

Anastasiia Salivon, Alain Lécureuil, Catherine Rameau, Sandrine Bonhomme  
 Université Paris-Saclay INRAE, AgroParisTech, Institut Jean-Pierre Bourgin, 78000 Versailles, France

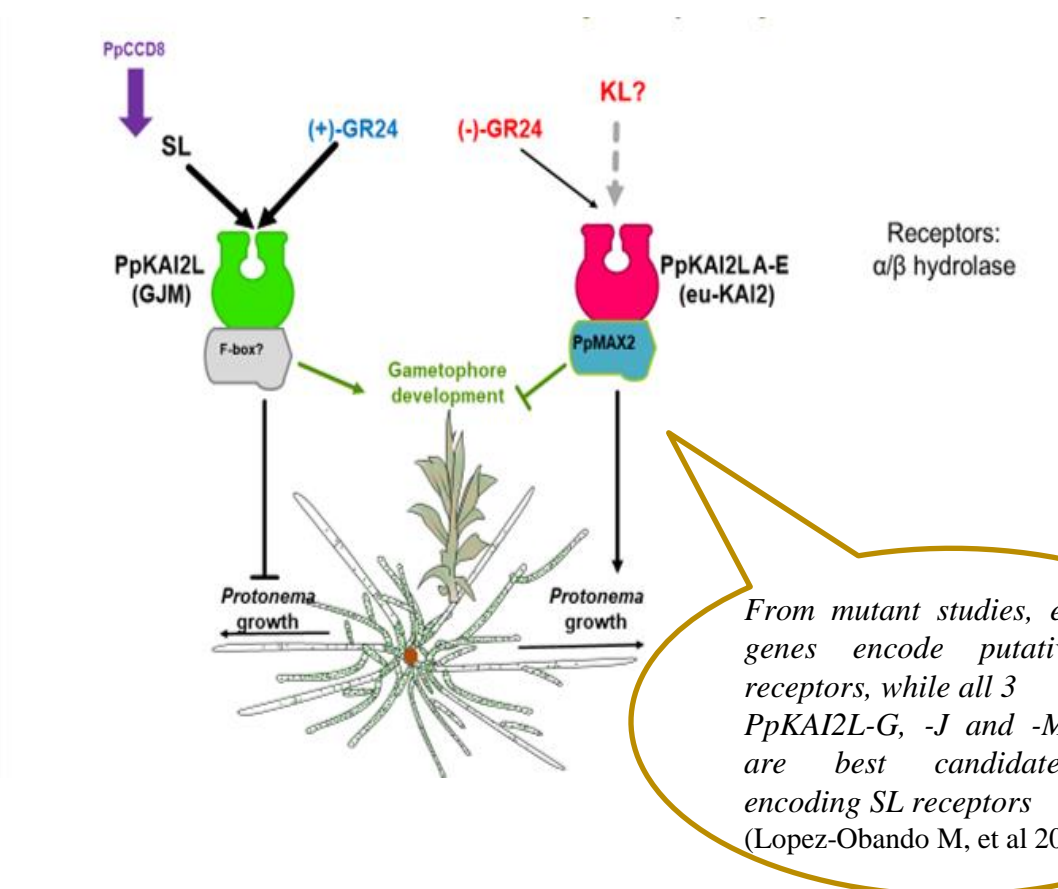
## Introduction: The Moss *Physcomitrium patens*

A) Geographic distribution

B) Example of an ecological habitat of *Physcomitrium patens* (i.e., lake floodplain in Yunnan China).

C) A gametophore with a single terminal mature sporophyte. Scale bar 5 mm.

## Two pathways, the SL and the KL pathways



## Strigolactones (SLs)

Strigolactones (SLs) are carotenoid-derived phytohormones with butenolide moieties.

Strigolactones (SLs) are plant hormones that were first identified as root-exudate products, exogenously indicating the vicinity of a host plant to parasitic plants such as *Striga*, and arbuscular mycorrhizal (AM) fungi

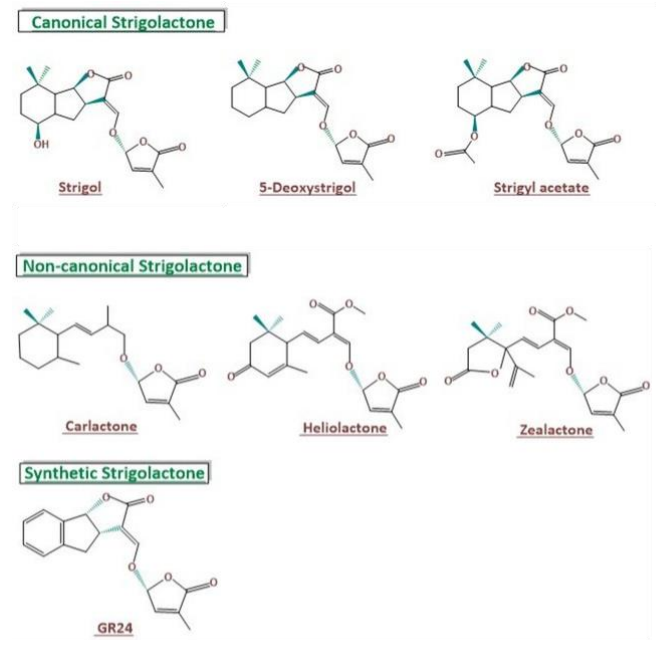
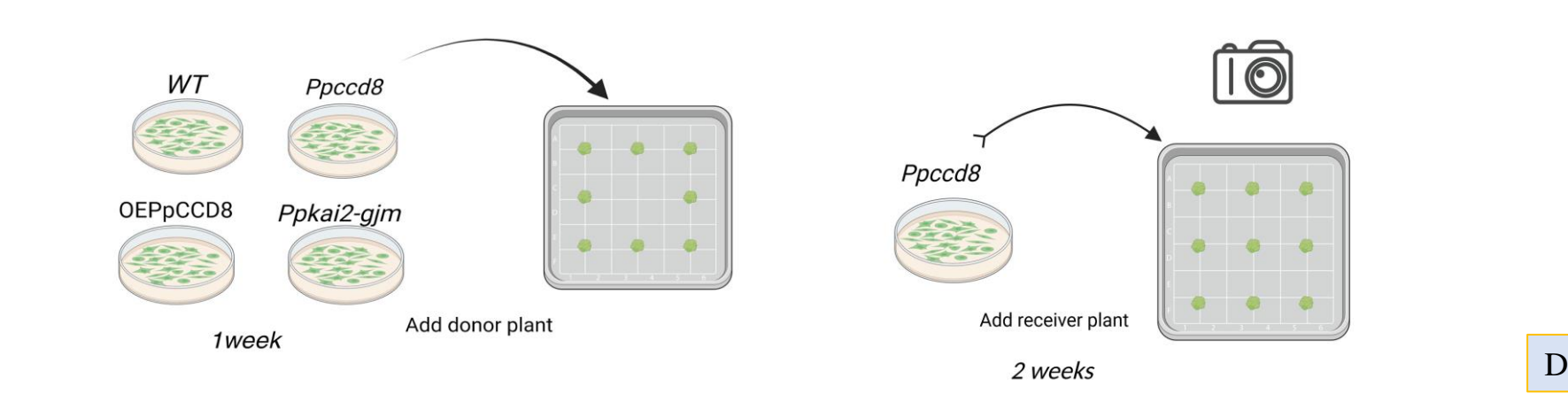
Roles for SLs in a range of endogenous developmental processes, including shoot branching and root architecture, were more recently described,

SLs are present in most land plants

Lopez-Obando M, et al 2021

## Challenge: To estimate the possibility of production of SL by the triple *Ppkai2l-gjm* mutant

## Design of experiment

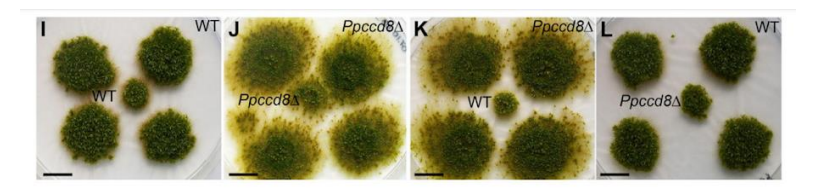
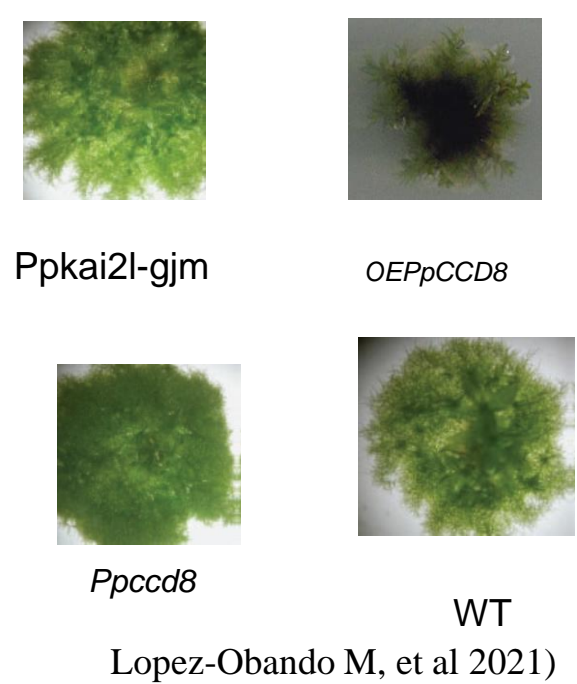
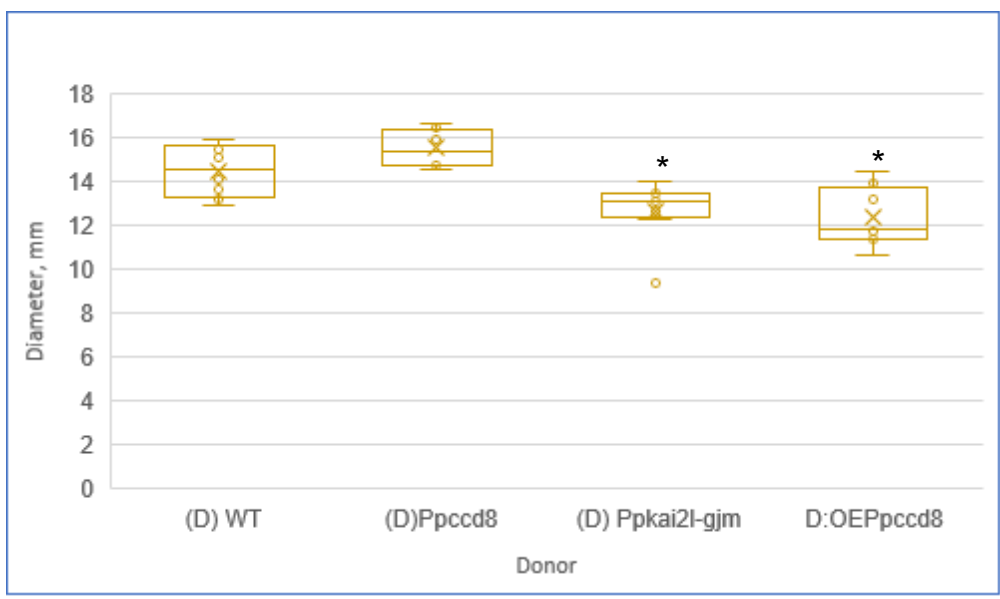


## Definition of alleopathy

The effect, direct or indirect, positive (growth promoting) or negative (growth inhibiting), of one plant on other plants through the release of chemical compounds in the environment

Elroy Rice (1984)

## The size evolution of a receiver plant *Ppccd8*



Proust H, et al 2011

## Conclusion: Ppkai2l-gjm produces similar amounts of metabolites as the over-expressor OEPpCCD8, presumably SLs.

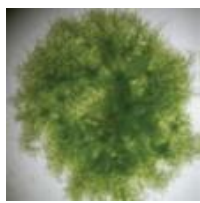
**Reference**

Proust H, Hoffmann B, Xie X, Yoneyama K, Schaefer DG, Nogue F, Rameau C (2011) Strigolactones regulate protonema branching and act as a quorum sensing-like signal in the moss *Physcomitrella patens*. *Development* 138: 1531–1539

Lopez-Obando M, Guillory A, Boyer FD, Cornu D, Hoffmann B, Le Bris P, Pouvreau JB, Delavault P, Rameau C, De Saint Germain A, Bonhomme S (2021). *The Physcomitrium (Physcomitrella) patens PpKAI2L receptors for strigolactones and related compounds function via MAX2-dependent and -independent pathways*. *Plant Cell*, 33(11):3487-3512

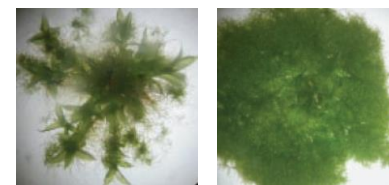
Trouiller B, Schaefer D, Charlot F and Nogué F (2006) MSH2 Is Essential for the Preservation of Genome Integrity and Prevents Homeologous Recombination in the Moss *Physcomitrella Patens*. *Nucleic Acids Research*, 34 (1):232-242

WT



# Looking for KAI2-Ligand (KL) synthesis mutants in the Moss *Physcomitrium patens* using UV mutagenesis

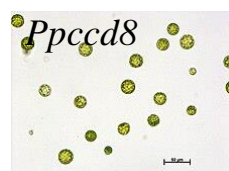
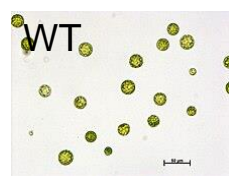
*Ppmax2.1* *Ppccd8*



*The purpose of this experiments is to set up the appropriate tools for a sequential screen for **KL synthesis mutants**, that will combine eye-visible phenotypic identification and use of specific fluorescent molecular markers.*

*The working hypothesis is that synthesis mutants will show a similar phenotype to KL signaling mutants i.e that of the *Ppmax2* mutants.*

Irradiate protoplast UV-B



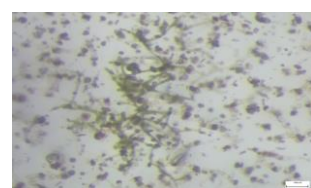
Not Irradiated protoplast



We select apt mutants to confirm that the mutagenesis conditions were appropriate.

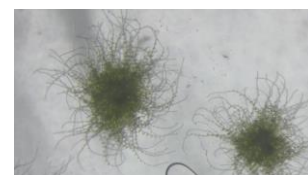
2 Fluoro-Adenine, 2-FA

WT



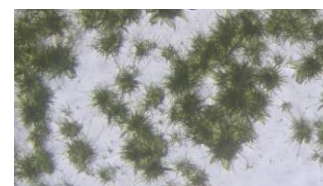
B medium

*Ppccd8*



B medium

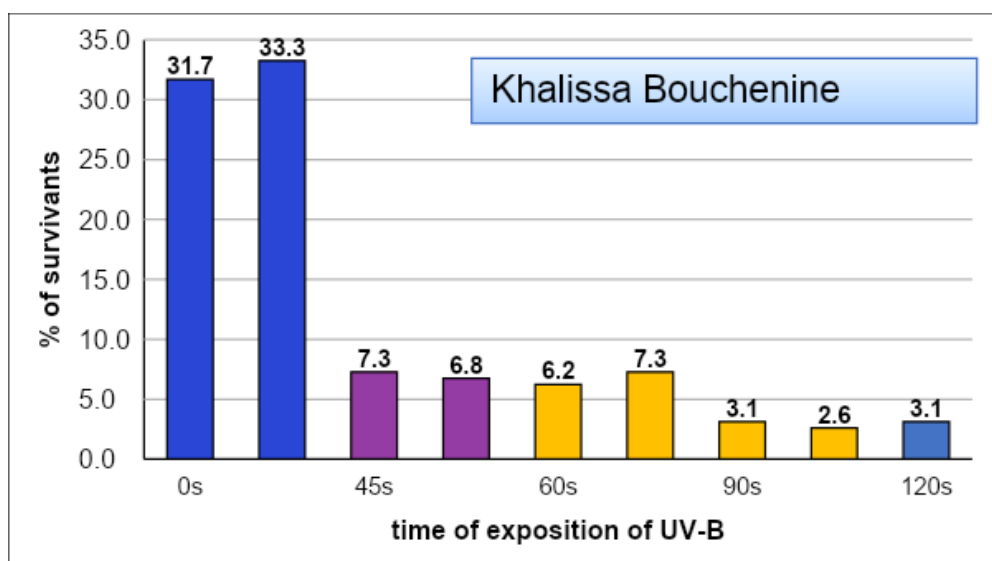
*Ppmax2*



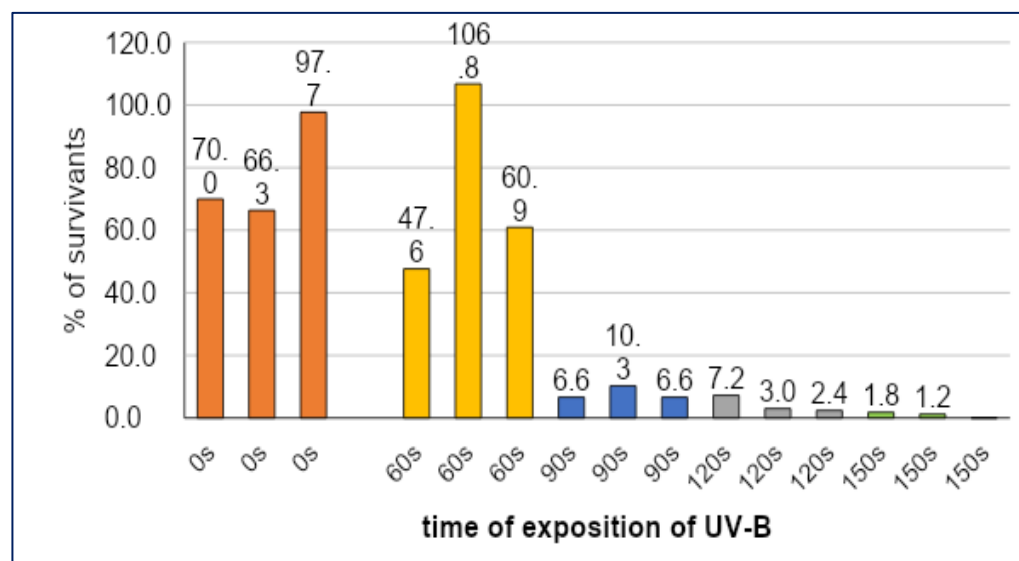
We obtained 35 *Ppapt* mutants out of  $1.68 \cdot 10^6$  regenerants

**Challenge: We are looking for the efficient UV dose to irradiate to get 10% surviving protoplasts**

## Number of surviving protoplasts WT



## Number of surviving protoplasts *Ppccd8*



The optimal irradiation length of WT protoplasts for UV-B mutagenesis was 60sec

The optimal irradiation length of *Ppccd8* protoplasts for UV-B mutagenesis was 90sec

**Planning** Using UV mutagenesis to identify eye-visible phenotype mutants.

The best team ever 😊



As part of MALKOM-X Project now we are going to use vermiculite culture and identify novel allelochemicals from in the Moss *Physcomitrium* and to adress the role of these compounds and their associated pathways in moss development and moss-moss interaction

Acknowledgements: The authors acknowledge the funding by PAUSE- Collège de France and ANR-Ukraine (ANR-22-PAUK-0067).