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UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI BIOSCIENZE

7 - The *ALOG* family members *OsG1L1* and *OsG1L2* regulate inflorescence branching in rice

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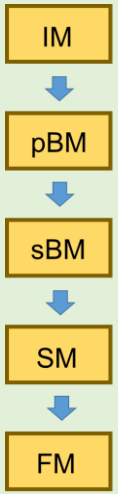
Human population growth makes of primary importance to find new ways to improve agricultural crop production and meet the increasing food demand. **Inflorescence architecture is one of the key agronomical traits which determines grain yield**; thus, it has been a major target for crop domestication and improvement.

In ***Oryza sativa***, **inflorescence architecture is established at early stages of reproductive development.**

The *ALOG* gene *TAWAWA1* (*TAW1*) has been shown to be a regulator of meristem activity [1]. Combining laser microdissection of rice inflorescence meristems with RNA-seq, we observed that other two members of the *ALOG* gene family, ***OsG1-like 1* (*OsG1L1*) and *OsG1L2***, present an expression profile similar to *TAW1*. Furthermore, the loss-of-function CRISPR mutants *osg1l1* and *osg1l2* present a phenotype similar to the *taw1* mutant, suggesting that **these three genes may act in related pathways controlling inflorescence development.**



1- Phenotypical analysis of panicle architecture of *osg1l1* and *osg1l2* mutants

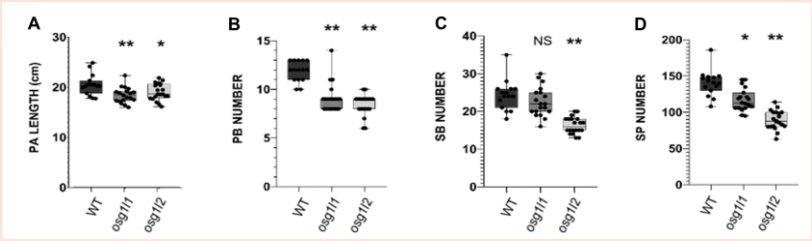


Figure 1. Graphs representing the comparison of: (A) Panicle (PA) Length, (B) Primary Branch (PB) number, (C) Secondary Branch (SB) number, (D) Spikelet/seed (SP) number in WT, *osg1l1* and *osg1l2* backgrounds. (n=20; One-Way ANOVA with Tukey test; **p<0,01; * p<0,05)

The main panicles of WT, *osg1l1* and *osg1l2* mutants were compared. Both **mutants showed** similar **aberrations in panicle architecture** although the phenotype of the ***osg1l2* mutant was more severe.**

2- RNA-seq analysis of *osg1l2* allowed the inference of a Gene Regulatory Network (GRN)

RNA-seq analysis was performed on early stages inflorescences of *osg1l2* and wt plants. The obtained list of differentially expressed genes (**DEGs**) was **used to infer a GRN** using a regression tree with random forest approach. We focused on the gene cluster in which *OsG1L2* is present. Interestingly, **other genes already known to be involved in inflorescence development (like *OsFC1* [2], *OsMADS34* [3], *OsESP* [4]) are present.**

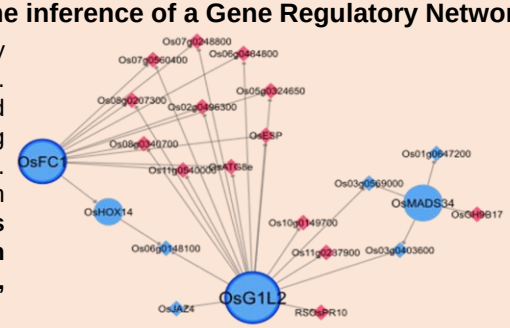


Figure 2. GRN: Transcription factors and other genes are represented in circles and diamonds, respectively. The interactions are represented by an arrow. Upregulated and downregulated genes are in magenta and blue, respectively.

3- Validation of GRN by qRT-PCR

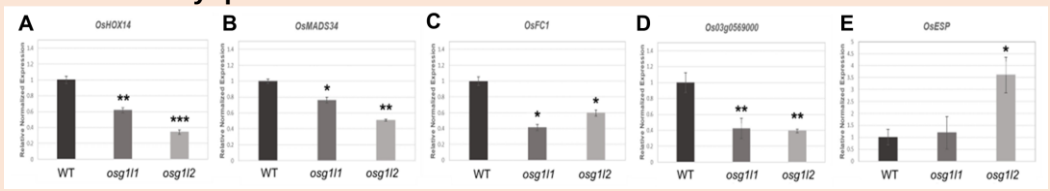
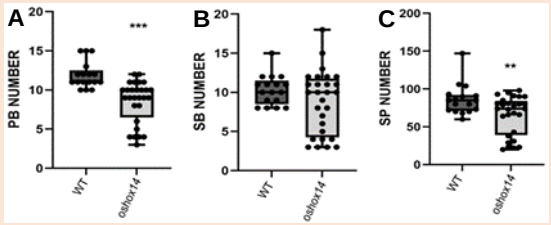


Figure 3. Expression analysis of *OsHOX14* (A), *OsMADS34* (B), *OsFC1* (C), *Os03g0569000* (D), *OsESP* (E) by qRT-PCR in wild type (WT), *osg1l1* and *osg1l2* mutants. The expression was normalized to that of *Elongation Factor 1* and the expression level of wild type was set to 1. The asterisks indicate: *** p< 0,001; **p<0,01; * p<0,05, student's t-test.

4- Gene regulatory network inference predicts a functional role for *OsHOX14*



Since for *OsHOX14* only an overexpression study is reported [5], we selected this gene for further studies to validate the GRN predicted involvement of this gene in inflorescence development. This analysis revealed that the *oshox14* mutants developed panicles with less PBs and spikelets/seeds than wild-type plants; and so that ***OsHOX14* plays a role in inflorescence branching as predicted by the GRN.**

Figure 4. Phenotypical analysis of panicle architecture in wild type and *oshox14* mutant. Graphs representing the comparison of: A, Primary Branch (PB) number; B, Secondary Branch (SB) number; C, Spikelet/seed (SP) number in WT and *oshox14* backgrounds. (n=5; *** = p< 0,001; ** = p<0,01; * = p<0,05, Student's t-test)

CONCLUSIONS:

- ***OsG1L1 and OsG1L2*** play an **important role in inflorescence development**;
- *OsG1L1* and *OsG1L2* **probably act in the same pathway**;
- *OsG1L2* seems to act in pathways that include ***OsMADS34*, *OsHOX14* and *OsFC1***;
- The functional analysis of *OsHOX14* indicates that the proposed **GRN promises to be of value for the identification of new players in the first stages of inflorescence development**.

REFERENCES:
1 Yoshida, A. et al.. 2013. TAWAWA1, a regulator of rice inflorescence architecture, functions through the suppression of meristem phase transition. Proceedings of the National Academy of Sciences of the United States of America, 110(2), 767–772.
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3 Gao, X. et al. 2010. The SEPALLATA-like gene OsMADS34 is required for rice inflorescence and spikelet development. Plant Physiology, 153(2), 728–740.
4 Luan, X. et al. 2019. Epigenetic modification of ESP, encoding a putative long noncoding RNA, affects panicle architecture in rice. Rice, 12(1), 20.
5 Shao, J. et al. (2018). Functional analysis of the HD-Zip transcription factor genes Oshox12 and Oshox14 in rice. PLOS ONE, 13(7), e0199248.