Interactomics for Rice Flowering: a Proximity Labelling Approach

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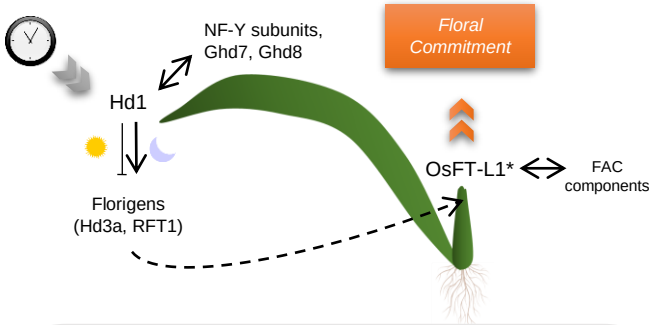
ABSTRACT

Variability in flowering-controlling genes has been fundamental over history for adapting rice cultivation to different latitudes. The molecular network controlling floral induction in rice is highly complex and it is the result of a stratification of interactions: transcriptional, epigenetic and post-translational. A relatively new technique called Proximity Labelling (PL) allows a high-throughput identification of protein interactors by molecular engineering, and has never been tested in rice plants. The goal of this research is to implement PL in rice, establishing efficient vectors and protocols while applying it to the identification of interactors of two rice flowering regulators: OsFT-L1 and Hd1. The application of PL to OsFT-L1 and Hd1 will deliver a list of the proteins laying in their contiguity. The method exploits a biotin-ligase fused to the the two proteins of interest. Exposure of tissues to biotin will lead to biotinylation of OsFT-L1- and Hd1- interacting proteins. Biotin tags will allow for selective precipitation of the proximal proteome followed by mass spectroscopy analysis.

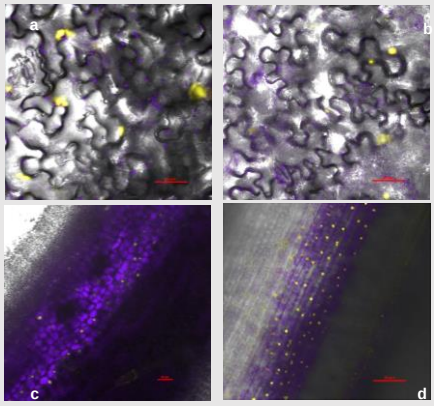


*Note: for further up-to-date information on OsFT-L1 function go to oral presentation: *"A triple florigen system is essential for flowering and panicle architecture in rice"* – Francesca Giaume (University of Milan)

CONFOCAL MICROSCOPY

a,b) Fluorophore-conjugated OsFT-L1 and Hd1 infiltrated in *N. benthamiana* leaves.

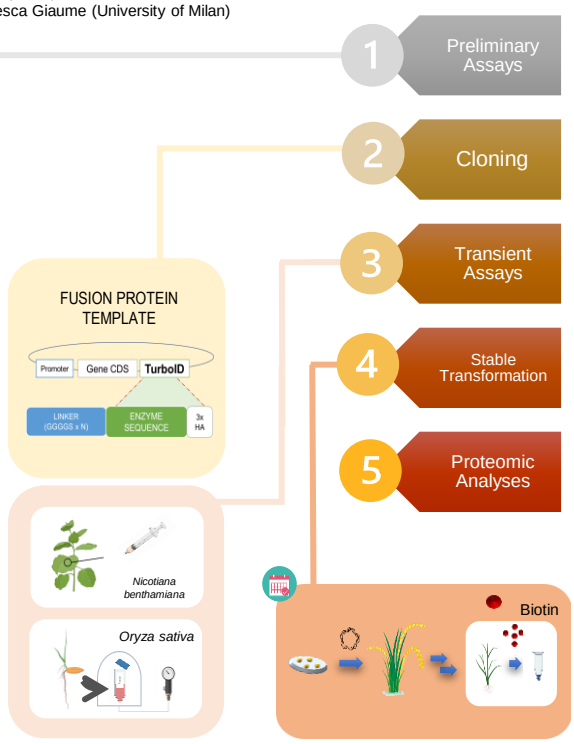
c, d) Fluorophore-conjugated Ghd8 and Hd1 transiently expressed in rice seedlings.



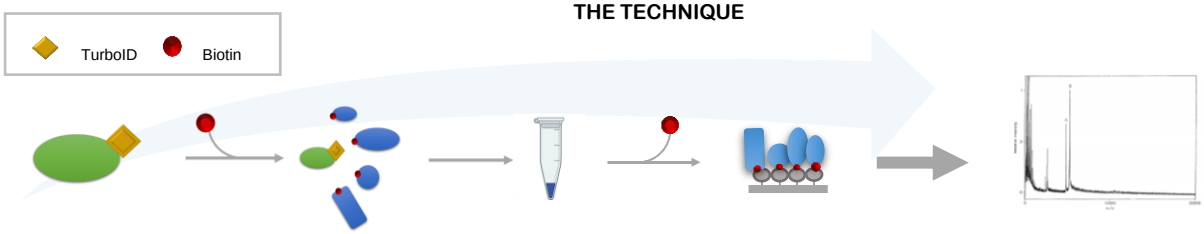
BIOCHEMICAL ANALYSES

Western Blot assays from agroinfiltrated *N. benthamiana* leaves (data not shown) indicate that both proteins are present, although Hd1 protein seems to be less stable than OsFT-L1 in *N. benthamiana*.

PROJECT WORKFLOW



THE TECHNIQUE



FUTURE PERSPECTIVES

This project will allow the implementation of a new proteomic approach in rice, providing a list of presumed interactors of Hd1 and OsFT-L1, and by this potentially unveiling undisclosed aspects of flowering regulation in rice based on post-translational control. Moreover, a recently described methodology for rice transformation by transient assays will be used for the study of flowering control, for protein analysis and for proteomics. The findings of this research could support rice breeding in view of yield improvement and acclimation to challenging environmental changes.

REFERENCES: Zhang et al. (2020); Burman et al. (2020); Du et al. (2017)