Frontiers in Plant Science, 9*, 247.

Guo, C., Xu, Y., Shi, M., Lai, Y., Wu, X., Wang, H., & Wu, G. (2017). Repression of miR156 by miR159 regulates the timing of the juvenile-to-adult transition in Arabidopsis. *The Plant Cell*, *29*(6), 1293-1304.

Han, S. H., Yoo, S. C., Lee, B. D., An, G., & Paek, N. C. (2015). Rice FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (OsFKF 1) promotes flowering independent of photoperiod. *Plant, Cell & Environment*, *38*(12), 2527-2540.

He, F., Long, R., Wei, C., Zhang, Y., Li, M., Kang, J., & Chen, L. (2022). Genome-wide identification, phylogeny and expression analysis of the SPL gene family and its important role in salt stress in Medicago sativa L. *BMC Plant Biology*, *22*(1), 1-13.

Hori, K., Matsubara, K., & Yano, M. (2016). Genetic control of flowering time in rice: integration of Mendelian genetics and genomics. *Theoretical and Applied Genetics*, *129*(12), 2241-2252.

Hori, K., Ogiso-Tanaka, E., Matsubara, K., Yamanouchi, U., Ebana, K., & Yano, M. (2013). H d16, a gene for casein kinase I, is involved in the control of rice flowering time by modulating the day-length response. *The Plant Journal*, *76*(1), 36-46.

Hossain, M. A., Lee, Y., Cho, J.-I., Ahn, C.-H., Lee, S.-K., Jeon, J.-S., & Park, P. B. (2010). The bZIP transcription factor OsABF1 is an ABA responsive element binding factor that enhances abiotic stress signaling in rice. *Plant Molecular Biology, 72*(4-5), 557-566.

Itoh, H., & Izawa, T. (2013). The coincidence of critical day length recognition for florigen gene expression and floral transition under long-day conditions in rice. *Molecular Plant, 6*(3), 635-649.

Kazan, K., & Lyons, R. (2016). The link between flowering time and stress tolerance. *Journal of Experimental Botany, 67*(1), 47-60.

Kinoshita, A., & Richter, R. (2020). Genetic and molecular basis of floral induction in *Arabidopsis thaliana*. *Journal of Experimental Botany, 71*(9), 2490-2504.

Kumar, A., Choudhary, A., Kaur, N., & Kaur, H. (2021). Wake Up: It's time to bloom. *Russian Journal of Plant Physiology, 68*(4), 579-595.

Lai, D., Fan, Y., Xue, G., He, A., Yang, H., He, C., & Cheng, J. (2022). Genome-wide identification and characterization of the SPL gene family and its expression in the various developmental stages and stress conditions in foxtail millet (*Setaria italica*). *BMC Genomics, 23*(1), 1-20.

Lee, S., & An, G. (2007). Diversified mechanisms for regulating flowering time in a short-day plant rice. *Journal of Plant Biology, 50*(3), 241-248.

Li, L., Xu, J., Yang, D., Tan, X., & Wang, H. (2010). Computational approaches for microRNA studies: A review. *Mammalian Genome, 21*(1), 1-12.

Long, J.-M., Liu, C.-Y., Feng, M.-Q., Liu, Y., Wu, X.-M., & Guo, W.-W. (2018). miR156-SPL modules regulate induction of somatic embryogenesis in citrus callus. *Journal of Experimental Botany, 69*(12), 2979-2993.

MacFarlane, L.-A., & R Murphy, P. (2010). MicroRNA: biogenesis, function and role in cancer. *Current Genomics, 11*(7), 537-561.

Makky, M., Cherie, D., Putri, R., & Hasan, A. (2021). *Chemical compounds identification of Rice cultivars in West Sumatra.* Paper presented at the IOP Conference Series: Earth and Environmental Science.

Manuela, D., & Xu, M. (2020). Juvenile leaves or adult leaves: Determinants for vegetative phase change in flowering plants. *International Journal of Molecular Sciences, 21*(24), 9753.

Nie, S., Xu, L., Wang, Y., Huang, D., Muleke, E. M., Sun, X., & Liu, L. (2015). Identification of bolting-related microRNAs and their targets reveals complex miRNA-mediated flowering-time regulatory networks in radish (*Raphanus sativus* L.). *Scientific Reports, 5*, 14034.

Preston, J. C., & Hileman, L. C. (2010). SQUAMOSA-PROMOTER BINDING PROTEIN 1 initiates flowering in Antirrhinum majus through the activation of meristem identity genes. *The Plant Journal, 62*(4), 704-712.

Rabinowitch, H. D. (2018). Physiology of flowering. In *Onions and allied crops* (pp. 113-134): CRC Press.

Ravoson, H. N., Harinoely, M., Andriamahenina, N. N., Andrianirinamanantsoa, N. N. F., Rasoazanany, E. O., Rakotozafy, L. V., & Raboanary, R. (2019). Quantitative Analysis of Nutrient Minerals in Malagasy Rice Species by EDXRF and Dietary Intake Assessment. *American Journal of Applied Chemistry, 7*(5), 137-144.

Sanan-Mishra, N., Kumar, V., Sopory, S. K., & Mukherjee, S. K. (2009). Cloning and validation of novel miRNA from basmati rice indicates cross talk between abiotic and biotic stresses. *Molecular Genetics and Genomics, 282*(5), 463.

Shafiq, S., Berr, A., & Shen, W. H. (2014). Combinatorial functions of diverse histone methylations in A rabidopsis thaliana flowering time regulation. *New Phytologist, 201*(1), 312-322.

Shi, J. (2022). Machine learning and bioinformatics approaches for classification and clinical detection of bevacizumab responsive glioblastoma subtypes based on miRNA expression. *Scientific Reports, 12*(1), 8685-8685.

Shi, J., Dong, A., & Shen, W.-H. (2015). Epigenetic regulation of rice flowering and reproduction. *Frontiers in Plant Science, 5*, 803.

Shim, J. S., & Jang, G. (2020). Environmental signal-dependent regulation of flowering time in rice. *International Journal of Molecular Sciences, 21*(17), 6155.

Song, Y. H., Ito, S., & Imaizumi, T. (2010). Similarities in the circadian clock and photoperiodism in plants. *Current Opinion in Plant Biology, 13*(5), 594-603.

Sridevi, V., & Chellamuthu, V. (2015). Impact of weather on rice–A review. *International Journal of Applied Research, 1*(9), 825-831.

Srivastava, A. K., Zhang, C., Yates, G., Bailey, M., Brown, A., & Sadanandom, A. (2016). SUMO is a critical regulator of salt stress responses in rice. *Plant Physiology, 170*(4), 2378-2391.

Stief, A., Altmann, S., Hoffmann, K., Pant, B. D., Scheible, W.-R., & Bäurle, I. (2014). Arabidopsis miR156 regulates tolerance to recurring environmental stress through SPL transcription factors. *The Plant Cell, 26*(4), 1792-1807.

Sun, C., Fang, J., Zhao, T., Xu, B., Zhang, F., Liu, L., & Chen, F. (2012). The histone methyltransferase SDG724 mediates H3K36me2/3 deposition at MADS50 and RFT1 and promotes flowering in rice. *The Plant Cell, 24*(8), 3235-3247.

Tanaka, N., Itoh, H., Sentoku, N., Kojima, M., Sakakibara, H., Izawa, T., & Nagato, Y. (2011). The COP1 ortholog PPS regulates the juvenile–adult and vegetative–reproductive phase changes in rice. *The Plant Cell, 23*(6), 2143-2154.

Teotia, S., & Tang, G. (2015). To bloom or not to bloom: role of microRNAs in plant flowering. *Molecular Plant, 8*(3), 359-377.

Todaka, D., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2015). Recent advances in the dissection of drought-stress regulatory networks and strategies for development of drought-tolerant transgenic rice plants. *Frontiers in Plant Science, 6*, 84.

Tsuji, H., Taoka, K.-i., & Shimamoto, K. (2011). Regulation of flowering in rice: two florigen genes, a complex gene network, and natural variation. *Current Opinion in Plant Biology, 14*(1), 45-52.

Vega Rodríguez, A., Rodríguez-Oramas, C., Sanjuán Velázquez, E., Hardisson de la Torre, A., Rubio Armendáriz, C., & Carrascosa Iruzubieta, C. (2022). Myths and realities about genetically modified food: A risk-benefit analysis. *Applied Sciences, 12*(6), 2861.

Verma, D. K., & Srivastav, P. P. (2020). Bioactive compounds of rice (*Oryza sativa* L.): review on paradigm and its potential benefit in human health. *Trends in Food Science & Technology, 97*, 355-365.

Wang, J.-W. (2014). Regulation of flowering time by the miR156-mediated age pathway. *Journal of Experimental Botany, 65*(17), 4723-4730.

Wang, J.-W., Czech, B., & Weigel, D. (2009). miR156-regulated SPL transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. *Cell, 138*(4), 738-749.

Wang, J., Hu, J., Qian, Q., & Xue, H.-W. (2013). LC2 and OsVIL2 promote rice flowering by photoperoid-induced epigenetic silencing of OsLF. *Molecular Plant, 6*(2), 514-527.

Wang, P., Gong, R., Yang, Y., & Yu, S. (2019). Ghd8 controls rice photoperiod sensitivity by forming a complex that interacts with Ghd7. *BMC Plant Biology, 19*(1), 1-14.

Xie, K., Shen, J., Hou, X., Yao, J., Li, X., Xiao, J., & Xiong, L. (2012). Gradual increase of miR156 regulates temporal expression changes of numerous genes during leaf development in rice. *Plant Physiology, 158*(3), 1382-1394.

Xie, Y., Liu, Y., Wang, H., Ma, X., Wang, B., Wu, G., & Wang, H. (2017). Phytochrome-interacting factors directly suppress MIR156 expression to enhance shade-avoidance syndrome in Arabidopsis. *Nature Communications, 8*(1), 1-11.

Xing, H., Li, Y., Ren, Y., Zhao, Y., Wu, X., & Li, H.-L. (2022). Genome-wide investigation of microRNAs and expression profiles during rhizome development in ginger (Zingiber officinale Roscoe). *BMC Genomics, 23*(1), 1-13.

Xu, F.-f., Liang, J., Huang, Y., Chuan, T., CHEN, Y.-l., & BAO, J.-s. (2016). Association mapping of quantitative trait loci for yield-related agronomic traits in rice (*Oryza sativa* L.). *Journal of Integrative Agriculture, 15*(10), 2192-2202.

Yang, Y., Fu, D., Zhu, C., He, Y., Zhang, H., Liu, T., & Wu, C. (2015). The RING-finger ubiquitin ligase HAF1 mediates heading date 1 degradation during photoperiodic flowering in rice. *The Plant Cell, 27*(9), 2455-2468.

Yu, N., Niu, Q. W., Ng, K. H., & Chua, N. H. (2015). The role of miR156/SPL s modules in Arabidopsis lateral root development. *The Plant Journal, 83*(4), 673-685.

Yu, S., Galvão, V. C., Zhang, Y.-C., Horrer, D., Zhang, T.-Q., Hao, Y.-H., & Wang, J.-W. (2012). Gibberellin regulates the Arabidopsis floral transition through miR156-targeted SQUAMOSA PROMOTER BINDING–LIKE transcription factors. *The Plant Cell, 24*(8), 3320-3332.

Zhang, C., Liu, J., Zhao, T., Gomez, A., Li, C., Yu, C., & Liu, B. (2016). A drought-inducible transcription factor delays reproductive timing in rice. *Plant Physiology, 171*(1), 334-343.

Zhao, J., Chen, H., Ren, D., Tang, H., Qiu, R., Feng, J., & Zhong, T. (2015). Genetic interactions between diverged alleles of Early heading date 1 (Ehd1) and Heading date 3a (Hd3a)/RICE FLOWERING LOCUS T1 (RFT 1) control differential heading and contribute to regional adaptation in rice (*Oryza sativa*). *New Phytologist, 208*(3), 936-948.

Zhao, J., Huang, X., Ouyang, X., Chen, W., Du, A., Zhu, L., & Li, S. (2012). OsELF3-1, an ortholog of Arabidopsis early flowering 3, regulates rice circadian rhythm and photoperiodic flowering. *PloS One, 7*(8), e43705.

Zheng, C., Ye, M., Sang, M., & Wu, R. (2019). A regulatory network for miR156-SPL module in Arabidopsis thaliana. *International Journal of Molecular Sciences, 20*(24), 6166.

Zheng, T., Sun, J., Zhou, S., Chen, S., Lu, J., Cui, S., & Zhu, S. (2019). Post-transcriptional regulation of Ghd7 protein stability by phytochrome and Os GI in photoperiodic control of flowering in rice. *New Phytologist, 224*(1), 306-320.

Zhou, S., Zhu, S., Cui, S., Hou, H., Wu, H., Hao, B., & Jiang, L. (2021). Transcriptional and post-transcriptional regulation of heading date in rice. *New Phytologist, 230*(3), 943-956.

Zhu, C., Peng, Q., Fu, D., Zhuang, D., Yu, Y., Duan, M., & Lian, X. (2018). The E3 ubiquitin ligase HAF1 modulates circadian accumulation of EARLY FLOWERING3 to control heading date in rice under long-day conditions. *The Plant Cell, 30*(10), 2352-2367.

Zong, W., Ren, D., Huang, M., Sun, K., Feng, J., Zhao, J., & Zhang, H. (2021). Strong photoperiod sensitivity is controlled by cooperation and competition among Hd1, Ghd7 and DTH8 in rice heading. *New Phytologist, 229*(3), 1635-1649.