5. CONCLUSIONS AND FUTURE PERSPECTIVES

During my PhD I directed my work in identifying, characterizing and defining the function of OsFT-L1. In fact, my goal was to position and place this gene within the known photoperiod pathway and to define its relationship with the other two rice florigens.

When I started my thesis there were no data on the subject, only a florigenic function was hypothesized for this molecule, since, if over-expressed, it led to early flowering.
Thanks to an RNA sequencing we managed to find that *FT-L1* was up regulated by floral commitment in the shoot apical meristem.
In my thesis I was able to demonstrate that *FT-L1* activation depends on the photoperiod and florigens, unveiling that the presence of functional *Hd3a* and *RFT1* is not only necessary but also sufficient for *FT-L1* induction in the SAM.
We further found that FT-L1 carries out its function only in the meristem. Expression in leaves was not negligible but after having mis-expressed *FT-L1* under vasculature and meristem specific promoters, we concluded that it is a floral transition promoter acting only at the meristem and not in leaves.

In order to define its function, I used mutants obtained following two different approaches and in different varieties. On the one hand I obtained CRISPR mutants in a Nipponbare (NB) background through genome editing and on the other mutants in Volano, obtained through chemical mutagenesis with sodium azide. In fact, firstly I generated lines of single and double mutants in combination with the other two florigens and I analyzed their flowering time in short- and long- day conditions. After this I was able to determine the additive effect at the level of flowering time that *FTL1* demonstrated with *Hd3a*, under short day and *RFT1*, under long day.
Once its effects on flowering were taken into account, I decided to focus on another cultivar to confirm and corroborate what we saw in NB.
To this end, I worked on mutants chemically obtained in an Italian variety (Volano, which is insensitive to photoperiod) by isolating three lines harboring amino acid substitutions in the sequence of the protein of interest.
On these mutants I conducted a flowering experiment, both under short day and natural long day, in the field, and I analyzed the panicle.

From these analyses I was able to confirm the effect that this gene has on flowering induction after commitment. In fact, also in this variety and in both conditions, the mutants show a strong delay in days to heading. I also found, after careful analysis of several traits, that mutants' panicle had a different architecture, with a significant increase in the number of secondary branches.
These data together suggest that FT-L1 is involved not only in flowering promotion but also in subsequent stages of inflorescence development.

Observing the panicle, it became immediately clear that the mutants had flowers that could not fill the grains properly. The flowers were often empty with very small or even absent caryopses.
Following a counting analysis on the number of flowers, I was able to conclude that the total number of flowers, although increased compared to the WT, was largely composed of the sterile fraction. The increase in the total number is consistent with the increase in the number of secondary branches while the increase in the sterile portion indicates that this gene plays a role in determining flower fertility.

In CRISPR lines I could not test the role that FT-L1 had at the level of the determination of the ramifications and of the spikelets. This is due to the forced conditions of growth in phytotrons because NB lines were transgenic. The NB variety used is not the optimal model for evaluating branching as it develops a small

panicle under our growth conditions that tend to reduce and often even abolish the differences between WT and mutants.

Based on all the phenotypic observations, FT-L1 seems to act by promoting the shift between secondary branches meristem and spikelet meristem.
In fact, a mutation in this gene causes a delay in the determination of spikelet identity showing a phenotype of reiteration of branch meristems. As a matter of fact, it is worth noting that only the number of secondary branches is increased, underlying that FT-L1 acts during the final stages of inflorescence determination.

Notably, therefore, we were able to identify and confirm the function of FT-L1 as both inducer of flowering together with the other two florigens and inducer of panicle determinacy.
This specific temporal and spatial activity during inflorescence development was also corroborated by the localization patterns obtained with in situ hybridizations and the marker lines.

In this thesis, in addition to defining what the function of FT-L1 is and in which tissues it performs it, we have proposed a mechanism of action according to which FT-L1 acts together with some bZIP-type transcription factors, forming alternative FAC complexes.

Among the bZIPs tested, the transcription factor OsFD7 is known to have a role in branching, and interaction data, tested with BiFC and FLIM, suggest that OsFD7 performs its function together with FT-L1.

Thus, we demonstrate the plasticity of the FAC complex which, depending on the variability dictated by its components, assembles itself in different tissues and moments of development.

Finally, I have shown that the transcriptional regulation of *FT-L1* depends, at least in part, on the specific binding to its promoter by the SPLs. *SPL14* and *SPL17* have a redundant function in reducing the expression of *FT-L1* during the development of the inflorescence when the branch meristems are forming, to increase branch number. These findings highlight FT-L1 function as an integrator of both the SPLs and photoperiodic pathways.

In conclusion, little was known about the role of the florigens in further steps of the rice inflorescence development and I think this work has unveiled new features for florigenic proteins thanks to my characterization of FT-L1 function in these phases. This work also helps establishing a new model of floral induction, in which the activity of the florigens is potentiated at the SAM by a florigen-like protein. Literature data indicates that similar florigen-like genes are expressed also in SAMs of wheat, barley and Brachypodium and perform functions like those of *FT-L1* in rice. Dicot species do not possess such activities, suggesting that it evolved in the monocots and possibly in the grasses only.
Furthermore, this work lays the foundations for interesting developments such as trying to identify which genes are downstream of FT-L1 to better understand its mechanism of action. Through RNA-sequencing performed on WT and *ft-l1* mutants' meristems - at different stages of development - it will be possible to evaluate which are its target genes. Finally, together with the analysis of the FTL1 interactome, clues could be obtained to investigate its function. Important applicability scenarios at this point would open as regards the possibility of determining panicle branching, to increase yield and satisfy the ever-increasing demand for food.

6. MATERIALS AND METHODS

In this section of Material and Methods, I describe those I personally performed during my thesis. The experiment run by our collaborators are not described but relative methods are reported in Material and Methods of papers and Manuscript attached. All primer used are listed in the Manuscript Supplementary Table2 (see *Appendix 5*).

Plant materials and growth conditions

During my thesis, we have used two *japonica rice* (Oryza sativa) cultivars: Nipponbare and Volano. The latter is an Italian variety insensible to photoperiod caused by a background natural mutation in the master regulator *Hd1,* as a result of adaptation to Northern latitudes[9,20].

Both Nipponbare and Volano were grown under different inductive and non-inductive photoperiodic conditions as SD=10h:14h, light : dark and LD=14.5h:9.5 h, light : dark for greenhouses and growth chambers. Only for Volano also Natural LD conditions were used for paddy field experiment. The plants were grown during summer period, inn fields located in Tortona, Italy, 44.89°N, 8.86°E.

For *Arabidopsis thaliana* the growth conditions used were only SD non inductive ones, with 10h:14h, light:dark.

For the **misexpressor lines** we have cloned *FT-L1* downstream of two different tissue-specific promoters: 1.5 Kb of p*OSH1* (a meristem- and stem-specific promoter) and 1.3 Kb *pRPP16* (a vasculature-specific promoter) were cloned in a Gateway vector. The *pOSH1:GW* destination vector has been described in [67]. The *pRPP16* promoter was amplified using primers pRPP16-FW and pRPP16-RV and cloned in a *pIndex4* backbone modified as described by[67,105]. First, we cloned 531 bps of *FT-L1* CDS in the *pDONR207*, then by LR recombination reaction we inserted *FT-L1* CDS in the *pOSH1:GW* destination vector.

Using *pOSH1:FT-L1* as a donor we cut out *OSH1* promoter using MunI and MluI restriction enzymes and we inserted *RPP16* promoter using the primers pRPP16-FW and pRPP16-RV, containing MunI and AscI restriction sites; we digested the fragment with MunI and AscI (an isoschizomer of MluI) and we ligated the promoter into the empty vector with T4 ligase obtaining *pRPP16:FT-L1*.

At the end, all constructs were transformed in Volano FT-L1^{P95S} calli.

For obtaining **transcriptional marker lines** we have cloned 2500pb of *FT-L1* promoter with primers pFT-L1+ attB1-FW and pFT-L1+ attB2-RV). p*FT-L1* was inserted into *pDONR207* using BP recombinase. The *pFT-L1* entry vector was LRII - recombined into the final vector *pGWB540*, carrying the fluorophore *eYFP.*

For the **translational marker lines,** we used a multisite gateway vector: p*FT-L1* was cloned into *pDONR221* P1-P5r (with primers pFT-L1+ attB1-FW and pFT-L1+ attB5r-RV) and the *FT-L1* CDS into *pDONR221* P5-P2 (with primers OsFT-L1+attB5-FW and OsFT-L1+attB2-RV).

These fragments were recombined into final *pGWB540* using LRII recombinase.

CRISPR-Cas9 Editing and rice transformation

CRISPR *ft-l1* mutant lines were generated according to[106] protocol and in three different backgrounds: Nipponbare wild type, Nipponbare CRISPR *hd3a* which carries a loss of function homozygous mutation for *Hd3a* gene, *hd3a-3*, with a T deletion in the coding region (*Mineri et al.*), and Nipponbare *CRISPR rft1* which carries a loss of function homozygous mutation for *RFT1* gene, *rft1-1*, with an A insertion in the coding region (*Mineri et al.*).

The CRISPR mutants were generated by expressing a double sg-RNA, which are selected in regions between an exon and an intron to be specific, taking into account the high homology with Hd3a and RFT1. For this experiment, OLIGO 1 is located between the first exon and the first intron, while OLIGO 2 is between the third exon and the third intron.

After having obtained the final vector, we have transformed Nipponbare WT calli and stable lines of CRISPR *hd3a* and CRISPR *rft1* calli.
For CRISPR *spl14* and *spl17*, instead, the cloning was based on [107] protocol and expressed in Nipponbare calli.

The selected oligo for both *SPL14* and *SPL17* is in a region that could be a target both for creating frame-shift mutations with *spl* loss-of-function phenotypes, and for targeting miR156 core region to obtain *SPL* gain-of-function phenotypes.
Nipponbare and Volano calli were obtained, transformed and selected on 50 mg/L and 100 mg/L hygromycin. For rice in vitro transformation it has been applied the protocol of [108] and EHA105 strain of Agrobacterium tumefaciens was used.

Functional assay in A. thaliana
This assay has been performed in *A. thaliana ft-10* background. Plants of 3- to 4- weeks old plants were transformed in a solution of Agrobacterium with the constructs and Silwet L-77.
The vector *pFD:GW* (*pLEELA* backbone) has been used, in which the three CDSs of *FT-L1*, *FT-L1*^{P95S} and *FT* were inserted under *FD* promoter, through Gateway recombination system.
After the recovery of 24h in the dark, plants were moved to growth in greenhouse for 1 month, until siliques turn brown. Once collected the T0 seeds and soiled again, they were selected with the herbicides BASTA. The resistant plants' flowering time were then recorded.

RNA isolation and analysis of gene expression
For quantification of gene expression, quantitative reverse transcription polymerase chain reaction (qRT-PCR) has been used. SAM samples and young panicles were collected at different days after the shift (DAS) of the plants from LD to SD conditions, for inducing flowering. At least two biological replicates were sampled for every time point. RNA was extracted with NucleoZOL (Macherey-Nagel) following the manufacturer's instructions. For clearing the RNA samples from DNA residues, a treatment with DNase I has been performed (Turbo™ DNase, Invitrogen). The cDNA was synthetized with ImProm-II™ Reverse Transcriptase (Promega), using 1 µg of total RNA as a starting template and a polyT primer. Quantification of transcripts was performed with the primers listed in Suppl Table 2, using the Maxima SYBR Green qPCR Master Mix (Thermofisher Scientific), with a RealPlex2 thermocycler (Eppendorf). The annealing temperature for all primer pairs was 60°C. All quantifications were calculated based on technical triplicates. The *Ubiquitin* gene was used to normalize expression levels. For statistical analysis, t-test has been used, with a p-value threshold of 0.05.

In situ hybridization
We have performed in situ hybridizations on Nipponbare WT meristems, collected at different time points to obtain sections of meristems at different stages such as vegetative, early inflorescence and full reproductive one, bearing flowers.
Microtome sections of 7 µm thickness were treated according to the protocol published by [109], using 60°C as temperature condition for the hybridization.
We used two different approaches for probes' preparation. One probe was designed by amplifying a template of about 200 bp from the genomic DNA of *FT-L1* plus the T7 polymerase tag promoter sequence. The same probe and a second new one were obtained by a TA-cloning with the *pGEM T-easy*. For all the probes designed we obtained the same staining and pattern signal, validating and corroborating the results.

Protein-protein interaction assays

For BiFC and FRET-FLIM assays, two- to three-week-old *Nicotiana benthamiana* plants grown at 20 degrees were transfected with the vectors expressing the fusion proteins of interest. pBAT TL-B sYFP-N and *pBAT TL-B sYFP-C* were used for BiFC while *pABind-GFP* and *pABind-mCHERRY* for FRET-FLIM.

After the agroinfiltration with an OD_{600} of 0.4 for BiFC and OD_{600} of 0.5 for FLIM, plants were incubated at 25°C in SD conditions for 2-5 days.

After this period plants were ready for the confocal for BiFC imaging.

For FLIM, plants were induced with β-estradiol with 20 μM as final concentration and 0.1% Tween 20 14 to 16 hours before the confocal analysis [110,111].

The GFP lifetime has been measured without and with the acceptor mCherry on an average of 10 transformed cells (nuclei and cytoplasm). All the interactions have been repeated twice.

Sample preparation and confocal imaging

For the marker lines, shoot apices of the transgenic plants were collected and embedded in 6% (w/v) agarose, sliced with Leica Vibratome VT1200S into 50 μm thick sections and then they were stained with 1:1000, Renaissance©:1xPBS.

For BiFC and transcriptional and translational marker lines were used Nikon Eclipse Ti2 inverted microscope, equipped with a Nikon A1R+ laser scanning device (http://www.nikon.com/) and the NIS software. For BiFC experiment, YFP was excited with the 488-nm laser and the emission was collected at 505–550 nm while the Chlorophyll was excited with the 488-nm laser and we collected the emission at 663–738 nm. For transcriptional and translational marker lines analysis, instead, the eYFP was excited with the 488-nm laser and the emission was collected at 505–550 nm while the Renaissance© excitation was set at 405 nm with an emission at 425-475 nm.

While for the FLIM assay a Leica Multi-Photon Falcon Dive was used with the software Leica Application Suite LAS X FLIM/FCS, Version 3.5.6.

To perform FLIM analyses we at first verified the presence and distribution of both GFP and mCherry fluorescence emissions in our samples. The excitation was performed with a Multi-Photon pulsed laser set at 930 nm for the GFP and set at 1045 nm for the mCherry. For the acquisition of GFP and mCherry emissions, the spectral detectors were set with 493-547 nm and 586-650 nm windows, respectively. For the FLIM analysis, only the 930 nm excitation was used and we picked up the photon decay lifetime of GFP emission with the Leica FALCON wizard.

Structure and stability of FT-L1 variants

3D structure of wild-type and mutated FT-L1 was obtained by homology modelling using the tool Swiss Model (https://swissmodel.expasy.org/interactive) and monomeric AtFT (PDB id 6igh.1) as a template. Figures of the structure were prepared with PyMOL (The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC). To understand whether mutations in FT-L1 could impair protein stability, the homology models were analyzed with DUET, http://structure.bioc.cam.ac.uk/duet.

P-TRAP

The phenotypical analyisis of rice panicle architecture was performed using open-source pTRAP software ("Panicle TRAit Phenotyping") [100] .

At least 10 panicles per genotype were collected at full maturity. Inflorescence were disposed on blank sheets in an open conformation, so that nodes, branches and seeds could be clearly distinguished. Pictures were taken in the same day and at the same frontal distance for each genotype, hence uploaded on the software.

Computer analysis allowed for quantification of number and length of primary to tertiary branches, as well as the length of the main axis.

Alignment

So as to carry out SPLs and FT-L1 alignment, MAFFT software has been used (https://mafft.cbrc.jp/alignment/software/) with the accession LOC_Os08g39890.1 and LOC_Os09g31438 for SPL14 and SPL17 respectively and LOC_Os01g11940.1, for FT-L1.

For this last, we noticed that the first 100 amino acids of FT-L1 had no homology with the other florigens while, starting from the second Methionine, the homology increases till 80% for RFT1, 79% for Hd3a and 72% for FT. This evidence suggested that *FT-L1* was misannotated (http://rice.uga.edu), for this reason we considered as translation start site the ATG at position 382 from the transcription start site of *FT-L1* gene.

Statistical Analysis

All data were plotted using GraphPad Prism 8 software (GraphPad Software Inc., San Diego, California). With the same software, all mean values and standard deviations have been calculated and statistical data analysis have been performed. T-test has been used to compare data between two different conditions, in particular we used an unpaired two-tailed Student's t test with Welch correction, to take into account unequal variances.

A p value < 0.05 was considered as statistically significant. n.s., $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.001$.

Every expression experiment was supported by two independent biological replicates and three technical replicates for each biological one. For each biological replicate five meristems from five independent plants were sampled as a pool.

Moreover, since meristems are sampled within a time course, the expression of the gene of interest follows a trend. Therefore, the rationale is also based on the assumption that this trend provides an internal control for each time point that is considered in relation to the previous and/or following one.

Furthermore, to confirm that the acquired data were replicable, all the experiments have been repeated three times independently and the same results were obtained.

Accession numbers

Rice MSU Genome Annotation Project **(http://rice.uga.edu**)

OsFT-L1 > LOC_Os01g11940.1, OsHd3a > LOC_Os06g06320.1, OsRFT1 > LOC_Os06g06300,

Gf14 b > LOC_Os04g38870, Gf14 c > LOC_Os08g33370

OsFD7 > LOC_07g48660, OsFD4 > LOC_Os08g43600, OsFD1 > LOC_Os09g36910

SPL14 > LOC_Os08g39890.1, SPL17 > LOC_Os09g31438

OsMADS 15 > LOC_Os07g01820.1, UBQ > LOC_Os03g13170.1

BIBLIOGRAPHY

1. Huang, X. *et al.* A map of rice genome variation reveals the origin of cultivated rice. *Nature* **490**, 497–501 (2012).

2. Garris, A. J., Tai, T. H., Coburn, J., Kresovich, S. & McCouch, S. Genetic structure and diversity in Oryza sativa L. *Genetics* **169**, 1631–1638 (2005).

3. RAM, P. C. Maclean, J.L., Dawe, D.C., Hardy, B. and Hettel, G.P. (eds) Rice almanac. 3rd edn. *Annals of Botany* **92**, 739–739 (2003).

4. Kobayashi, K., Maekawa, M., Miyao, A., Hirochika, H. & Kyozuka, J. PANICLE PHYTOMER2 (PAP2), encoding a SEPALLATA subfamily MADS-box protein, positively controls spikelet meristem identity in rice. *Plant and Cell Physiology* **51**, 47–57 (2010).

5. Wang, L. *et al.* Bract suppression regulated by the miR156/529-SPLs-NL1-PLA1 module is required for the transition from vegetative to reproductive branching in rice. *Molecular Plant* **14**, 1168–1184 (2021).

6. Kellogg, E. A. & Danforth, D. Genetic control of branching patterns in grass inflorescences. doi:10.1093/plcell/koac080/6544599.

7. Itoh, J. I. *et al.* Rice plant development: From zygote to spikelet. *Plant and Cell Physiology* vol. 46 23–47 (2005).

8. Shrestha, R., Gómez-Ariza, J., Brambilla, V. & Fornara, F. Molecular control of seasonal flowering in rice, arabidopsis and temperate cereals. *Annals of Botany* vol. 114 1445–1458 (2014).

9. Gómez-Ariza, J. *et al.* Loss of floral repressor function adapts rice to higher latitudes in Europe. *Journal of Experimental Botany* **66**, 2027–2039 (2015).

10. Butler, W. L., Norris, K. H., Siegelman, H. W. & Hendricks, S. B. *DETECTION, ASSA Y, AND PRELIMINARY PURIFICATION OF THE PIGMENT CONTROLLING PHOTORESPONSIVE DEVELOPMENT OF PLANTS*.

11. Brambilla, V. & Fornara, F. Molecular Control of Flowering in Response to Day Length in Rice. *Journal of Integrative Plant Biology* **55**, 410–418 (2013).

12. Putterill, J., Robson, F., Lee, K., Simon, R. & Coupland, G. *The CONSTANS Gene of Arabidopsis Promotes Flowering and Encodes a Protein Showing Similarities to Zinc Finger Transcription Factors*. *Cell* vol. 80 (1995).

13. An, H. *et al.* CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. *Development* **131**, 3615–3626 (2004).

14. Fowler, S. *et al. GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in Arabidopsis and encodes a protein with several possible membrane-spanning domains*. *The EMBO Journal* vol. 18 (1999).

15. Griffiths, S., Dunford, R. P., Coupland, G. & Laurie, D. A. The evolution of CONSTANS-like gene families in barley, rice, and Arabidopsis. *Plant Physiology* **131**, 1855–1867 (2003).

16. Kojima, S. *et al.* Hd3a, a rice ortholog of the ArabidopsisFT Gene, promotes transition to flowering downstream of Hd1 under short-day conditions. *Plant and Cell Physiology* **43**, 1096–1105 (2002).

17. Hayama, R., Yokoi, S., Tamaki, S., Yano, M. & Shimamoto, K. Adaptation of photoperiodic control pathways produces short-day flowering in rice. *Nature* **422**, 719–722 (2003).

18. Izawa, T. *et al.* Phytochrome mediates the external light signal to repress FT orthologs in photoperiodic flowering of rice. *Genes and Development* **16**, 2006–2020 (2002).

19. Doi, K. *et al.* Ehd1, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of Hd1. *Genes and Development* **18**, 926–936 (2004).

20. Goretti, D. *et al.* Transcriptional and Post-transcriptional Mechanisms Limit Heading Date 1 (Hd1) Function to Adapt Rice to High Latitudes. *PLoS Genetics* **13**, (2017).

21. Kardailsky et al., 1999.

22. Kobayashi, Y., Kaya, H., Goto, K., Iwabuchi, M. & Araki, T. *A Pair of Related Genes with Antagonistic Roles in Mediating Flowering Signals*. https://www.science.org.

23. Chautard, H., Jacquet, M., Schoentgen, F., Bureaud, N. & Bénédetti, H. Tfs1p, a member of the PEBP family, inhibits the Ira2p but not the Ira1p Ras GTPase-activating protein in Saccharomyces cerevisiae. *Eukaryotic Cell* **3**, 459–470 (2004).

24. Banfield, M. J., Barker, J. J., Perry, A. C. & Leo Brady, R. *Function from structure? The crystal structure of human phosphatidylethanolamine-binding protein suggests a role in membrane signal transduction*. http://biomednet.com/elecref/0969212600601245.

25. Yeung, K. *et al. Suppression of Raf-1 kinase activity and MAP kinase signalling by RKIP*. www.nature.com (1999).

26. Hanzawa, Y., Money, T. & Bradley, D. *A single amino acid converts a repressor to an activator of flowering*. vol. 102 www.pnas.orgcgidoi10.1073pnas.0500932102 (2005).

27. Xi, W., Liu, C., Hou, X. & Yu, H. MOTHER OF FT and TFL1 regulates seed germination through a negative feedback loop modulating ABA signaling in Arabidopsis. *Plant Cell* **22**, 1733–1748 (2010).

28. Song, S. *et al.* OsMFT1 increases spikelets per panicle and delays heading date in rice by suppressing Ehd1, FZP and SEPALLATA-like genes. *Journal of Experimental Botany* **69**, 4283–4293 (2018).

29. Ahn, J. H. *et al.* A divergent external loop confers antagonistic activity on floral regulators FT and TFL1. *EMBO Journal* **25**, 605–614 (2006).

30. Danilevskaya, O. N. *et al.* Involvement of the MADS-box gene ZMM4 in floral induction and inflorescence development in maize. *Plant Physiology* **147**, 2054–2069 (2008).

31. Taoka, K. I., Ohki, I., Tsuji, H., Kojima, C. & Shimamoto, K. Structure and function of florigen and the receptor complex. *Trends in Plant Science* vol. 18 287–294 (2013).

32. Nakamura, Y. *et al.* High-Resolution Crystal Structure of Arabidopsis FLOWERING LOCUS T Illuminates Its Phospholipid-Binding Site in Flowering. *iScience* **21**, 577–586 (2019).

33. Chardon, F. & Damerval, C. Phylogenomic analysis of the PEBP gene family in cereals. *Journal of Molecular Evolution* **61**, 579–590 (2005).

34. Komiya, R., Ikegami, A., Tamaki, S., Yokoi, S. & Shimamoto, K. Hd3a and RFT1 are essential for flowering in rice. *Development* **135**, 767–774 (2008).

35. Corbesier, L. *et al.* FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. *Science (1979)* **316**, 1030–1033 (2007).

36. Shojiro Tamaki, Shoichi Matsuo, Hann Ling Wong, Shuji Yokoi, K. S. Hd3a Protein Is a Mobile. **316**, 1033–1037 (2007).

37. Komiya, R., Yokoi, S. & Shimamoto, K. A gene network for long-day flowering activates RFT1 encoding a mobile flowering signal in rice. *Development* **136**, 3443–3450 (2009).

38. Eom, J. S., Choi, S. B., Ward, J. M. & Jeon, J. S. The mechanism of phloem loading in rice (Oryza sativa). *Molecules and Cells* vol. 33 431–438 (2012).

39. Liu, C. *et al.* A Conserved Genetic Pathway Determines Inflorescence Architecture in Arabidopsis and Rice. *Developmental Cell* **24**, 612–622 (2013).

40. Song, S. *et al.* OsFTIP1-mediated regulation of florigen transport in rice is negatively regulated by the ubiquitin-like domain kinase OsUbDKγ4. *Plant Cell* **29**, 491–507 (2017).

41. Adrian, J. *et al.* Cis-regulatory elements and chromatin state coordinately control temporal and spatial expression of FLOWERING LOCUS T in arabidopsis. *Plant Cell* **22**, 1425–1440 (2010).

42. Abe, M. *et al.* FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science (1979)* **309**, 1052–1056 (2005).

43. Wigge, P. A. Integration of Spatial and Temporal Information During Floral Induction in Arabidopsis. *Science (1979)* **309**, 1056–1059 (2005).

44. Jaeger, K. E. & Wigge, P. A. FT Protein Acts as a Long-Range Signal in Arabidopsis. *Current Biology* **17**, 1050–1054 (2007).

45. Zhang, L. *et al.* Three CCT domain-containing genes were identified to regulate heading date by candidate gene-based association mapping and transformation in rice. *Scientific Reports* **5**, (2015).

46. Tamaki, S., Matsuo, S., Wong, H. L., Yokoi, S. & Shimamoto, K. Hd3a protein is a mobile flowering signal in rice. *Science* **316**, 1033–6 (2007).

47. Tsuji, H. *et al.* Hd3a promotes lateral branching in rice. *Plant Journal* **82**, 256–266 (2015).

48. Tamaki, S. *et al.* FT-like proteins induce transposon silencing in the shoot apex during floral induction in rice. *Proc Natl Acad Sci U S A* **112**, E901–E910 (2015).

49. Aki, T., Shigyo, M., Nakano, R., Yoneyama, T. & Yanagisawa, S. Nano scale proteomics revealed the presence of regulatory proteins including three FT-like proteins in phloem and xylem saps from rice. *Plant and Cell Physiology* **49**, 767–790 (2008).

50. Gómez-Ariza, J. *et al.* A transcription factor coordinating internode elongation and photoperiodic signals in rice. *Nature Plants* **5**, 358–362 (2019).

51. Furutani, I., Sukegawa, S. & Kyozuka, J. Genome-wide analysis of spatial and temporal gene expression in rice panicle development. *Plant Journal* **46**, 503–511 (2006).

52. Komiya, R., Ikegami, A., Tamaki, S., Yokoi, S. & Shimamoto, K. Hd3a and RFT1 are essential for flowering in rice. *Development* **135**, 767–774 (2008).

53. Kaur, A., Nijhawan, A., Yadav, M. & Khurana, J. P. OsbZIP62/OsFD7, a functional ortholog of FLOWERING LOCUS D, regulates floral transition and panicle development in rice. *Journal of Experimental Botany* **72**, 7826–7845 (2021).

54. Alvarez, J., Guli, C. L., Yu, X.-H. & Smyth, D. R. *terminal flower: a gene affecting inflorescence development in Arabidopsis thaliana*. *The Plant Journal* vol. 992.

55. Benlloch, R., Berbel, A., Serrano-Mislata, A. & Madueño, F. Floral initiation and inflorescence architecture: A comparative view. *Annals of Botany* vol. 100 659–676 (2007).

56. Kaneko-Suzuki, M. *et al.* TFL1-Like Proteins in Rice Antagonize Rice FT-Like Protein in Inflorescence Development by Competition for Complex Formation with 14-3-3 and FD. *Plant and Cell Physiology* **59**, 458–468 (2018).

57. Nakagawa, M., Shimamoto, K. & Kyozuka, J. *Overexpression of RCN1 and RCN2, rice TERMINAL FLOWER 1/CENTRORADIALIS homologs, confers delay of phase transition and altered panicle morphology in rice*.

58. Kim, Y. *et al.* ELF4 Regulates GIGANTEA Chromatin Access through Subnuclear Sequestration. *Cell Reports* **3**, 671–677 (2013).

59. Xue, W. *et al.* Natural variation in Ghd7 is an important regulator of heading date and yield potential in rice. *Nature Genetics* **40**, 761–767 (2008).

60. Taoka, K. I. *et al.* 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. *Nature* **476**, 332–335 (2011).

61. Purwestri, Y. A., Ogaki, Y., Tamaki, S., Tsuji, H. & Shimamoto, K. The 14-3-3 protein GF14c acts as a negative regulator of flowering in rice by interacting with the florigen Hd3a. *Plant and Cell Physiology* **50**, 429–438 (2009).

62. Pnueli, L. *et al.* Tomato SP-Interacting Proteins Define a Conserved Signaling System That Regulates Shoot Architecture and Flowering. *The Plant Cell* **13**, 2687–2702 (2001).

63. Cooper, B. *et al. A network of rice genes associated with stress response and seed development*. www.pnas.org.

64. Chen, F., Li, Q., Sun, L. & He, Z. The rice 14-3-3 gene family and its involvement in responses to biotic and abiotic stress. *DNA Research* **13**, 53–63 (2006).

65. Zhao, J. *et al.* Genetic interactions between diverged alleles of Early heading date 1 (Ehd1) and Heading date 3a (Hd3a)/ RICE FLOWERING LOCUS T1 (RFT1) control differential heading and contribute to regional adaptation in rice (Oryza sativa). *New Phytologist* **208**, 936–948 (2015).

66. Tsuji, H., Nakamura, H., Taoka, K. I. & Shimamoto, K. Functional diversification of FD transcription factors in rice, components of florigen activation complexes. *Plant and Cell Physiology* **54**, 385–397 (2013).

67. Brambilla, V. *et al.* Antagonistic transcription factor complexes modulate the floral transition in rice. *Plant Cell* **29**, 2801–2816 (2017).

68. Cerise, M. *et al.* OsFD4 promotes the rice floral transition via florigen activation complex formation in the shoot apical meristem. *New Phytologist* **229**, 429–443 (2021).

69. Tilman, D., Balzer, C., Hill, J. & Befort, B. L. Global food demand and the sustainable intensification of agriculture. *Proc Natl Acad Sci U S A* **108**, 20260–20264 (2011).

70. Sun, Q. *et al.* Overexpression of Loose Plant Architecture 1 increases planting density and resistance to sheath blight disease via activation of PIN-FORMED 1a in rice. *Plant Biotechnology Journal* vol. 17 855–857 (2019).

71. Liu, Q., Harberd, N. P. & Fu, X. SQUAMOSA Promoter Binding Protein-like Transcription Factors: Targets for Improving Cereal Grain Yield. *Molecular Plant* vol. 9 765–767 (2016).

72. Lu, Z. *et al.* Genome-wide binding analysis of the transcription activator IDEAL PLANT ARCHITECTURE1 reveals a complex network regulating rice plant architecture. *Plant Cell* **25**, 3743–3759 (2013).

73. Wang, J. *et al.* Tissue-specific ubiquitination by IPA1 INTERACTING PROTEIN1 modulates IPA1 protein levels to regulate plant architecture in rice. *Plant Cell* **29**, 697–707 (2017).

74. Duan, E. *et al.* Osshi1 regulates plant architecture through modulating the transcriptional activity of ipa1 in rice. *Plant Cell* **31**, 1026–1042 (2019).

75. Wang, L. *et al.* Coordinated regulation of vegetative and reproductive branching in rice. *Proc Natl Acad Sci U S A* **112**, 15504–15509 (2015).

76. Yan, Y. *et al.* MiR529a controls plant height, tiller number, panicle architecture and grain size by regulating SPL target genes in rice (Oryza sativa L.). *Plant Science* **302**, (2021).

77. Jiao, Y. *et al.* Regulation of OsSPL14 by OsmiR156 defines ideal plant architecture in rice. *Nature Genetics* **42**, 541–544 (2010).

78. Miura, K. *et al.* OsSPL14 promotes panicle branching and higher grain productivity in rice. *Nature Genetics* **42**, 545–549 (2010).

79. Li, M. *et al.* Reassessment of the four yield-related genes Gn1a, DEP1, GS3, and IPA1 in rice using a CRISPR/Cas9 system. *Frontiers in Plant Science* **7**, (2016).

80. Zhang, D. & Yuan, Z. Molecular control of grass inflorescence development. *Annual Review of Plant Biology* vol. 65 553–578 (2014).

81. Harrop, T. W. R. *et al.* Gene expression profiling of reproductive meristem types in early rice inflorescences by laser microdissection. *Plant Journal* **86**, 75–88 (2016).

82. Zong, J. *et al.* A rice single cell transcriptomic atlas defines the developmental trajectories of rice floret and inflorescence meristems. *New Phytologist* **234**, 494–512 (2022).

83. Kobayashi, K. *et al.* Inflorescence meristem identity in rice is specified by overlapping functions of three AP1/FUL-Like MADS box genes and PAP2, a SEPALLATA MADS Box gene. *Plant Cell* **24**, 1848–1859 (2012).

84. Yoshida, A. *et al.* TAWAWA1, a regulator of rice inflorescence architecture, functions through the suppression of meristem phase transition. *Proc Natl Acad Sci U S A* **110**, 767–772 (2013).

85. Morita, Y. & Kyozuka, J. Characterization of OsPID, the rice ortholog of PINOID, and its possible involvement in the control of polar auxin transport. *Plant and Cell Physiology* **48**, 540–549 (2007).

86. Wu, H. M., Xie, D. J., Tang, Z. S., Shi, D. Q. & Yang, W. C. PINOID regulates floral organ development by modulating auxin transport and interacts with MADS16 in rice. *Plant Biotechnology Journal* **18**, 1778–1795 (2020).

87. Komatsu, K. *et al. LAX and SPA: Major regulators of shoot branching in rice*. www.pnas.orgcgidoi10.1073pnas.1932414100 (2003).

88. Wang, L. *et al.* NECK LEAF 1, a GATA type transcription factor, modulates organogenesis by regulating the expression of multiple regulatory genes during reproductive development in rice. *Cell Research* **19**, 598–611 (2009).

89. Giaume, F. & Fornara, F. SPL transcription factors prevent inflorescence reversion in rice. *Molecular Plant* **14**, 1041–1043 (2021).

90. Komatsu, M., Chujo, A., Nagato, Y., Shimamoto, K. & Kyozuka, J. Frizzy panicle is required to prevent the formation of axillary meristems and to establish floral meristem identity in rice spikelets. *Development* **130**, 3841–3850 (2003).

91. Zheng, K. *et al.* The effect of RNA polymerase V on 24-nt siRNA accumulation depends on DNA methylation contexts and histone modifications in rice. **118**, 2100709118 (2021).

92. Higo, A. *et al.* DNA methylation is reconfigured at the onset of reproduction in rice shoot apical meristem. *Nature Communications* **11**, (2020).

93. Aoyama, T. & Chua, N.-H. *A glucocorticoid-mediated transcriptional induction system in transgenic plants*. *The Plant Journal* vol. 11 (1997).

94. Naruse, M., Takahashi, H., Kurata, N. & Ito, Y. Cytokinin-induced expression of OSH1 in a shoot-regenerating rice callus. *Plant Biotechnology* **35**, 267–272 (2018).

95. Asano, T. *et al. Construction of a specialized cDNA library from plant cells isolated by laser capture microdissection: toward comprehensive analysis of the genes expressed in the rice phloem*.

96. Miki, D., Zhang, W., Zeng, W., Feng, Z. & Zhu, J. K. CRISPR/Cas9-mediated gene targeting in Arabidopsis using sequential transformation. *Nature Communications* **9**, (2018).

97. Miki, D. *et al.* Gene Targeting Facilitated by Engineered Sequence-Specific Nucleases: Potential Applications for Crop Improvement. *Plant and Cell Physiology* vol. 62 752–765 (2021).

98. Kojima, S. Hd3a, a Rice Ortholog of the Arabidopsis FT Gene, Promotes Transition to Flowering Downstream of Hd1 under Short-Day Conditions. *Plant and Cell Physiology* **43**, 1096–1105 (2002).

99. Rigola, D. *et al.* High-throughput detection of induced mutations and natural variation using KeyPoint™ technology. *PLoS ONE* **4**, (2009).

100. Al-Tam, F. *et al. P-TRAP: a Panicle Trait Phenotyping tool*. *BMC Plant Biology* vol. 13 http://www.biomedcentral.com/1471-2229/13/122SOFTWARE (2013).

101. Lv, B. *et al.* Characterization of Flowering Locus T1 (FT1) gene in Brachypodium and wheat. *PLoS ONE* **9**, (2014).

102. Shaw, L. M. *et al.* FLOWERING LOCUS T2 regulates spike development and fertility in temperate cereals. *Journal of Experimental Botany* **70**, 193–204 (2019).

103. Gauley, A. & Boden, S. A. Stepwise increases in FT1 expression regulate seasonal progression of flowering in wheat (Triticum aestivum). *New Phytologist* **229**, 1163–1176 (2021).

104. Yamaguchi, T. & Hirano, H. Y. Function and diversification of MADS-box genes in rice. *TheScientificWorldJournal* vol. 6 1923–1932 (2006).

105. Ouwerkerk, P. B., de Kam, R. J., Hoge, H. J. & Meijer, A. H. Glucocorticoid-inducible gene expression in rice. *Planta* **213**, 370–378 (2001).

106. Lowder, L. G. *et al.* A CRISPR/Cas9 toolbox for multiplexed plant genome editing and transcriptional regulation. *Plant Physiology* **169**, 971–985 (2015).

107. Miao, J. *et al.* Targeted mutagenesis in rice using CRISPR-Cas system. *Cell Research* vol. 23 1233–1236 (2013).

108. Sahoo, K. K., Tripathi, A. K., Pareek, A., Sopory, S. K. & Singla-Pareek, S. L. An improved protocol for efficient transformation and regeneration of diverse indica rice cultivars. *Plant Methods* **7**, (2011).

109. Toriba, T. *et al.* BLADE-ON-PETIOLE genes temporally and developmentally regulate the sheath to blade ratio of rice leaves. *Nature Communications* **10**, (2019).

110. Bleckmann, A., Weidtkamp-Peters, S., Seidel, C. A. M. & Simon, R. Stem cell signaling in Arabidopsis requires CRN to localize CLV2 to the plasma membrane. *Plant Physiology* **152**, 166–176 (2010).

111. Rast-Somssich, M. I. *et al.* Alternate wiring of a KNOXI genetic network underlies differences in leaf development of A. thaliana and C. hirsuta. (2015) doi:10.1101/gad.269050.