The Rice CHD3/Mi-2 Chromatin Remodeling Factor Rolled Fine Striped Promotes Flowering Independent of Photoperiod

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Abstract: Genetic studies have revealed that chromatin modifications affect flowering time, but the underlying mechanisms by which chromatin remodeling factors alter flowering remain largely unknown in rice (Oryza sativa). Here, we show that Rolled Fine Striped (RFS), a chromodomain helicase DNA-binding 3 (CHD3)/Mi-2 subfamily ATP-dependent chromatin remodeling factor, promotes flowering in rice. Diurnal expression of RFS peaked at night under short-day (SD) conditions and at dawn under long-day (LD) conditions. The rfs-1 and rfs-2 mutants (derived from different genetic backgrounds) displayed a late-flowering phenotype under SD and LD conditions. Reverse transcription-quantitative PCR analysis revealed that among the flowering time-related genes, the expression of the major floral repressor Grain number and heading date 7 (Ghd7) was mainly upregulated in rfs mutants, resulting in downregulation of its downstream floral inducers, including Early heading date 1 (Ehd1), Heading date 3a (Hd3a), and Rice FLOWERING LOCUS T 1 (RFT1). The rfs mutation had pleiotropic negative effects on rice grain yield and yield components, such as plant height and fertility. Taking these observations together, we propose that RFS participates in multiple aspects of rice development, including the promotion of flowering independent of photoperiod.

Keywords: Rolled Fine Striped (RFS); flowering time; rice (Oryza sativa); ATP-dependent chromatin remodeling factor; epigenetics; histone methylation

1. Introduction

In rice (Oryza sativa), flowering time is a critical yield-related trait that is important for seasonal and regional adaptation worldwide. The transition from the vegetative to the reproductive phase is promoted by two florigen genes in rice, Heading date 3a (Hd3a) and Rice FLOWERING LOCUS T 1 (RFT1), which are first expressed in the leaves [1,2]. Hd3a and RFT1 act as the major mobile signals, moving to the shoot apical meristem and triggering the transition from vegetative to reproductive growth. Heading date 1 (Hd1) acts as a floral inducer or repressor depending on the length of the photoperiod [3]. Hd1 upregulates the expression of Hd3a and RFT1 under short-day (SD) conditions and downregulates them under long-day (LD) conditions. Early heading date 1 (Ehd1) encodes a B-type response regulator that upregulates the expression of Hd3a and RFT1 [4]. A number of regulators upstream of Ehd1 have been identified. For instance, Grain number and heading date 7 (Ghd7), encoding a CCT domain protein, downregulates Ehd1 expression in LDs [5]. Early heading date 2 (Ehd2)/Oryza sativa Indeterminate 1 (OsID1)/Rice Indeterminate 1 (RID1) promotes flowering by upregulating Ehd1 expression in both SD and LD conditions [6–8]. Early heading date 3 (Ehd3), encoding a PHD finger protein, downregulates Ghd7 expression in LDs and upregulates Ehd1 expression in LD and SD...
conditions [9]. The rice gene CONSTANS-LIKE 4 (OsCOL4) acts as a down-regulator of Ehd1 independent of the photoperiod [10].

Histone methylation is mediated by the histone methyltransferase (HMT) catalytic activity of SET DOMAIN GROUP (SDG) proteins, and the resulting alterations of chromatin structure affect the expression of flowering genes in rice [11]. For instance, methylation of histone H3 lysine 4 (H3K4) or lysine 36 (H3K36) on the chromatin of the flowering-related genes Hd3a, RFT1, and Ehd1 is required for promotion of flowering, but methylation of histone H3 lysine 27 (H3K27) represses the expression of flowering genes. The knockdown of SET DOMAIN GROUP 708 (SDG708) by RNA interference decreases mono-, di-, and trimethylation of histone H3K36 (H3K36me1/me2/me3) on the Hd3a, RFT1, and Ehd1 loci, leading to late flowering [12]. SDG701 trimethylates H3K4 (H3K4me3) in Hd3a and RFT1 chromatin to activate the floral transition [13]. A rice Trithorax group protein, OsTrx1, recruits the WD40 protein OsWDR5a and SDG723/OsTrx1/OsSET33 Interaction Protein 1 (SIPI) to deposit H3K4me3 on the Ehd1 locus [14–16]. SDG724 mediates the deposition of H3K36me2/me3 to upregulate the expression of OsMADS50 and RFT1 [17]. Mutation of SDG725 results in late flowering via a reduction of H3K36me2/me3 marks on several flowering genes including Ehd2, Ehd3, OsMADS50, Hd3a, and RFT1 [18,19]. Polycomb repressive complex 2 (PRC2) downregulates the expression of two genes encoding flowering repressors, rice Late Flowering (OsLF) and LEAFY COTYLEDON 2 and FLUSA3 3-LIKE 1 (OslFL1), via trimethylation of histone H3 lysine 27 (H3K27me3) [20–22].

In addition to epigenetic regulation of SDG proteins, ATP-dependent chromatin remodeling factors are also involved in altering chromatin structure to modulate flowering time. In Arabidopsis thaliana, the putative sucrose non-fermenting (SNF2)-like ATPase subunit PICKLE (PKL) plays roles in the floral transition by regulating the expression of the floral meristem identity gene LEAFY (LFY) and gibberellic acid (GA)-regulated genes [23,24]. PKL physically interacts with CONSTANS (CO) to facilitate binding to FLOWERING LOCUS T (FT) chromatin. PKL mediates deposition of H3K4me3 on the FT locus by associating with ARABIDOPSIS HOMOLOG OF TRITHORAX 1 (ATX1) to antagonize Polycomb group (PcG)-mediated repression of FT [25,26]. CHROMATIN REMODELING 4 (CHR4) interacts with transcription factors involved in floral meristem identity to regulate the expression of key floral regulators [27].

In rice, Rolled Fine Striped (RFS), which encodes a chromodomain helicase DNA-binding 3 (CHD3)/Mi-2 chromatin-remodeling factor, is involved in various aspects of rice development such as crown root development, seedling development, and leaf morphogenesis [28–30]. Genome-wide analysis revealed that the rfs mutant shows a reduction of H3K4me3 and H3K27me3 [31]. These results demonstrate that RFS can modulate both active and repressive epigenetic marks on histones. Our previous report showed that RFS controls reactive oxygen species (ROS) homeostasis by modulating H3K4me3 levels on ROS-related genes [32].

Here, we found that two rfs mutants (rfs-1 and rfs-2) derived from different genetic backgrounds exhibited late flowering independent of the photoperiod, implying that RFS is involved in flowering-time regulation in rice. Although numerous genes that control rice flowering have been reported, few epigenetic regulator that modulate flowering has not been identified in rice. To reveal the regulatory function of RFS in rice flowering, we analyzed the expression and histone methylation levels of flowering-time genes in rfs-2 mutant. Reverse transcription-quantitative PCR (RT-qPCR) showed that RFS promotes flowering by downregulating Ghd7 in LDs and upregulating Ehd1 in SD and LD conditions. Our results suggest a new molecular function of RFS as a floral regulator in rice.

2. Results
2.1. The rfs Mutants Exhibit a Late Flowering Phenotype Independent of the Photoperiod

To elucidate the involvement of RFS in flowering-time regulation in rice, we investigated the flowering time of two rfs mutants [32] under different photoperiod conditions. The rfs-1 mutant (which was isolated from a gamma-ray mutagenesis) flowered around 8 d
later than its parental *japonica* cultivar ‘Seolak’ (SL), which flowered at 92 days after sowing (DAS) under natural long-day (NLD) conditions in the field (37° N latitude, Suwon, Korea). In the growth chambers, the *rfs-1* mutant flowered around 8 d and 11 d later than SL under SD (10 h light/14 h dark), and LD (14.5-h light/9.5-h dark) conditions, respectively (Figure 1a,c).

Figure 1. Mutation of *RFS* results in delayed rice flowering independent of the photoperiod. (a,b) Flowering phenotypes of the *rfs-1* (a) and *rfs-2* (b) mutants were compared with their parental *japonica* cultivars ‘Seolak’ (SL), and ‘Hwayoung’ (HY), respectively. Rice plants were grown in the paddy field under natural long-day (NLD) (~14 h light/day) conditions until the parental lines flowered. White scale bars = 10 cm. (c,d) Days to flowering of the *rfs-1* (c) and *rfs-2* (d) mutants and their parental lines (SL and HY) were determined in short-day (SD) (10 h light/day), NLD, and long-day (LD) (14.5 h light/day) conditions. (e,f) Comparison of leaf emergence rates between HY and *rfs-2* plants under SD (e) and LD (f) conditions during plant development. The leaf emergence rate was scored according to the method of Itoh et al. (1998). Red arrows indicate the average flowering date of HY. Means and standard deviations were obtained from 20 plants (c,d) and 10 plants (e,f) of each genotype.

To further confirm whether mutation of *RFS* delays rice flowering, we observed the flowering time of the *rfs-2* knockout mutant, which harbors a T-DNA fragment in the 8th
intron of RFS [32]. Similar to the rfs-1 mutant, the flowering time of the rfs-2 mutant was delayed by 19 d compared with its parental japonica cultivar ‘Hwayoung’ (HY), which flowered at 102 DAS. In addition, the rfs-2 mutant flowered around 15 d and 21 d later than HY under SD, and LD conditions, respectively (Figure 1b,d). These observations suggest that RFS is involved in the promotion of flowering, independent of photoperiod.

To test whether growth retardation causes the late flowering in rfs mutants, we measured the number of emerged leaves in HY and rfs-2 plants under SD and LD conditions [33]. The leaf emergence rates of the rfs-2 mutant were indistinguishable from those of HY under both conditions (Figure 1e,f). Therefore, the late flowering of rfs mutants was mainly due to a delayed floral transition, not to growth retardation or prolonged plastochron.

2.2. Expression Pattern of RFS

The RFS transcript levels were measured every 2 weeks from 4 to 14 weeks after sowing (WAS) at zeitgeber time (ZT) 1 by RT-qPCR analysis. RFS was constitutively expressed during plant growth, implying that RFS has roles throughout plant development (Figure 2a). We also analyzed the diurnal expression of RFS under SD and LD conditions. The leaf blades of HY and rfs-2 plants were harvested every 3 h during a 24-h period at 25 DAS under SD conditions and at 80 DAS under LD conditions; these timepoints were approximately 30 days before flowering in HY. RFS transcripts were not detectable in rfs-2 leaf blades, but in HY, RFS showed diurnal expression patterns with a peak at night under SD conditions and at dawn under LD conditions (Figure 2b,c).

2.3. Expression Patterns of Hd3a and RFT1 in the rfs Mutants

We monitored the transcript levels of two rice florigen genes (Hd3a and RFT1) in developing leaves of HY and rfs-2 plants harvested at ZT1 throughout the vegetative stages, until the HY plants flowered. Hd3a and RFT1 transcripts gradually increased in HY with a peak at 8 WAS under SD and 15 WAS under LD conditions to trigger the transition to reproductive growth (Figure 3a–d) [34,35]. However, the expression of Hd3a and RFT1 remained low in the rfs-2 mutant throughout the experiment. The expression of Ehd1 was similar to that of the florigen genes in HY and rfs-2 plants (Figure 3e,f). These results suggest that mutation of RFS downregulates the expression of flowering genes, leading to late flowering in rice.

2.4. Expression Analysis of Flowering-Time Genes in the rfs Mutants

To reveal the regulatory effect of RFS on flowering pathways in rice, we examined diurnal changes of the expression of flowering-time genes in the leaf blades of HY and rfs-2 plants that were harvested during diurnal cycles, as shown in Figure 2b,c. The expression of Hd3a and RFT1 was mostly suppressed in the rfs-2 mutant, unlike the diurnal expression in HY under both SD and LD conditions (Figure 4a–d). Next, we measured the expression levels of Ehd1 and Hd1, which encode upstream regulators of Hd3a and RFT1 [3,4]. The expression level of Hd1 in the rfs-2 mutant was similar to that in HY under both photoperiods (Figure 4e,f). By contrast, Ehd1 transcript levels were consistently lower in rfs-2 leaves compared to HY at ZT21 and ZT0 in SD and LD conditions, respectively (Figure 4g,h).

Previous genetic studies have revealed that diurnal expression of Ehd1 is delicately regulated by various upstream regulators. We therefore examined the transcript levels of genes encoding upstream regulators of Ehd1: OsGIGANTEA (OsGI) [36], Ghd7 [5], Ehd2 [6–8], Ehd3 [9], and rice FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (OsFKF1) [37]. The expression of Ghd7, a negative regulator of Ehd1, was higher in the rfs-2 mutant than in HY under LDs (Figure 4i,j). However, the transcript levels of positive regulators of Ehd1, including OsGI, Ehd2, Ehd3, and OsFKF1, were not altered in either photoperiod condition (Figure 4k–r). In addition, the expression of rice EARLY FLOWERING 3 (OsELF3), a repressor of Ghd7, did not show any differences between HY and the rfs-2 mutant (Figure 4s,t). These results suggest that RFS can repress the expression of Ghd7 in LDs.
and activate the expression of *Ehd1* in both SD and LD conditions, thereby upregulating florigen genes in rice.

**Figure 2.** Expression profile of *RFS*. (a) Expression levels of *RFS* during plant development in HY under LD conditions. Total RNA was isolated from the leaves harvested every 2 weeks from 4 weeks after sowing (WAS) to 14 WAS at ZT1. The red arrow indicates the average flowering date of HY. (b,c) Diurnal change of *RFS* expression in HY and *rfs-2* plants under SD (b) and LD (c) conditions. Total RNA was extracted from HY and *rfs-2* leaf blades harvested every 3 h during a 24-h period from plants at 25 days after sowing (DAS) in SDs and 80 DAS in LDs. The open and filled bars at the top of graphs represent light and dark periods, respectively. Transcript levels of *RFS* were determined by RT-qPCR and normalized to that of *OsUBQ5* (*LOC_Os01g22490*). Means and standard deviations were obtained from three biological replicates. Experiments were repeated three times with similar results. ZT, zeitgeber time (hours after dawn).
We further investigated whether RFS affects the expression of other epigenetic regulators including SDG701 [13], SDG708 [12], OsTrx1 [14–16], SDG724 [17], SDG725 [18,19], and rice VIN3-LIKE 2 (OsVIL2) [20,21]. These genes encode regulators that mediate histone methylation to promote rice flowering. The RT-qPCR analysis was performed on HY and rfs-2 leaf blades sampled at ZT0 at 4 WAS and at ZT12 at 8 WAS in SD and LD conditions, respectively. There were no significant differences in the transcript levels of these genes between HY and rfs-2 plants in either photoperiod condition (Figure S1).

2.5. Histone Methylation Levels of Ehd1 and Ghd7 in the rfs Mutants

A previous study reported that the rfs mutant showed global reductions of histone H3K4me3 and H3K27me3 by about 56% and 23%, respectively [31]. Therefore, we conducted a chromatin immunoprecipitation (ChIP) assay to determine whether RFS modifies the histone methylation level of flowering-time genes. The leaf blades of HY and rfs-2 plants were collected at ZT1 at 9 WAS under LD conditions in the growth chamber. We speculated that downregulation of Ehd1 in the rfs-2 mutant might be caused by reduced levels of the activating mark H3K4me3 and upregulation of Ghd7 in the rfs-2 mutant might be caused by reduced levels of the repressive mark H3K27me3.
Similar results. ZT, zeitgeber time (hours after dawn). Means and standard deviations were obtained from three biological replicates. The experiments were repeated three times with similar results. ZT, zeitgeber time (hours after dawn).

Diurnal expression of flowering genes in the rfs-2 mutant. Rice plants were grown for 25 days after sowing (DAS) in SDs (a,c,e,g,i,k,m,o,q,s) and 80 DAS in LDs (b,d,f,h,j,l,n,p,r,t). Total RNA was isolated from the leaf blades collected every 3 h during a 24-h period. Transcript levels of *Hd3a* (a,b), *RFT1* (c,d), *Hd1* (e,f), *OsGl* (g,h), *Ehd1* (i,j), *Ghd7* (k,l), *Ehd2* (m,n), *Ehd3* (o,p), *OsFKF1* (q,r), and *OsELF3* (s,t) were determined by RT-qPCR and normalized to that of *OsUBQ5* (LOC_Os01g22490). The open and filled bars at the top of graphs represent light and dark periods, respectively. Means and standard deviations were obtained from three biological replicates. The experiments were repeated three times with similar results. ZT, zeitgeber time (hours after dawn).

To test these hypotheses, we examined the enrichment of modified histone proteins on *Ehd1* and *Ghd7* chromatin using specific antibodies against H3K4me3 and H3K27me3. We used *Hd3a* and *OsLF* as positive controls for methylated histone-enriched loci. SDG701 deposits H3K4me3 on the *Hd3a* locus to activate *Hd3a* transcription [13]. The PRC2 complex targets the *OsLF* locus and deposits H3K27me3 to repress *OsLF* transcription [20,22]. We found that while H3K4me3 was highly enriched in the promoter region of *Ehd1* compared to the intergenic region, the H3K4me3 level in *Ehd1* did not differ between HY and the rfs-2 mutant (Figure 5a,b). H3K27me3 in *Ghd7* was higher around the transcription start site than in the intergenic region. However, there was no significant difference in enrichment of H3K27me3 at the *Ghd7* locus between HY and the rfs-2 mutant (Figure 5c,d).
Histone modification patterns of differentially expressed genes in the rfs-2 mutant. (a,c) Schematic representation of Ehd1 (a) and Ghd7 (c) loci. White and black boxes indicate untranslated regions and exons, respectively. Thick and thin black bars represent promoter and intron regions, respectively. Short red lines with numbers (1–9 for Ehd1 and 1–7 for Ghd7) represent genomic DNA regions eluted from the protein–DNA complexes. The primers used for qPCR analysis of fragments are listed in Supplemental Table S1. Black scale bars = 1 kb. (b,d) Chromatin immunoprecipitation (ChIP) analysis of the H3K4me3 level on Ehd1 (b) and the H3K27me3 level on Ghd7 (d) in the 9-week-old HY and rfs-2 plants grown in LD conditions at ZT1. Hd3a and OsLF were used as positive controls for the H3K4me3-enriched locus and the H3K27me3-enriched locus, respectively [18,25,27]. Means and standard deviations were obtained from three biological replicates. Experiments were repeated three times with similar results.

2.6. Agronomic Traits of the rfs Mutants

To identify whether the rfs mutation affects grain yield and yield components, we evaluated agronomic traits, including plant height, number of panicles per plant, length of panicles, number of panicle branches per panicle, number of grains per panicle, spikelet fertility, 500-grain weight, and yield per plant, in HY and rfs-2 plants grown in the paddy field under NLD conditions [38]. Plant height and panicle length of the rfs-2 mutant were shorter than those of HY (Figure 6a,b). The rfs-2 mutant had more panicles per plant compared to HY, but had fewer primary and secondary branches per panicle (Figure 6c–e,j). In addition, the number of grains per panicle, spikelet fertility, and 500-grain weight were significantly lower in the rfs-2 mutant compared to HY (Figure 6f–h). The reduction of spikelet fertility in the rfs-2 mutant might be due to delayed flowering, which results in later grain filling, when conditions tend to be unfavorable due to lower temperatures. Consequently, total grain yield was lower in the rfs-2 mutant due to the reduction of yield components, including spikelet fertility, grain number, and 500-grain weight, although the mutant plants had more panicles per plant compared with HY (Figure 6i).
3. Discussion

3.1. Regulatory Roles of RFS in Flowering-Time Pathways

Studies of various mutant alleles of RFS have revealed that RFS functions in multiple aspects of plant development, including crown root development, seedling development, and leaf morphogenesis [28–30]. In this study, we found that RFS also affects rice flowering time. Mutation of RFS delayed flowering under SD and LD conditions (Figure 1). Moreover, expression of two rice florigen genes, Hd3a and RFT1, maintained at low levels in rfs-2 mutant whereas their transcript levels were elevated in HY throughout plant growth (Figure 3). In HY, RFS expression showed a diurnal rhythm with a peak at ZT15 and ZT0 under SD, and LD conditions, respectively (Figure 2b,c). The expression of Ehd1 subsequently peaked at ZT18 in SDs and at ZT0 in LDs (Figure 4g,h). Finally, Hd3a and RFT1 transcript levels were strongly upregulated at ZT21 and ZT3 under SD and LD conditions, respectively (Figure 4a–d). Our findings strongly suggest that RFS is a floral inducer in rice.

Mutation of RFS decreased the expression of the florigen genes Hd3a and RFT1, which are rice orthologs of Arabidopsis FT, under SD and LD conditions (Figure 4a–d). In Arabidopsis, the CHD3 protein PKL promotes flowering through activation of the FT locus, suggesting that CHD3 chromatin-remodeling factors have conserved functions in flowering plants. However, among the upstream regulators of Hd3a and RFT1, there were no differences in OsGI and Hd1 expression between HY and rfs-2 plants (Figure 4e,f,k,l), but the transcript levels of Ghd7 and Ehd1 in the rfs-2 mutant differed from those in HY

Figure 6. Agronomic traits assessed in the rfs-2 mutant. Nine agronomic traits were examined and compared in the HY and rfs-2 plants after harvest in the autumn. Plant height (a), panicle length (b), number of panicles per plant (c), number of primary branches per panicle (d), number of secondary branches per panicle (e), number of grains per panicle (f), spikelet fertility (g), 500-grain weight (h), yield per plant (i), and phenotype of panicles (j). Values are shown as means (n = 5) and error bars indicate standard deviation. Differences between means were compared using two-tailed Student's t-tests (*p < 0.05, **p < 0.01, ***p < 0.001).
These observations implied that RFS does not participate in the OsGI–Hd1–Hd3a regulatory pathway, which is similar to the Arabidopsis GI–CO–FT module. Instead, RFS may regulate flowering time through rice-specific flowering pathways involving Ghd7 and Ehd1 (Figure 7). Although CHD3 chromatin remodeling factors are involved in the regulation of flowering in rice and Arabidopsis, the regulatory pathways might differ between the two species.

Figure 7. Proposed model of the flowering regulatory pathway controlled by RFS. RFS downregulates Ghd7 in LDs and upregulates Ehd1 in LD and SD conditions to induce the floral transition. Arrows represent upregulation, and lines ending with bars represent downregulation. Solid and dashed lines indicate direct and indirect regulation, respectively.

3.2. RFS May Control Expression of Flowering-Time Genes via Histone Modification

In Arabidopsis, PKL mediates deposition of H3K27me3 to repress target genes [39–42], and mutation of CHR4 changes H3K27me3 and H3K4me3 levels [27]. Moreover, RFS is responsible for modification of H3K27me3 and H3K4me3 to control gene expression [31,32,43]. We therefore speculated that RFS controls the expression of Ghd7 and Ehd1 through H3K27me3, and H3K4me3, respectively. However, our ChIP analysis showed no apparent differences in H3K4me3 levels of Ehd1 or H3K27me3 levels of Ghd7 between HY and the rfs-2 mutant (Figure 5). One possible explanation for this is that RFS regulates Ehd1 and Ghd7 via other types of modifications, such as histone acetylation. In mammalian cells, CHD3 is a component of the nucleosome remodeling and deacetylase (NURD) complex, which is associated with histone deacetylation, resulting in transcriptional repression [44,45]. In addition, RFS might control an unidentified upstream regulator of Ehd1 and Ghd7. A previous study proposed that OsTrx1 may be an upstream regulator of Ghd7 [14]. In the absence of OsTrx1, which activates target genes by adding the H3K4me3 mark, the expression of Ghd7 increased, suggesting that Ghd7 expression might be indirectly controlled by OsTrx1. Therefore, further analysis and exploration will be required to elucidate the involvement of RFS in flowering.

ATP-dependent chromatin remodeling factors act as multimeric complexes in various organisms. In this study, we could not observe significant alteration in transcript levels of other epigenetic regulators that promote rice flowering, including SDG701 [13], SDG708 [12], OsTrx1 [14–16], SDG724 [17], SDG725 [18,19], and OsVIL2 [20,21] between HY and rfs-2 (Figure S1). These results suggest that RFS is not directly involved in the transcriptional regulation of these epigenetic regulators. Therefore, we could not exclude the possi-
bility that RFS recruits these regulators to modify the histone methylation of flowering-time genes. Recent biochemical approaches using immunoprecipitation and mass spectrometry have identified the components of ISWI and SWI/SNF chromatin remodeling complexes in plants [46,47]. In Arabidopsis, PKL and CHR4 recruit several transcription factors and cofactors for proper epigenetic regulation during development [25–27,48]. In rice, the components of complexes that associate with CHD3 have not yet been elucidated. Therefore, it will be interesting to find the components that physically interact with RFS during rice development, including flowering, crown root development, seedling development, and leaf morphogenesis.

3.3. RFS Is Involved in Inflorescence Development

CHR4 plays diverse roles in the inflorescence meristem to promote flowering in Arabidopsis [27]. CHR4-deficient plants show an accelerated transition from the vegetative phase to bolting, but a delay in the formation of floral primordia. CHR4 interacts with MADS, SQUAMOSA promoter-binding protein-like (SPL), and APETALA2 (AP2)-type transcription factors, which regulate the floral transition and floral meristem identity [24,49]. In addition, RNA-sequencing and ChIP sequencing have revealed that CHR4 mediates the response to endogenous flowering pathways in the inflorescence meristem by controlling the expression of floral regulators [27]. In rice, overexpression of ABERRANT PANICLE ORGANIZATION 1 (APO1), which encodes an F-box protein, causes a precocious transition of the inflorescence meristem to the spikelet meristem, reducing the number of panicle branches [50]. The rfs-2 mutant had significantly decreased grain yield compared to HY (Figure 6i). Based on these findings, we speculated that RFS might control other developmental processes, as well as flowering. Among the agronomic traits that affect grain yield, the number of secondary panicle branches was markedly reduced in the rfs-2 mutant (Figure 6e), implicating RFS in inflorescence meristem fate. Because the inflorescence architecture of rice is closely related to yield, the role of RFS in the transition from the vegetative meristem to the inflorescence meristem will be an interesting topic for future studies.

4. Materials and Methods

4.1. Plant Materials and Growth Conditions

The rfs-1 and rfs-2 mutants used in this study were reported previously [32]. Rice plants were grown in the paddy field under NLD conditions (≥14 h sunlight/day, 37° N latitude) in Suwon, Republic of Korea. To perform experiments under controlled day-length conditions, rice plants were grown in growth chambers under LD (14.5-h light and 9.5-h dark) or SD (10-h light and 14-h dark) conditions at 30 °C in the light and 24 °C in the dark.

4.2. RNA Extraction and Reverse Transcription-Quantitative PCR (RT-qPCR) Analysis

Total RNA was isolated using an RNA extraction kit (MG Med, Seoul, Korea) according to the manufacturer’s manual. After RNA quantification, cDNA was synthesized from 2 µg of total RNA by M-MLV reverse transcriptase (Promega, Madison, WI, USA) at 42 °C for 1 h after priming with oligo(dT) (Promega, Madison, WI, USA) at 70 °C for 5 min. Prepared cDNAs were diluted to 100 µL with distilled water and then used as templates for RT-qPCR. RT-qPCR was performed with a LightCycler 480 (Roche, Basel, Switzerland) using 2× GoTaq master mix (Promega, Madison, WI, USA) in a 20-µL reaction volume. Rice UBIQUITIN 5 (LOC_Os01g22490) served as an internal control for relative quantification. The primer sequences used for RT-qPCR are listed in Supplemental Table S1.

4.3. Chromatin Immunoprecipitation (ChIP) Assay

ChIP was performed as previously described [51]. Leaf blades of 63-d-old plants grown in the LD growth chamber were harvested at ZT1. One gram of leaves was crosslinked in 1% formaldehyde under vacuum. After isolation of nuclei, chromatin was sheared into 500–1000 bp in length by sonication and then immunoprecipitated with
anti-H3K4me3 (Millipore, Temecula, CA, USA) or anti-H3K27me3 (Millipore, Temecula, CA, USA) antibodies. The immunoprecipitated products and 5% of input chromatin were reverse-crosslinked at 65 °C and eluted with QIAquick PCR purification kit (Qiagen, Hilden, Germany). Finally, the precipitated DNA was quantified by qPCR with the primers listed in Supplemental Table S1.

4.4. Measurement of Agronomic Traits

To investigate agronomic traits, HY and rfs-2 plants were grown in the paddy field under NLD conditions. Plant height was measured just after heading. The other traits, such as panicle length, the number of panicle branches, the number of grains, fertility, 500-grain weight, and yield per plant, were examined after harvest. The panicles of main tillers were used to analyze panicle length, the number of panicle branches, and the number of grains.

5. Conclusions

In this study, we show that a CHD3/Mi-2 chromatin remodeling factor, RFS, is involved in the regulation of flowering induction in rice. RFS loss-of-function mutants exhibited a late-flowering phenotype under SD and LD conditions. RFS promotes the expression of two rice florigen genes, Hd3a and RFT1, through downregulation of Ghd7 and upregulation of Ehd1, thereby promoting floral induction. This study, thus, provides evidence that a chromatin remodeling factor plays crucial roles in rice flowering.

Supplementary Materials: Supplementary materials can be found at https://www.mdpi.com/1422-0067/22/3/1303/s1, Figure S1. Expression level of epigenetic regulators of rice flowering in the rfs-2 mutant; Table S1. List of primers used in this study.

Author Contributions: K.K., S.-C.Y. and N.-C.P. designed and supervised the research. H.Y. and Y.S. performed the experiments and analyzed data. H.Y., S.-C.Y., K.K., and N.-C.P. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

RFS Rolled Fine Striped
SD Short day
LD Long day
NLD Natural long day
H3K4me3 Trimethylation of histone H3 lysine 4
H3K27me3 Trimethylation of histone H3 lysine 27
SL Seolak
HY Hwayoung
ZT Zeitgeber time
DAS Days after sowing
RT-qPCR Reverse transcription quantitative PCR
ChIP Chromatin Immunoprecipitation
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