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Poster Communication Abstract – PH.41

HYPOXIA SIGNALLING MECHANISMS: FROM PLANTS TO YEAST

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Flooding events are one of the main causes linked to crop losses worldwide. Flooded plants suffer from hypoxia, which leads to a metabolic crisis that most crop plants cannot overcome. Sensing the environmental availability of oxygen is essential to most organisms on Earth. Various mechanisms enable different species to perceive oxygen and regulate its usage accordingly. Plants exploit the cysteine branch of the N-degron pathway to attune selective proteolysis to oxygen availability. This does not occur in the yeast *Saccharomyces cerevisiae* as its proteome lacks a key regulatory enzyme to oxidize N-terminal cysteine to Cys-sulfinic acid. Hence, yeast holds great potential as a chassis to engineer, study and optimize orthogonally the oxygen hypoxia signalling mechanisms from plants. We therefore developed an orthogonal reporter for oxygen levels, namely the Dual Luciferase Oxygen Reporter (DLOR). This synthetic construct consists of two bioluminescent enzymes, renilla and firefly luciferases, separated by an ubiquitin monomer to ensure post-translational cleavage of the two units. The firefly luciferase is equipped with an N-terminal degron, from the plant transcription factor RAP2.12, which confers oxygen-dependent instability when its N-terminal Cys is oxidized. In the present work, we show that the expression in yeast of Plant Cysteine Oxidase enzymes (PCOs) is sufficient to enable the N-degron dependent proteolysis of firefly luciferase. Furthermore, we characterized the dynamics and oxygen sensitivity of the DLOR-PCO cuple. The activity of the five *Arabidopsis thaliana* PCO isoforms shows a degree of correlation with the studies performed *in vitro*. The effect of point-mutations on PCO enzymes can be reproduced in our setup in yeast. Our system holds promising potential for detailed and high-throughput studies of the components of the cysteine branch of the N-degron pathway, crucial not only for plants, but also several other organisms.