

CONTROLS OF STEM ELONGATION BY THE FLOWERING PATHWAY IN RICE

VICENTINI G.*, BERTAGNON G.*, GIAUME F.**, FORNARA F.**, BRAMBILLA V.*

*) Università degli Studi di Milano, Dipartimento di Scienze Agrarie e Ambientali

**) Università degli Studi di Milano, Dipartimento di Bioscienze

stem elongation, rice flowering, stem gibberellin sensitivity, PREMATURE INTERNODE ELONGATION 1 (PINE1)

Environmental factors, particularly photoperiod, play a crucial role in rice flowering. Short daylength triggers the synthesis of florigens in the leaves, which are then transported through the phloem to the shoot apical meristem. The arrival of florigens induces the transition from a vegetative meristem to an inflorescence. Flowering also causes other architectural changes, in particular florigens are responsible to initiate stem elongation, which is essential for panicle heading and seed dispersion.

The process of flowering and stem elongation needs thus to be coordinated, and a genetic link between the two processes has been found in the gene *PREMATURE INTERNODE ELONGATION 1 (PINE1)*. *PINE1* is a transcription factor expressed during the vegetative growth. It's responsible for the maintenance of an unelongated stem. With the perception of short days and the arrival of the florigens, *PINE1* expression is inhibited, permitting stem elongation. As such, *pine1* knockout mutant obtained with CRISPR/Cas9 displays a strong phenotype of premature internode elongation, which start from a seedling stage and is uncoupled from the flowering pathway. Conversely, overexpression of *PINE1* results in a dwarf phenotype, confirming its role as an inhibitor of stem elongation.

In order to study the molecular mechanism of action of *PINE1*, we studied its interactors. We determined through Yeast two-hybrid that *PINE1* can interact with TOPLESS-corepressor proteins, and we observed that the interaction is dependent on the presence of EAR motifs in *PINE1* sequence. Furthermore, the same TOPLESS can interact with an HISTONE DEACETYLASE (HDAC). This data suggests the existence of the complex *PINE1*-TOPLESS-HDAC

which could modify downstream gene expression by chromatin compaction. We also demonstrated the significance of these EAR MOTIFS for proper PINE1 function, as transgenic expression of PINE1 clones with alterations in these motifs failed to complement the *pine1* knockout mutant phenotype. Through RNA-sequencing we are trying to determine which genes are under the control of PINE1, and the study of some of those genes is currently ongoing.

Finally, stem elongation is a critical agronomical trait for cereals due to its association with lodging. Shorter stems contribute to increased lodging resistance. To utilize *PINE1* as a dwarfing gene, we generated plants with various modifications in the *PINE1* promoter, using a CRISPR/Cas9 multiplex approach, targeting eight sites in *PINE1* promoter region. Some deletions effectively prevented PINE1 inhibition during flowering, resulting in overexpression of *PINE1* and significantly shorter stems.