A common set of genes regulated by florigenic and photoperiodic induction at the shoot apical meristem of rice

DIPARTIMENTO DI BIOSCIENZE

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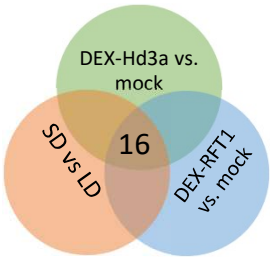
Introduction

Higher plants flower when the florigenic signal, expressed in the leaf blades, reaches the shoot apical meristem (SAM). It induces a phase shift through the formation of a florigen activation complex (FAC) which grants the transcription of floral identity genes, such as *OsMADS15*, *18* and *34*. In rice such signal is carried by the proteins encoded by the homologous genes *Hd3a* and *RFT1* upon induction in short day (SD) conditions. Many proteins involved in the process remain unknown. We created inducible lines in either *Hd3a* or *RFT1* allowing the expression of only one florigen at a time in non-inductive conditions using Dexamethasone (DEX)

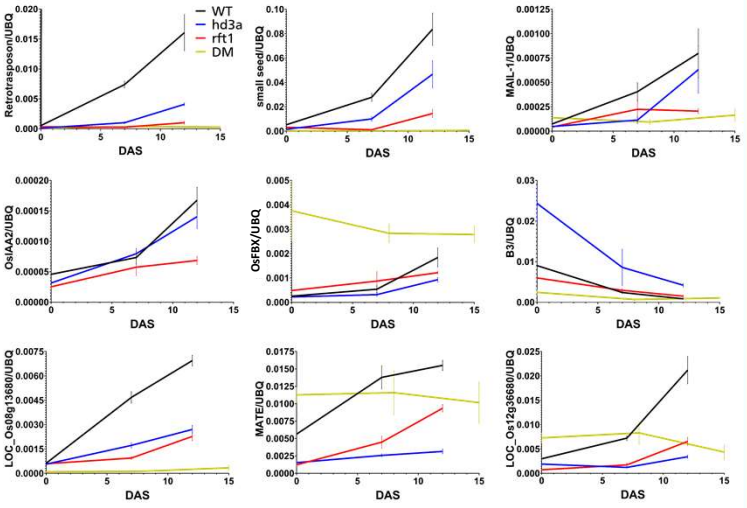
The datasets to unravel new players in the flowering pathway



Visual representation of the three datasets which have been crossed

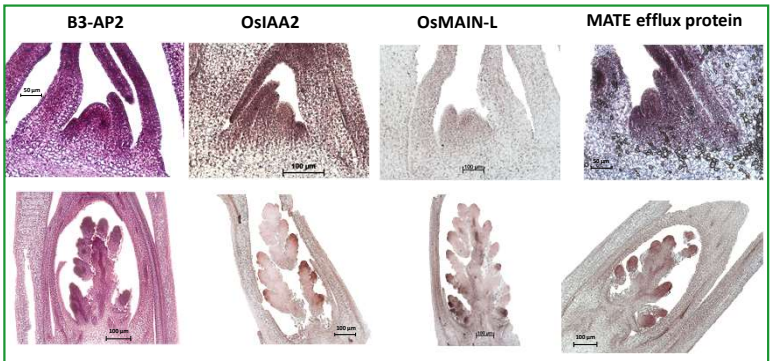
Three independent RNA-seq experiment were carried out to obtain differentially expressed genes in as many conditions. The first two datasets came from the comparison of the transcriptome of induced plants (either *Hd3a* or *RFT1*) and plants treated with a mock solution. Both batches were kept in long day (LD) conditions to minimize endogenous expression. The last one compared the expression in LD grown plants, and SD grown plants (the natural inductive condition). Crossing these datasets and imposing stringent conditions we found 16 genes of potential interest.

RNA-seq genes are regulated by the florigens



qRT-PCR showing the expression of 9 genes from the datasets in wild type plants and in mutants where either Hd3a, RFT1 or both has been knocked down through CRISPR-Cas9. Ubq was used for normalization. DAS=Days after shift (from LD to SD to induce flowering). n=3. DM = double mutants

qRT-PCR were performed on SAM tissues to assess the expression during the floral transition of 9 still uncharacterized genes taken from the dataset. The trends confirmed the RNA-seq data, showing a milder change in the expression levels along the transition in mutant plants, even stronger in double mutants where the WT trend is abolished. This means that in the absence of florigens these genes are de-regulated. Interestingly, it seems that the expression is most affected in *rft1* lacking plants, rather than *hd3a*.



In situ hybridization of four genes from the dataset, in two different timepoints (inflorescence meristem IM in the upper row and primary branches PB in the lower).

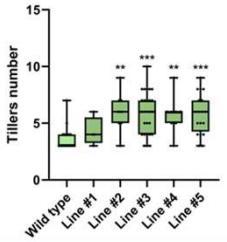
The expression pattern is once again in agreement with RNA-seq observation, with «*B3-AP2*» levels being more clear and detectable in the IM phase. As for the other three genes, the signal in the IM is absent or negligible, while in the PB is strong and clear in the primary and secondary branches tips.

F-box mutant line #5 have a wider tillering angle

Mutant lines in one of the genes (*OsFBX*) were created through CRISPR-Cas9 technique. Plants have been grown in LD (16H) for two months. The seedlings show a slowed growth and a paler look, which begins to recover after two weeks from sprouting. At one month the plants have started tillering and the tillers of line #5 started to spread, in contrast to WT plants and other lines. At two months, all tillers are out and line #5 show a peculiar architecture.

Wild type and f-box mutant plants at different stages of growth: A) 6 days old seedlings, B) one month old plants and C) two months old plants

F-BOX mutant plants have a higher tiller number



Plants have been grown in LD for 2 months, and tillers have been counted in 5 lines, each carrying homozygous independent alleles. All mutated lines showed an increase in the tiller number when compared to wild type plants

Tiller number of 2 months old plants grown in LD.

Conclusions

We identified a common set of genes which integrate the florigenic response and the photoperiod at the shoot apical meristem, and among them an F-BOX protein encoding a gene who is responsible for the number of tillers and their architecture.

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