

A NOVEL OXYGEN SENSOR FOR USE IN PLANTA TO MONITOR BIOTIC AND ABIOTIC STRESS

PANICUCCI G.*, IACOPINO S.****, DE MEO E.**, PERATA P.***, WEITS D.A.***

*) Biology Department, University of Pisa, Pisa (Italy)

**) Department of Pharmacy and Biotechnology, University of Bologna (Italy)

***) Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa (Italy)

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Oxygen levels in plant tissues are strongly affected by environmental availability and pathological processes. Indeed, oxygen availability is heavily reduced upon natural phenomena such as flooding and waterlogging, in which case the depletion of molecular oxygen causes the activation of adaptive responses, such as fermentation. On another note, plant defense mechanisms can be induced upon the establishment of hypoxic niches within aerobic tissues following infection of pathogenic organisms such as *Botrytis cinerea*. Moreover, adaptive responses of the plant to hypoxia are required for the continued growth of *Agrobacterium* and *Plasmodiophora* induced hypoxic galls. Therefore, monitoring hypoxia signaling and oxygen gradients within plant tissues can provide insights on adaptive responses and defense mechanisms employed by plants facing both biotic and abiotic stresses. In this work, we set out to develop and optimize a set of genetically encoded oxygen biosensors for use in plants. We developed a promoter based on a five times repetition of the hypoxia responsive promoter element (HRPE) fused to the 5' Ω leader sequence of the tobacco mosaic virus (TMV) and we employed it to drive expression of various reporter proteins. We were able to show that our sensor was activated within two hours of externally imposed hypoxia, and stronger activation was observed progressively as external O₂ concentration dropped below 5% v/v. Moreover, we employed the recently characterized oxygen-independent UnaG fluorescent protein coupled to an oxygen-dependent mCherry fluorophore driven by our 5xHRPE- Ω promoter to create a ratiometric sensor able to distinguish between different degrees of tissue hypoxia. Additionally, we show that a constitutively expressed Unag-mCherry fusion can be used to directly measure O₂ concentration, therefore uncoupling the sensor from oxygen signaling. Next, we aim to use these tools to characterize the role of O₂ dynamics in abiotic and biotic stresses.