

Plant Communications, Volume 4

Supplemental information

Environmental control of rice flowering time

Giulio Vicentini, Marco Biancucci, Lorenzo Mineri, Daniele Chirivì, Francesca Giaume, Yiling Miao, Junko Kyo-zuka, Vittoria Brambilla, Camilla Betti, and Fabio Fornara

Supplementary Table 1. List of rice flowering time genes. The list includes a brief description of the function of genes not discussed in the main text. References used to elaborate the description are also indicated. Genes are listed in alphabetical order.

Name	Description	Source
Ehd2/RID1/OsID1 - Os10g0419200 SID1/OsIDD4 - Os02g0672100	Ehd2 is an IDD domain containing transcription factor. <i>SID1</i> encodes for a zinc finger transcription factor. <i>Ehd2</i> promotes flowering through <i>Ehd1</i> both in LD and SD. Knockout mutants of <i>Ehd2</i> never flower, but if <i>SID1</i> is overexpressed, then flowering is recovered. However, overexpression of <i>Ehd1</i> in the <i>ehd2</i> mutant background does not restore the never-flowering phenotype. In a WT background <i>SID1</i> overexpressors show no phenotypes. Both Ehd2 and SID1 can bind to the same location in florigens promoters.	(Matsubara et al., 2008; Park et al., 2008; Cai et al., 2014; Deng et al., 2017)
Ehd3 - Os08g0105000	PHD finger motifs containing protein expressed in developing leaves and in the shoot apex. It promotes flowering both in SD and LD. In SD, it promotes <i>Ehd1</i> expression while in LD it is a key repressor of <i>Ghd7</i> . Knockout of <i>Ehd3</i> prevents flowering in LD due to constitutive high levels of <i>Ghd7</i> .	(Matsubara et al., 2011)
Ehd4 - Os03g0112700	CCCH-type zinc finger protein. Its knockout mutant is late flowering both in LD and SD, with null expression of <i>Ehd1</i> and the florigens. The effect of Ehd4 on <i>Ehd1</i> transcription is indirect.	(Gao et al., 2013)
EL1/CK1 - Os03g0793500	The <i>el1</i> loss of function mutant is early flowering only in LD. It represses <i>Ehd1</i> possibly by activating Ghd7 protein and maybe other flowering repressors via phosphorylation.	(Hori et al., 2013)
HGW - Os06g0160400	UBA domain protein involved in ubiquitination. It promotes flowering under field conditions by promoting <i>OsGI</i> , <i>Hd1</i> and <i>Hd3a</i> expression.	(Li et al., 2012)
OsCONSTANS-like genes	Many CONSTANS-like genes have been characterized having effects on flowering time and including <i>OsCO3</i> , <i>OsCOL10</i> , <i>OsCOL13</i> , <i>OsCOL15</i> and <i>OsCOL16</i> . All have been shown to delay flowering when overexpressed both in LD and SD by repressing <i>Ehd1</i> . Frequently, knockout mutants don't have phenotypes, possibly because of redundancy among them. The <i>oscol4</i> knockout shows early flowering in both LD and SD and increased levels of <i>Ehd1</i> transcription. Expression of <i>OsCOL4</i> is high during vegetative growth but it progressively reduces over time, permitting <i>Ehd1</i> mRNA levels to rise. <i>OsCOL4</i> overexpression leads to late flowering in both photoperiods.	(Kim et al., 2008; Lee et al., 2016; Lee et al., 2010; Tan et al., 2016; Sheng et al., 2016; Tan et al., 2017; Wu et al., 2017; Wu et al., 2018)
OsDOF12 - Os03g0169600	<i>OsDOF12</i> encodes for a zinc finger transcriptional activator. Its overexpression leads to higher <i>Hd3a</i> levels in LD, causing early flowering.	(Li et al., 2009)
OsELF3 - Os01g0566100	Knockout mutants are late flowering under LD. Its overexpressor are early flowering in LD but late flowering in SD. It acts by inhibiting <i>OsGI</i> , that in turn results in higher amounts of <i>Hd1</i> in the <i>oself3</i> mutant. OsELF3 can also inhibit the red light-induced expression of Ghd7.	(Saito et al., 2012; Zhao et al., 2012; Yang et al., 2013a)
OsEMF2b - Os09g0306800	<i>OsEMF2b</i> encodes for a zinc finger protein homolog of Arabidopsis EMF2, which is part of a polycomb complex acting to silence gene expression by histone methylation. The <i>OsEMF2b</i> loss of function mutant is late flowering both in LD	(Xie et al., 2015)

	and SD. OsEMF2b is responsible for methylating OsLFL1, leading to its silencing and thus promoting flowering.	
OsFKF1 - Os11g0547000	Homolog of FKF1 and encoding for a blue light photoreceptor with F-box domain typical of E3 ubiquitin ligase. The knockout is late flowering both in LD and SD. OsFKF1 promotes flowering by inducing <i>Ehd2</i> expression, that results in <i>Ghd7</i> inhibition and finally an activation of the <i>Ehd1</i> pathway.	(Han et al., 2015)
OsLFL1 - Os01g0713600	The overexpressor is late flowering because OsLFL1 can bind to the <i>Ehd1</i> promoter to prevent its expression. It can also bind to the <i>OsMFT1</i> promoter to activate its expression.	(Peng et al., 2007; Peng et al., 2008; Song et al., 2018)
OsMADS50 – Os03g0122600 OsMADS56 – Os10g0536100	Knockout mutants of <i>OsMADS50</i> are late flowering only in LD while knockout mutants of <i>OsMADS56</i> have no phenotype; however, <i>OsMADS56</i> overexpressors are late flowering only in LD. So <i>OsMADS50</i> and <i>OsMADS56</i> are specific LD flowering promoter and repressor respectively. Both <i>osmads50</i> mutant and <i>OsMADS56</i> overexpressor show upregulation of <i>LFL1</i> , explaining their phenotype. They can form homodimers or heterodimers between each other.	(Ryu et al., 2009)
OsMADS51 - Os01g0922800	Acts as a flowering promoter in SD by activating the <i>Ehd1</i> pathway. <i>OsMADS51</i> expression is promoted by <i>OsGI</i> .	(Kim et al., 2007)
OsMFT1 - Os06g0498800	Belongs to the family of phosphatidylethanolamine-binding proteins. In LD the overexpressor is late flowering while the knockout mutant is slightly early. Its expression is positively affected by <i>Ghd7</i> , and <i>OsMFT1</i> acts by reducing <i>Ehd1</i> expression.	(Song et al., 2018)
OsNF-YB9 - Os06g0285200 OsNF-YC12 - Os05g0304800	OsNF-YB9 and OsNF-YC12 are subunit of a heterotrimeric transcription factor. These 2 subunits interact together and each one is also able to bind <i>OsGI</i> . Their overexpressors are late flowering.	(Das et al., 2019)
OsVIL2 - Os02g0152500	The knockout mutant is late flowering in both LD and SD. <i>OsVIL2</i> seems to positively regulate <i>Ehd1</i> expression by reducing transcription of the flowering inhibitor <i>OsLFL1</i> .	(Yang et al., 2013b)
OsVIL3 - Os05g0145400	The <i>osvil3</i> mutant is late flowering only in SD. <i>OsVIL3</i> can bind to the promoter of <i>OsLF</i> (a minor flowering repressor) to reduce its expression.	(Wang et al., 2013)
RCN1 - Os03g0281900 RCN2 - Os02g0531600	Transcription factors homologs of TFL1/CEN. <i>RCN1</i> is expressed in developing panicles; <i>RCN2</i> both in vegetative and reproductive meristems. Overexpressors show delayed heading and altered panicle morphology, sometimes plants are incapable of concluding panicle development.	(Nakagawa et al., 2002)
RFL - Os04g0598300	Transcription factor homolog to LFY. It promotes flowering by increasing <i>OsMADS50</i> expression. It is also fundamental for a correct panicle architecture.	(Rao et al., 2008)
SDG724 – Os09g0307800	<i>SDG724</i> encodes for a histone methyltransferase which promotes flowering in LD. The loss-of-function mutant flowers late in LD and with only a slight flowering delay in SD. <i>SDG724</i> promotes H3K36me2/3 methylation of <i>OsMADS50</i> and <i>RFT1</i> , and enhances the expression of both florigens.	(Sun et al., 2012)
SDG725 - Os02g0554000 MRG702 – Os11g0545600	<i>SDG725</i> encodes for a histone methyltransferase. Knockdown plants show brassinosteroid-deficiency phenotypes, and are late flowering both in LD and SD due to altered methylation in many flowering related genes which leads to increased	(Sui et al., 2012; Sui et al., 2013; Jin et al., 2015)

	expression of the florigens. MRG702 encodes for a reader protein of H3K4me3 and H3K36me3. Knock-down plants show brassinosteroids-deficiency phenotypes and late flowering phenotypes, just as <i>SDG725</i> knock-downs. Indeed, MRG702 is able to bind DNA methylated by SDG725 and promote expression of flowering promoters, even though the precise mechanism is still unclear.	
Se14 - Os03g0151300	It encodes an H3K4 demethylase. It represses flowering in LD by inhibiting <i>Ehd1</i> and <i>RFT1</i> expression. The effect on <i>RFT1</i> is due to the demethylation activity on the <i>RFT1</i> promoter and gene, while the effect on <i>Ehd1</i> is indirect.	(Yokoo et al., 2014)
miR172 SNB - Os07g0235800 OsIDS1 - Os03g0818800	<i>miR172</i> is a flowering promoter inhibited by PHYB. It targets the two AP2-like genes <i>SNB</i> and <i>OsIDS1</i> . These genes are flowering repressors positively regulated by <i>Hd1</i> and <i>OsCOL4</i> . Overexpressors of <i>SNB</i> and <i>OsIDS1</i> have decreased <i>Ehd1</i> expression both in LD and SD. Expression of <i>miR172</i> increase with the age of the plant, facilitating the repression of <i>SNB</i> and <i>OsIDS1</i> with aging.	(Lee et al., 2014)
SPIN1 - Os03g0815700 SPL11 - Os12g0570000	SPIN1 is an E3-ubiquitin ligase involved in the degradation of the flowering inhibitor SPL11, which suppress <i>Hd3a</i> expression.	(Vega-Sánchez et al., 2008)
OsTrx1 - Os09g0134500 SIP1 - Os09g0560900	OsTrx1 is a histone methyltransferase expressed mainly in leaf blades. It promotes flowering in LD by promoting <i>Ehd1</i> expression. SIP1 is a C2H2 zinc finger transcription factor which can bind to OsTrx1 and to the <i>Ehd1</i> promoter, effectively directing the action of OsTrx1 to the <i>Ehd1</i> locus.	(Choi et al., 2014; Jiang et al., 2018)

References

- Cai, Y., Chen, X., Xie, K., Xing, Q., Wu, Y., Li, J., Du, C., Sun, Z., and Guo, Z. (2014). Dlf1, a WRKY Transcription Factor, Is Involved in the Control of Flowering Time and Plant Height in Rice. *PLoS One* 9:e102529.
- Choi, S. C., Lee, S., Kim, S. R., Lee, Y. S., Liu, C., Cao, X., and An, G. (2014). Trithorax group protein *Oryza sativa* trithorax1 controls flowering time in rice via interaction with early heading date3. *Plant Physiol.* 164:1326–1337.
- Das, S., Parida, S. K., Agarwal, P., and Tyagi, A. K. (2019). Transcription factor OsNF-YB9 regulates reproductive growth and development in rice. *Planta* 250:1849–1865.
- Deng, L., Li, L., Zhang, S., Shen, J., Li, S., Hu, S., Peng, Q., Xiao, J., and Wu, C. (2017). Suppressor of rid1 (SID1) shares common targets with RID1 on florigen genes to initiate floral transition in rice. *PLOS Genet.* 13:e1006642.
- Gao, H., Zheng, X.-M., Fei, G., Chen, J., Jin, M., Ren, Y., Wu, W., Zhou, K., Sheng, P., Zhou, F., et al. (2013). Ehd4 Encodes a Novel and *Oryza*-Genus-Specific Regulator of Photoperiodic Flowering in Rice. *PLOS Genet.* 9:e1003281.
- Han, S.-H., Yoo, S.-C., Lee, B.-D., An, G., and Paek, N.-C. (2015). Rice FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (OsFKF1) promotes flowering independent of photoperiod. *Plant. Cell Environ.* 38:2527–2540.

- Hori, K., Ogiso-Tanaka, E., Matsubara, K., Yamanouchi, U., Ebana, K., and Yano, M. (2013). Hd16, a gene for casein kinase I, is involved in the control of rice flowering time by modulating the day-length response. *Plant J.* **76**:36–46.
- Jiang, P., Wang, S., Zheng, H., Li, H., Zhang, F., Su, Y., Xu, Z., Lin, H., Qian, Q., and Ding, Y. (2018). SIP1 participates in regulation of flowering time in rice by recruiting OsTrx1 to Ehd1. *New Phytol.* **219**:422–435.
- Jin, J., Shi, J., Liu, B., Liu, Y., Huang, Y., Yu, Y., and Dong, A. (2015). MORF-RELATED GENE702, a reader protein of trimethylated histone H3 lysine 4 and histone H3 lysine 36, is involved in brassinosteroid-regulated growth and flowering time control in rice. *Plant Physiol.* **168**:1275–1285.
- Kim, S. L., Lee, S., Kim, H. J., Nam, H. G., and An, G. (2007). OsMADS51 Is a Short-Day Flowering Promoter That Functions Upstream of Ehd1, OsMADS14, and Hd3a. *Plant Physiol.* **145**:1484–1494.
- Kim, S.-K., Yun, C.-H., Lee, J. H., Jang, Y. H., Park, H.-Y., and Kim, J.-K. (2008). OsCO3, a CONSTANS-LIKE gene, controls flowering by negatively regulating the expression of FT-like genes under SD conditions in rice. *Planta* **228**:355–365.
- Lee, Y.-S., Lee, D.-Y., Cho, L.-H., and An, G. (2014). Rice miR172 induces flowering by suppressing OsIDS1 and SNB, two AP2 genes that negatively regulate expression of Ehd1 and florigens. *Rice* **7**:1–13.
- Li, D., Yang, C., Li, X., Gan, Q., Zhao, X., and Zhu, L. (2009). Functional characterization of rice OsDof12. *Planta* **229**:1159–69.
- Li, J., Chu, H., Zhang, Y., Mou, T., Wu, C., Zhang, Q., and Xu, J. (2012). The Rice HGW Gene Encodes a Ubiquitin-Associated (UBA) Domain Protein That Regulates Heading Date and Grain Weight. *PLoS One* **7**:e34231.
- Matsubara, K., Yamanouchi, U., Wang, Z.-X., Minobe, Y., Izawa, T., and Yano, M. (2008). Ehd2, a Rice Ortholog of the Maize INDETERMINATE1 Gene, Promotes Flowering by Up-Regulating Ehd1. *Plant Physiol.* **148**:1425–1435.
- Matsubara, K., Yamanouchi, U., Nonoue, Y., Sugimoto, K., Wang, Z. X., Minobe, Y., and Yano, M. (2011). Ehd3, encoding a plant homeodomain finger-containing protein, is a critical promoter of rice flowering. *Plant J.* **66**:603–612.
- Nakagawa, M., Shimamoto, K., and Kyojuka, J. (2002). Overexpression of RCN1 and RCN2, rice Terminal Flower 1/Centroradialis homologs, confers delay of phase transition and altered panicle morphology in rice. *Plant J.* **29**:743–750.
- Park, S. J., Kim, S. L., Lee, S., Je, B. Il, Piao, H. L., Park, S. H., Kim, C. M., Ryu, C.-H., Park, S. H., Xuan, Y., et al. (2008). Rice Indeterminate 1 (OsId1) is necessary for the expression of Ehd1 (Early heading date 1) regardless of photoperiod. *Plant J.* **56**:1018–1029.
- Peng, L. T., Shi, Z. Y., Li, L., Shen, G. Z., and Zhang, J. L. (2007). Ectopic expression of OsLFL1 in rice represses Ehd1 by binding on its promoter. *Biochem. Biophys. Res. Commun.* **360**:251–256.
- Peng, L. T., Shi, Z. Y., Li, L., Shen, G. Z., and Zhang, J. L. (2008). Overexpression of transcription factor OsLFL1 delays flowering time in *Oryza sativa*. *J. Plant Physiol.* **165**:876–885.
- Rao, N. N., Prasad, K., Kumar, P. R., and Vijayraghavan, U. (2008). Distinct regulatory role for RFL, the rice LFY homolog, in determining flowering time and plant architecture. *Proc. Natl. Acad. Sci.* **105**:3646–3651.
- Ryu, C. H., Lee, S., Cho, L. H., Kim, S. L., Lee, Y. S., Choi, S. C., Jeong, H. J., Yi, J., Park, S. J., Han, C. D., et al. (2009). OsMADS50 and OsMADS56 function antagonistically in regulating long day (LD)-dependent flowering in rice. *Plant, Cell Environ.* **32**:1412–1427.

- Saito, H., Ogiso-Tanaka, E., Okumoto, Y., Yoshitake, Y., Izumi, H., Yokoo, T., Matsubara, K., Hori, K., Yano, M., Inoue, H., et al. (2012). Ef7 Encodes an ELF3-like Protein and Promotes Rice Flowering by Negatively Regulating the Floral Repressor Gene Ghd7 under Both Short- and Long-Day Conditions. *Plant Cell Physiol.* **53**:717–728.
- Sheng, P., Wu, F., Tan, J., Zhang, H., Ma, W., Chen, L., Wang, J., Wang, J., Zhu, S., Guo, X., et al. (2016). A CONSTANS-like transcriptional activator, OsCOL13, functions as a negative regulator of flowering downstream of OsphyB and upstream of Ehd1 in rice. *Plant Mol. Biol.* **92**:209–222.
- Song, S., Wang, G., Hu, Y., Liu, H., Bai, X., Qin, R., and Xing, Y. (2018). OsMFT1 increases spikelets per panicle and delays heading date in rice by suppressing Ehd1, FZP and SEPALLATA-like genes. *J. Exp. Bot.* **69**:4283–4293.
- Sui, P., Jin, J., Ye, S., Mu, C., Gao, J., Feng, H., Shen, W. H., Yu, Y., and Dong, A. (2012). H3K36 methylation is critical for brassinosteroid-regulated plant growth and development in rice. *Plant J.* **70**:340–347.
- Sui, P., Shi, J., Gao, X., Shen, W.-H., and Dong, A. (2013). H3K36 Methylation Is Involved in Promoting Rice Flowering. *Mol. Plant* **6**.
- Sun, C., Fang, J., Zhao, T., Xu, B., Zhang, F., Liu, L., Tang, J., Zhang, G., Deng, X., Chen, F., et al. (2012). The Histone Methyltransferase SDG724 Mediates H3K36me_{2/3} Deposition at MADS50 and RFT1 and Promotes Flowering in Rice. *Plant Cell* **24**:3235–3247.
- Tan, J., Jin, M., Wang, J., Wu, F., Sheng, P., Cheng, Z., Wang, J., Zheng, X., Chen, L., Wang, M., et al. (2016). OsCOL10, a CONSTANS-like gene, functions as a flowering time repressor downstream of Ghd7 in rice. *Plant Cell Physiol.* **57**:798–812.
- Tan, J., Wu, F., and Wan, J. (2017). Flowering time regulation by the CONSTANS-Like gene OsCOL10. *Plant Signal. Behav.* **12**:e1267893.
- Vega-Sánchez, M. E., Zeng, L., Chen, S., Leung, H., and Wang, G.-L. (2008). SPIN1, a K Homology Domain Protein Negatively Regulated and Ubiquitinated by the E3 Ubiquitin Ligase SPL11, Is Involved in Flowering Time Control in Rice. *Plant Cell* **20**:1456–1469.
- Wang, J., Hu, J., Qian, Q., and Xue, H. W. (2013). LC2 and OsVIL2 Promote Rice Flowering by Photoperoid-Induced Epigenetic Silencing of OsLF. *Mol. Plant* **6**:514–527.
- Wu, W., Zheng, X. M., Chen, D., Zhang, Y., Ma, W., Zhang, H., Sun, L., Yang, Z., Zhao, C., Zhan, X., et al. (2017). OsCOL16, encoding a CONSTANS-like protein, represses flowering by up-regulating Ghd7 expression in rice. *Plant Sci.* **260**:60–69.
- Wu, W., Zhang, Y., Zhang, M., Zhan, X., Shen, X., Yu, P., Chen, D., Liu, Q., Sinumporn, S., Hussain, K., et al. (2018). The rice CONSTANS-like protein OsCOL15 suppresses flowering by promoting Ghd7 and repressing RID1. *Biochem. Biophys. Res. Commun.* **495**:1349–1355.
- Xie, S., Chen, M., Pei, R., Ouyang, Y., and Yao, J. (2015). OsEMF2b acts as a regulator of flowering transition and floral organ identity by mediating H3K27me₃ deposition at OsLFL1 and OsMADS4 in rice. *Plant Mol. Biol. Report.* **33**:121–132.
- Yang, Y., Peng, Q., Chen, G. X., Li, X. H., and Wu, C. Y. (2013a). OsELF3 Is Involved in Circadian Clock Regulation for Promoting Flowering under Long-Day Conditions in Rice. *Mol. Plant* **6**:202–215.
- Yang, J., Lee, S., Hang, R., Kim, S.-R., Lee, Y.-S., Cao, X., Amasino, R., and An, G. (2013b). OsVIL2 functions with PRC2 to induce flowering by repressing OsLFL1 in rice. *Plant J.* **73**:566–578.
- Yokoo, T., Saito, H., Yoshitake, Y., Xu, Q., Asami, T., Tsukiyama, T., Teraishi, M., Okumoto, Y., and Tanisaka, T. (2014). Se14, Encoding a JmjC Domain-Containing Protein, Plays Key Roles in Long-Day Suppression of Rice Flowering through the Demethylation of H3K4me₃ of RFT1. *PLoS One* **9**:e96064.

Zhao, J., Huang, X., Ouyang, X., Chen, W., Du, A., Zhu, L., Wang, S., Deng, X. W., and Li, S. (2012). OsELF3-1, an Ortholog of Arabidopsis EARLY FLOWERING 3, Regulates Rice Circadian Rhythm and Photoperiodic Flowering. *PLoS One* 7:e43705.