Identification and functional characterization of abiotic stress-responsive RING E3-Ubiquitin ligases in rice

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Background and Objectives

Rice is one of the most important crops worldwide and a traditionally important crop in Portugal. However, its production is highly affected by various abiotic stresses, such as drought and high salinity. Over the last decades, an enormous effort has been made to identify key-players regulating stress responses in rice (mainly transcriptional regulators and functional genes). However, little is known how the proteome pool is affected under stress, especially by the action of Ubiquitin-Proteasome system (UPS). The modulation of key components of the UPS has been established as a potential candidate for crop improvement programs towards abiotic stress tolerance (Melo et al., 2021). Among the different components of the UPS, the E3-ubiquitin ligases are the most abundant group. In rice, still a large number of E3-ubiquitin ligases remain uncharacterized. In our group, we are following a candidate gene approach to identify and functionally characterize E3-ubiquitin ligases involved in abiotic stress response. We selected 16 putative RING E3-ligases showing differential gene expression under stress, and validated their gene expression profile by RT-qPCR using in-house stress assays. Four of these RING E3-ubiquitin ligases were selected for a deepen functional characterization using a transgenic approach either overexpressing or knock-out the gene through CRISPR, and identifying its targets by Yeast-Two hybrid. At the moment, we have observed several interesting phenotypes putatively related to abiotic stress tolerance such as erect leafs, long lateral roots or heat-tolerance at the germination stage, and identified several putative targets (such as heat-shock proteins) which are being validated (BiFC, degradation assays, etc.). Taken together, this work will enhance our comprehension of the molecular and physiological mechanisms underlying the function of these E3-ubiquitin ligases to develop stress tolerant crops.

Fig. 1. A candidate-gene approach identified 16 putative stress-responsive E3-ubiquitin ligases using in silico tools.
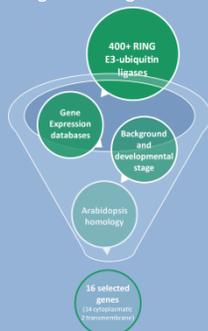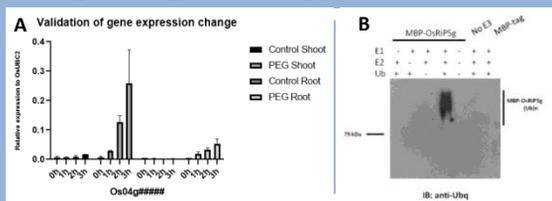

Fig. 2. After in-house assays for gene expression validation (A), 4 genes were selected for further functional characterization, including auto-ubiquitination assay (B).

PEG – 30% Polyethylene glycol 6000;
Control – no PEG treatment

Fig. 3. We also aimed to identify putative targets of our selected E3-ubiquitin ligases through Yeast Two-hybrid (4A) and validating those interactions by BiFC (4B).
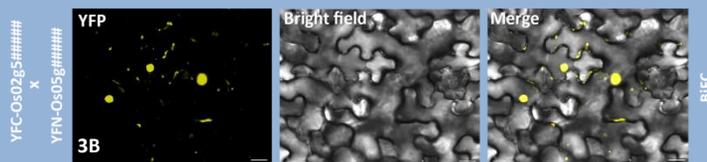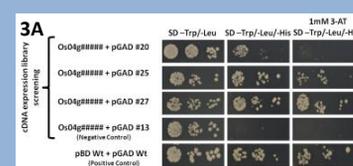

Fig. 4. Overexpression (OX) and Knock-out (KO) lines were produced for the 4 genes selected, which are being characterized in response to abiotic stress; Transgenic plants showing phenotypes. A – the transgenic plant (left) shows erect leaves; B – the transgenic plant (left) shows long primary root; C – KO seedlings show better development under heat-stress.

Acknowledgments

Bibliography

Melo, F.V., Oliveira, M.M., Saibo, N.J.M., Lourenço, T.F. (2021) Modulation of Abiotic Stress Responses in Rice by E3-Ubiquitin Ligases: A Promising Way to Develop Stress-Tolerant Crops. Frontiers Plant Sci. 12: p. 368. DOI:10.3389/fpls.2021.640193