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Use of glycans metabolic engineering for studying plant cell wall biosynthesis and functions

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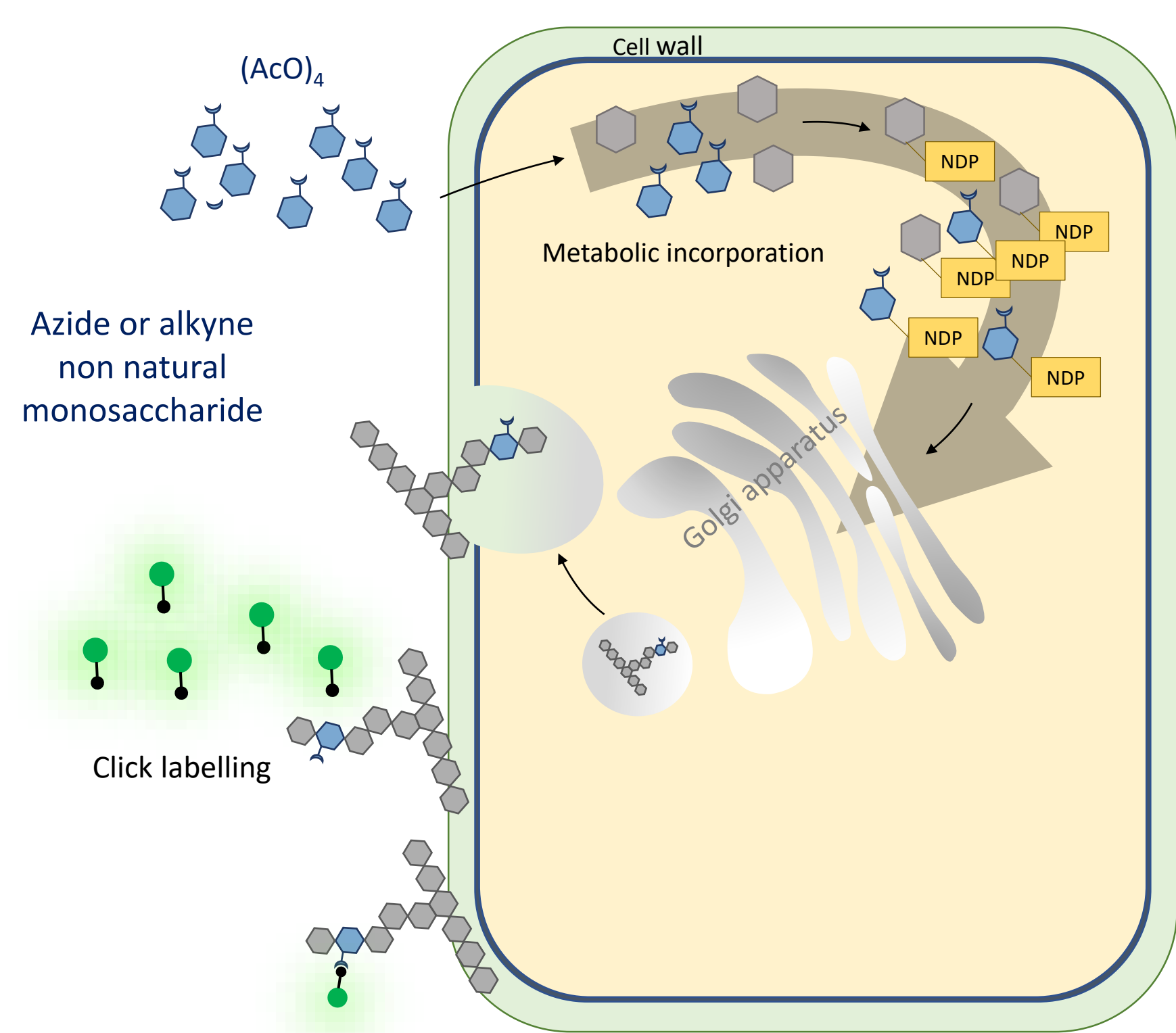


ABSTRACT

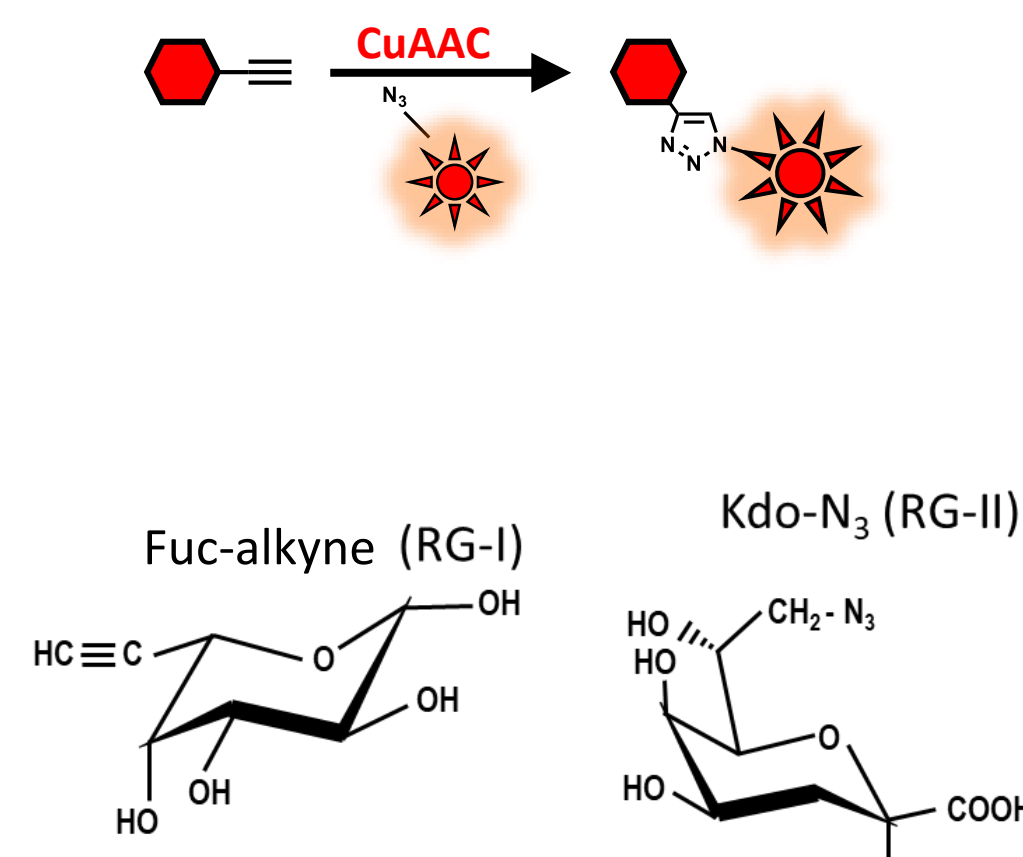
Glycans metabolic engineering is a powerful tool for studying the glycosylation of living cells [1, 2]. The use of modified monosaccharides, such as deoxy or fluorinated sugars, has been reported as a powerful pharmacological approach for studying carbohydrate metabolism and plant cell wall biosynthesis [3]. Moreover, non-natural metabolite derivatives that carry functions enabling bio-orthogonal ligations are now widely used for glycomolecules imaging in living organism. In both cases, these derivatives must cross the cell membrane and be accepted by the biosynthetic machinery of the cell to produce nucleotide-sugars that will be taken in charge by the enzymatic machinery to build complex glycomolecule such as cell wall polysaccharides in plant. Here, we illustrate the use of glycan metabolic engineering, photo release of caged monosaccharide derivative and click-chemistry, for studying the plant cell wall biosynthesis and function with an emphasis of the pectic domain rhamnogalacturonan II [4, 5].

Metabolic Labeling

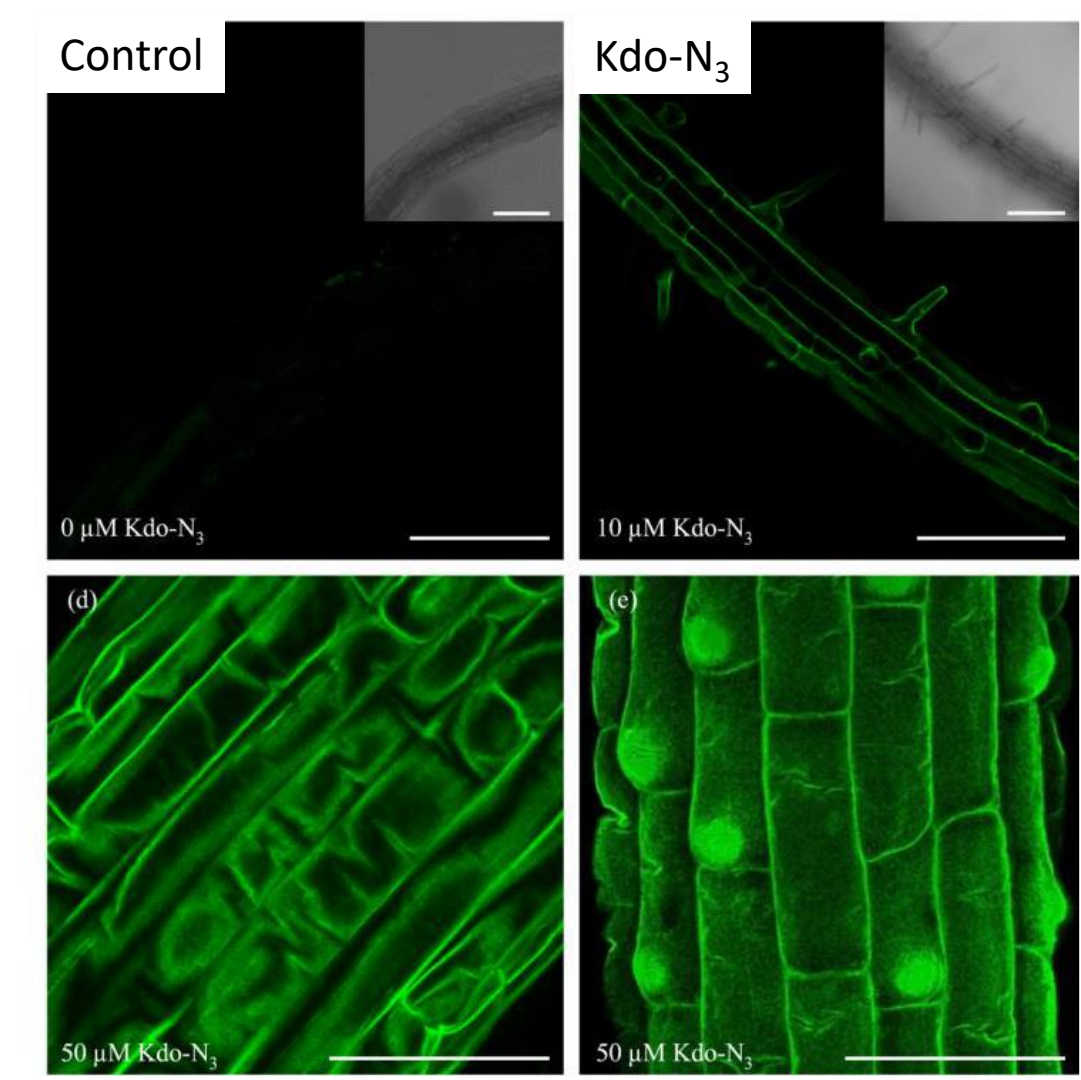
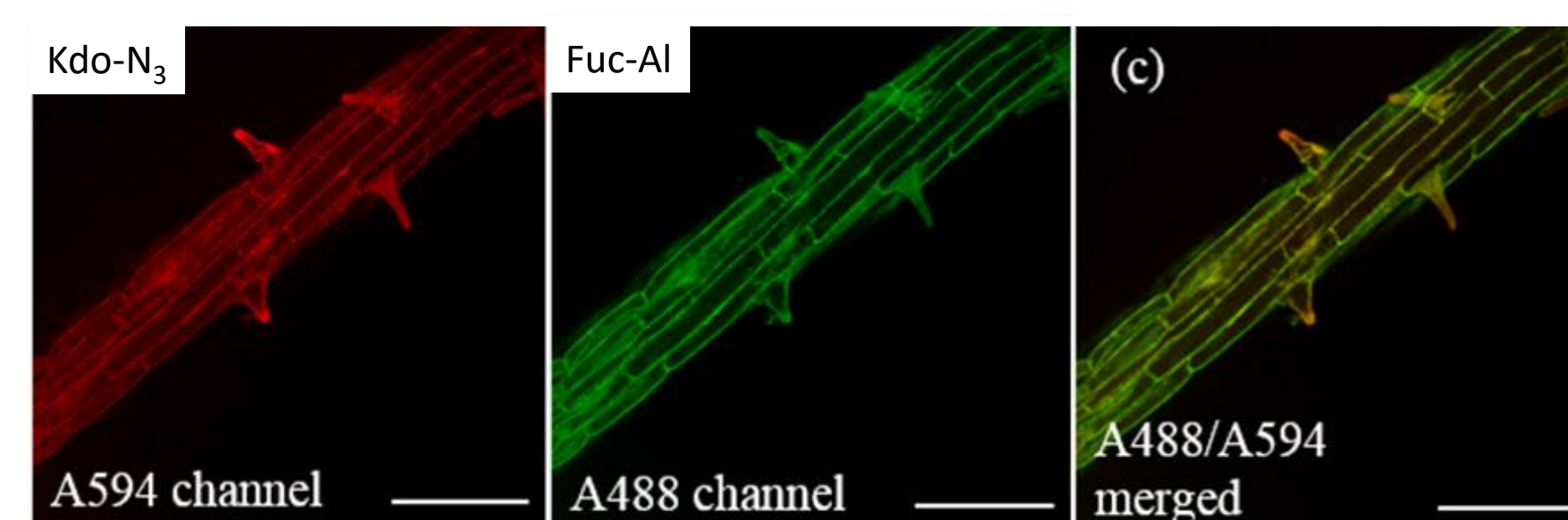
Plant cells are feeded with acetylated non-natural sugar derivatives carrying azide or alkyne function. Monosaccharides are incorporated during the biosynthesis of plant cell wall polysaccharide before being visualized through a bioorthogonal reaction with a fluorophore.



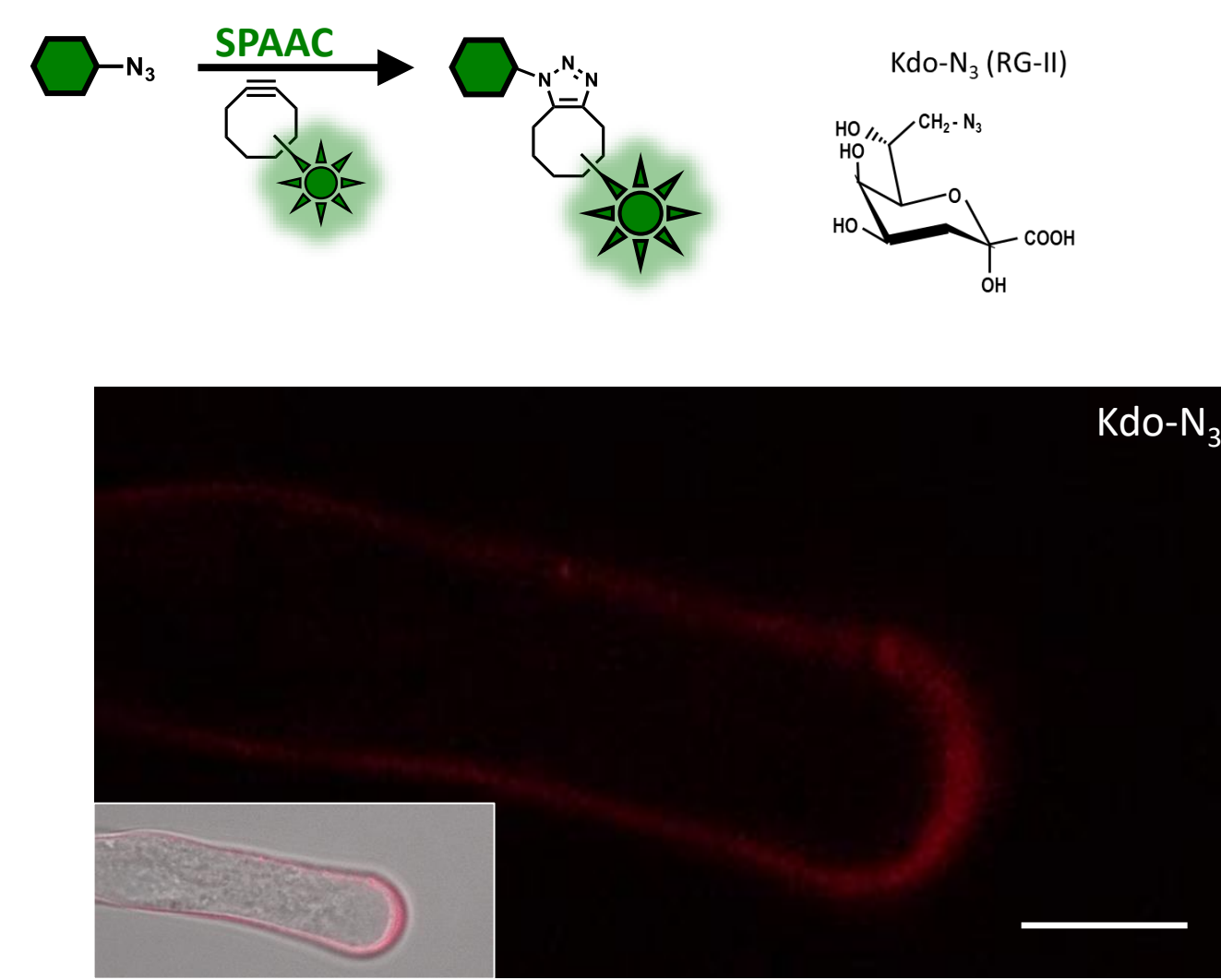
CuAAC : Labeling of pectic polysaccharide in plant cell wall using Fucose and Kdo derivatives



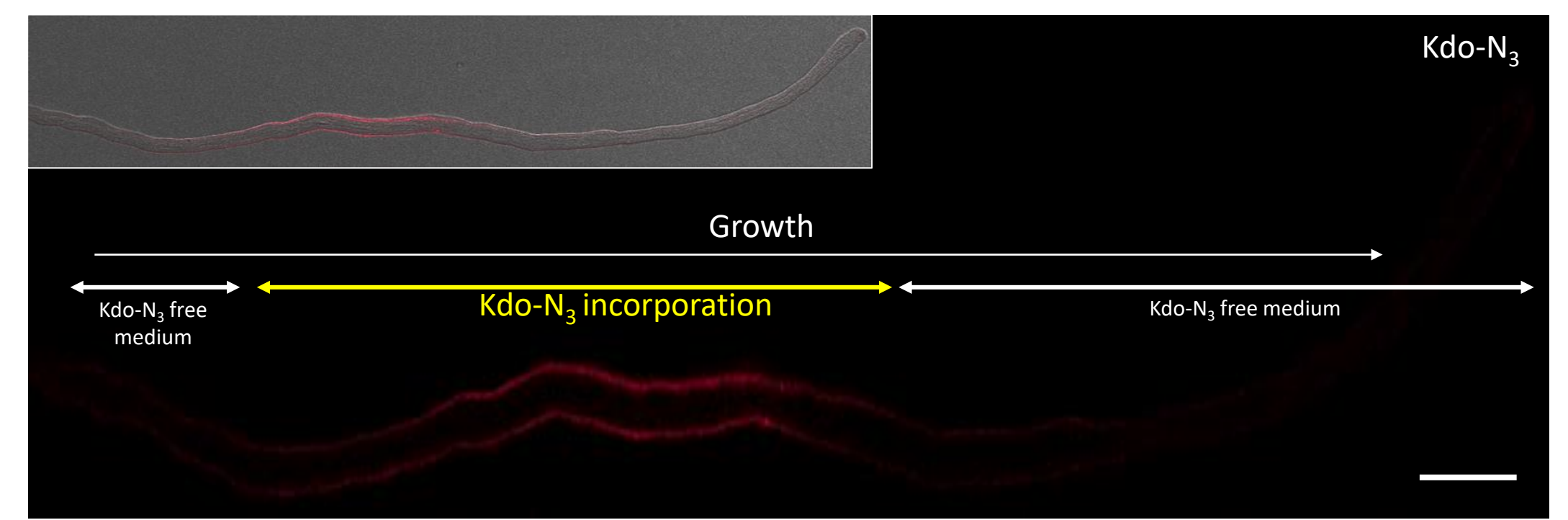
Kdo-N₃ and Fuc-Al are incorporated respectively in rhamnogalacturonan I and rhamnogalacturonan II [3,6]. CuAAC and SPAAC are two complementary technics that enable the double labeling of cell wall carbohydrate (CuAAC) or the labeling of living and elongating cells (SPAAC). SPAAC offers the possibility for live-imaging of polysaccharides deposition and recycling during cell growth [7].



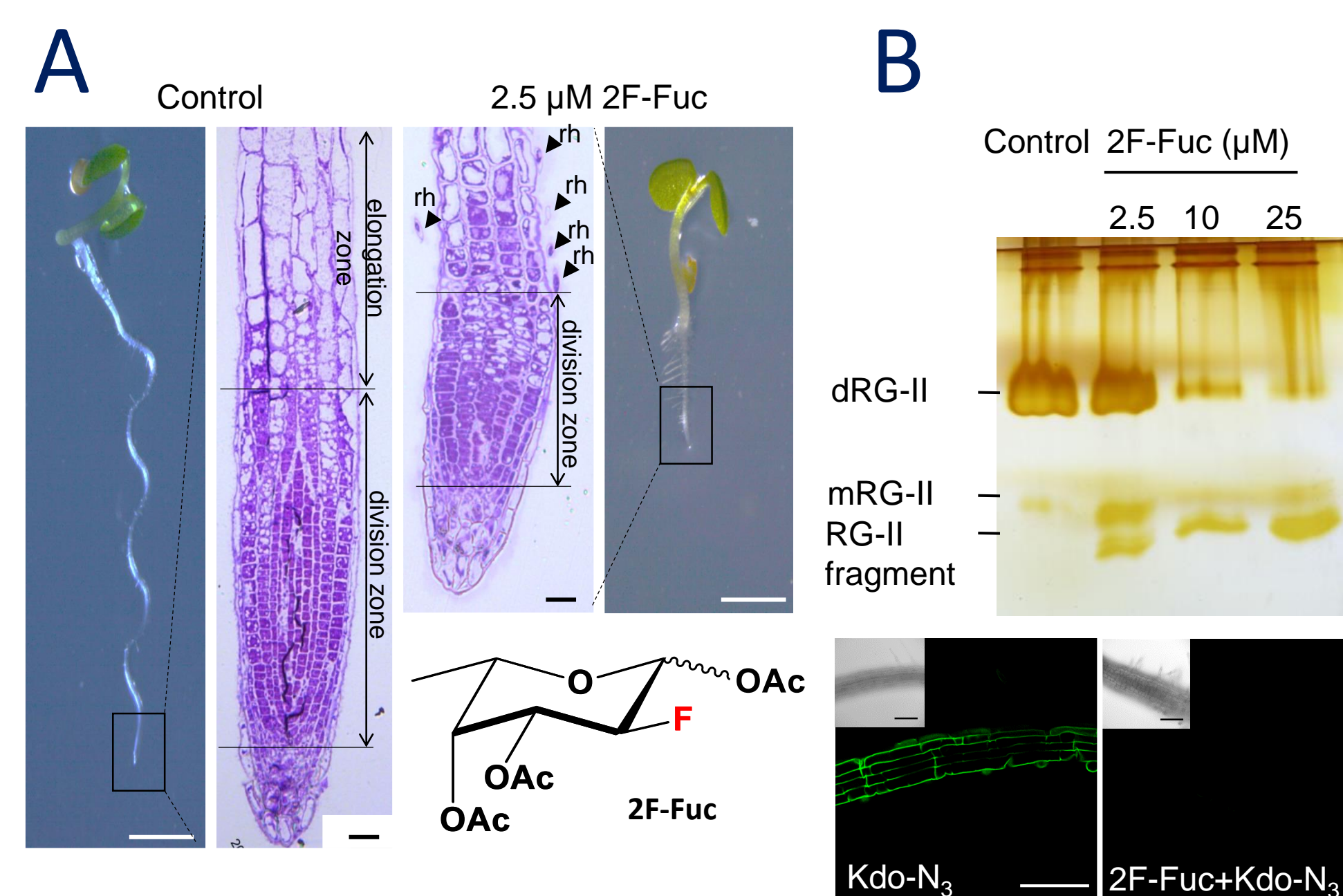
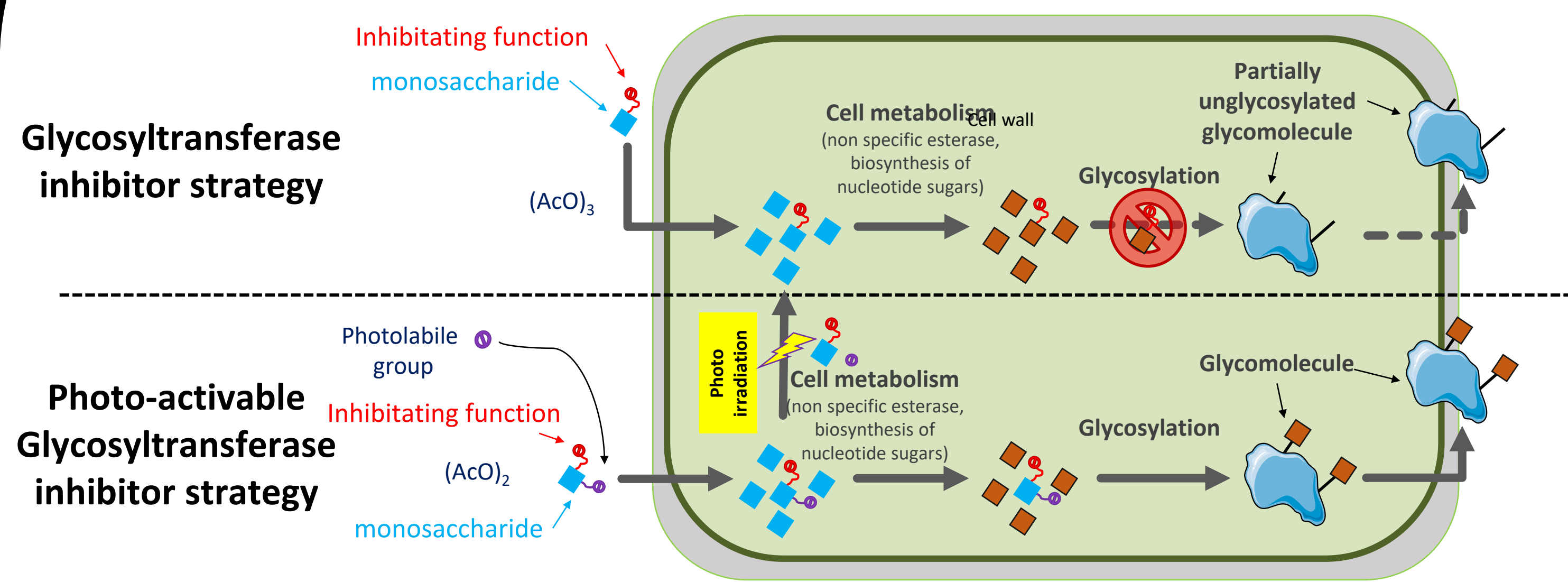
SPAAC : Labeling of pectic polysaccharide in LIVING plant cell wall using Kdo derivatives



Metabolic labeling of rhamnogalacturonan II in living and elongating tobacco pollen tubes

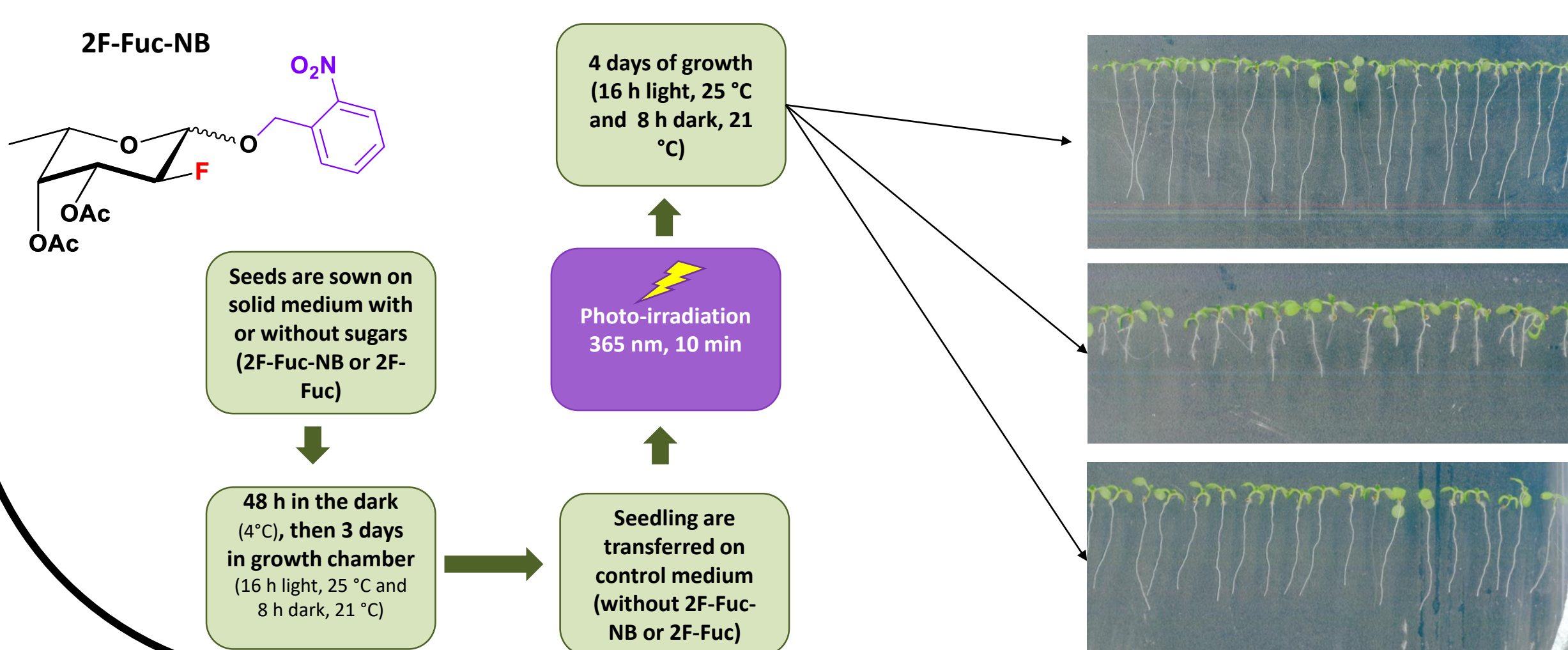


Glycosyltransferases inhibitors

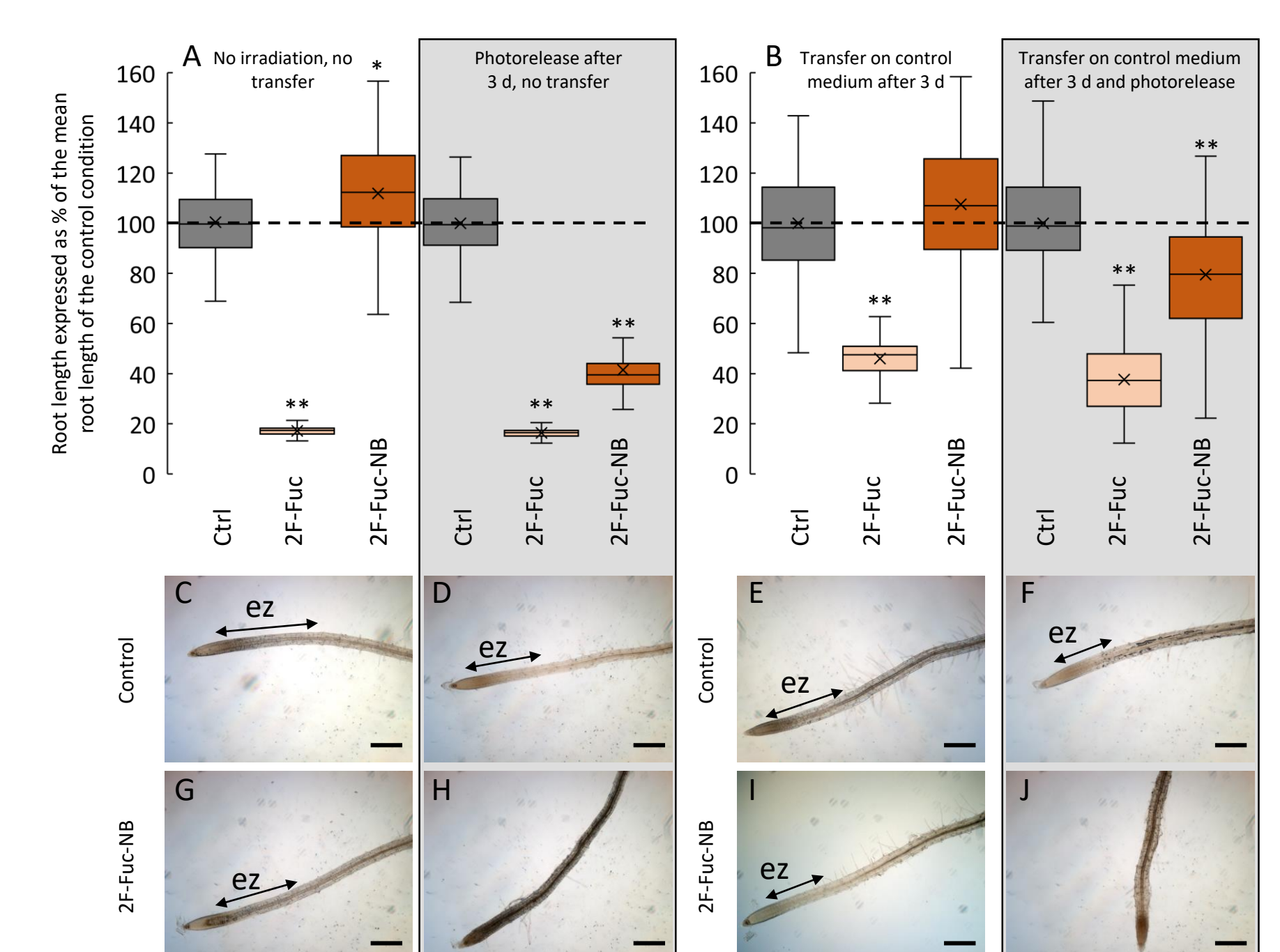


2F-Fuc is incorporated and inhibits root elongation (A) by disrupting the pectic domain rhamnogalacturonan II (RG-II) synthesis and dimerization (B)[4]. RG-II were separated by gel electrophoresis and RG-II was imaged using metabolic labeling followed by CuAAC with Alexa fluor 488 (B).

Photoirradiation of 2-fluoro sugar caged by an ortho-nitrobenzyl alcohol (2F-Fuc-NB) on the anomeric carbon results in the release of free 2-fluoro monosaccharide and ortho-nitrosobenzaldehyde through aci-nitro intermediates.



The photorelease of the trapped 1-OH-2F-Fuc was performed under a 365 nm LED illumination. We demonstrated that the *in planta* elimination by photoexcitation of the photolabile group releases free 2F-Fuc in plant cells, which in turn inhibits in a dose-dependent manner and, reversibly, the root cell elongation [8]. 2F-Fuc was used as positive control.



Conclusion: The glycans metabolic engineering has the potential to contribute to the study of glycosylation of growing plants. The modification of glycoconjugates by a chemical reporter or by inhibition of part of their biosynthetic pathways during the growth of the plant can help to expand knowledge on the dynamics of formation of plant walls.

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