New details on OsFTL1 florigenic protein revealed by Proximity Labelling in rice

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The transition to the reproductive stage is a fundamental step of the plant life cycle. In angiosperms, a set of proteins, called florigens, belonging to the family of phosphatidylethanolamine-binding proteins (PEBPs), induce the differentiation of cells at the shoot apical meristem (SAM) to prompt the development of floral organs at specific photoperiodic conditions. The downstream molecular pathway is highly complex, resulting from a series of transcriptional, epigenetic and post-translational events.

In rice (*Oryza sativa*), the variability of flowering-controlling genes has been essential over history to increase the crop yield and adapt its cultivation to different latitudes. Nevertheless, the extent of cooperation between flowering regulators is far from being completely understood, as the circumstances of protein-protein interactions remain mostly unknown.

OsFTL1, a PEBP protein, is a key player in rice reproductive development, controlling the acquisition of meristem identities during the flower differentiation process. OsFTL1 also influences traits of agronomic interest, such as panicle branching and floret fertility.

We established, for the first time in stably transformed rice plants, the Proximity Labelling (PL) technique, to analyse the interactome of OsFTL1. PL exploits an optimized biotin-ligase fused to the protein of interest allowing the biotinylation of proteins laying in its contiguity and in specific organs (here, the SAM). The subsequent selective precipitation of its proximal proteome, followed by mass spectrometry and bioinformatic analysis, returns a list of the potential interactors of the target protein. Differently from other proteomic techniques, the *in vivo* labelling allows the identification of feeble and transient interactions, as well as tracing the cell localization of the target protein.

The protocol application on rice flower meristems has delivered a list of about 3000 proteins, 12 of which have been statistically identified as highly probable OsFTL1 interactors in two distinct stages of reproductive differentiation. Some of these proteins are transcription factors which had never been reported to control flower development. Others are organelle-specific candidates highlighting new functional properties of OsFTL1, which may be common to florigen-like proteins of other crop species. A set of experiments supporting the biological validity of the proteomic analysis are also here presented.

This research underlines the importance of omic approaches to the study of post-translational molecular networks for disclosing new components of plant developmental processes. Furthermore, our research can provide selectable genetic markers and gene-editing targets for improving yield and environmental adaptability of cultivated rice varieties.