

# Identification of new targets for improving abiotic stress tolerance in plants

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## SUMMARY

Water availability is one of the main limitations for crop productivity. One strategy to improve plant's drought tolerance is to develop chemical compounds able to manipulate ABA signalling, thus promoting stomata closure and reducing water consumption<sup>[1]</sup>.

Our main goal is to find **new targets** other than ABA receptors that contribute to plant stress tolerance, and which can be regulated by small molecules in order to **activate drought resistance** in crops.

A **chemical screening** will be used to find interesting compounds, followed by a characterization of their *in vitro* and *in vivo* activity. The molecular targets of the selected compounds will then be identified through a genetic screening using a **mapping-by-sequencing** strategy.

## 1 Identification of small molecules able to trigger stress response

### A Pilot chemical screening

Luciferase reporter assay<sup>[2]</sup>

*pMAPKKK18::LUC*<sup>+</sup> stress-inducible reporter line

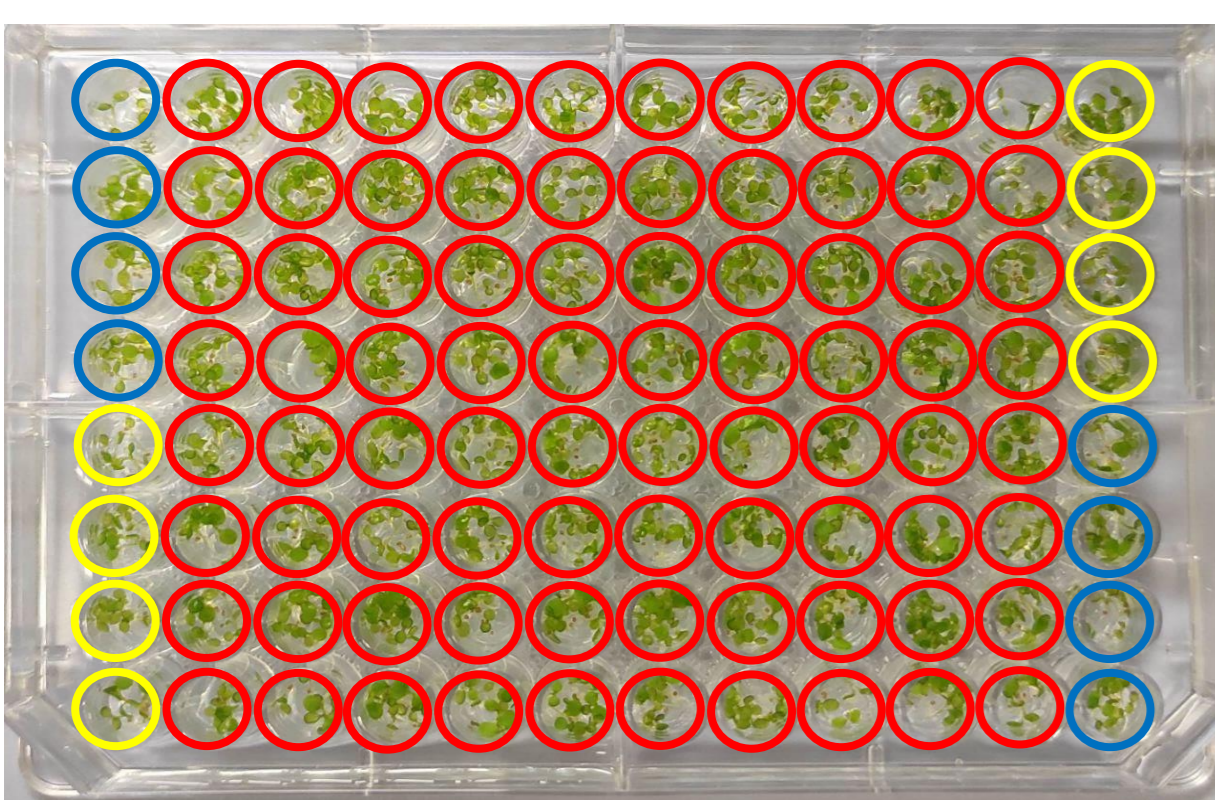
# of compounds

1200 OTAVA<sup>TM</sup> natural product-like library

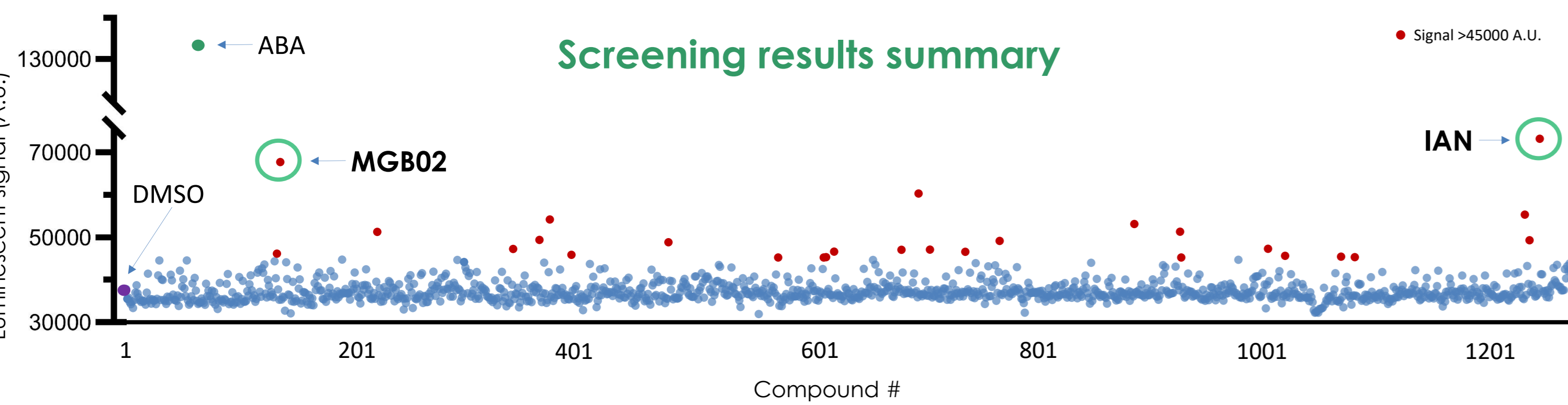
46 Indole library

**MAPKKK18** is highly induced by **ABA**

LAS-3000 luminescent image analyzer  
ImageJ Rainbow LUC LUT



○ DMSO ○ ABA (25 μM) ○ Test compound (100 μM)

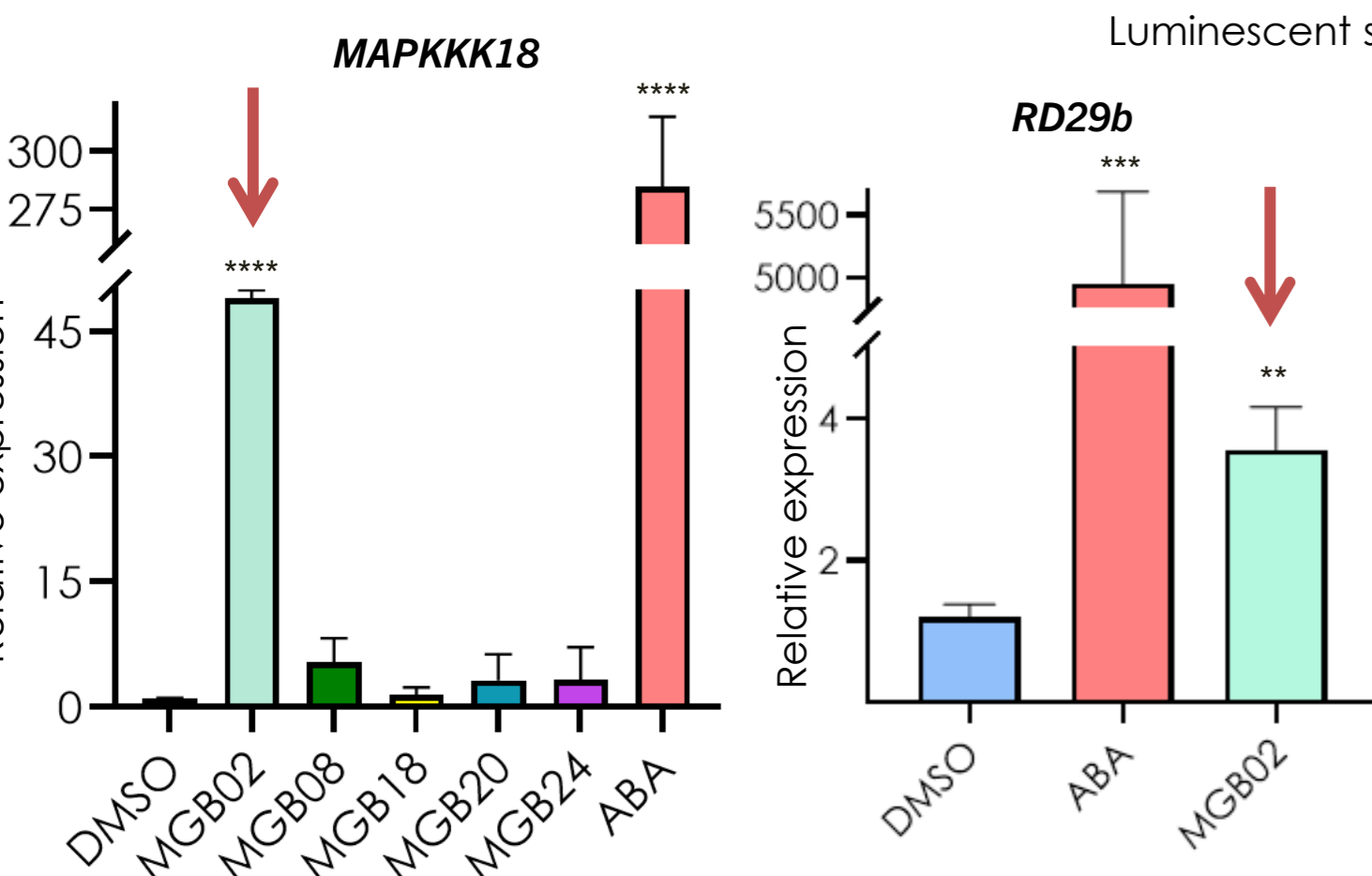
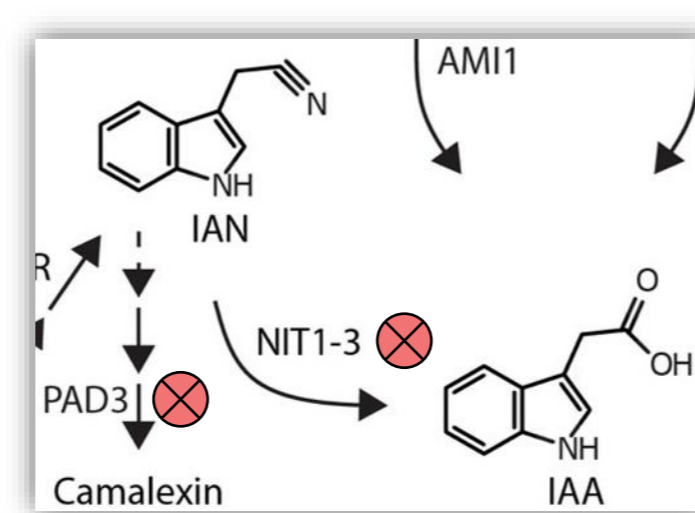
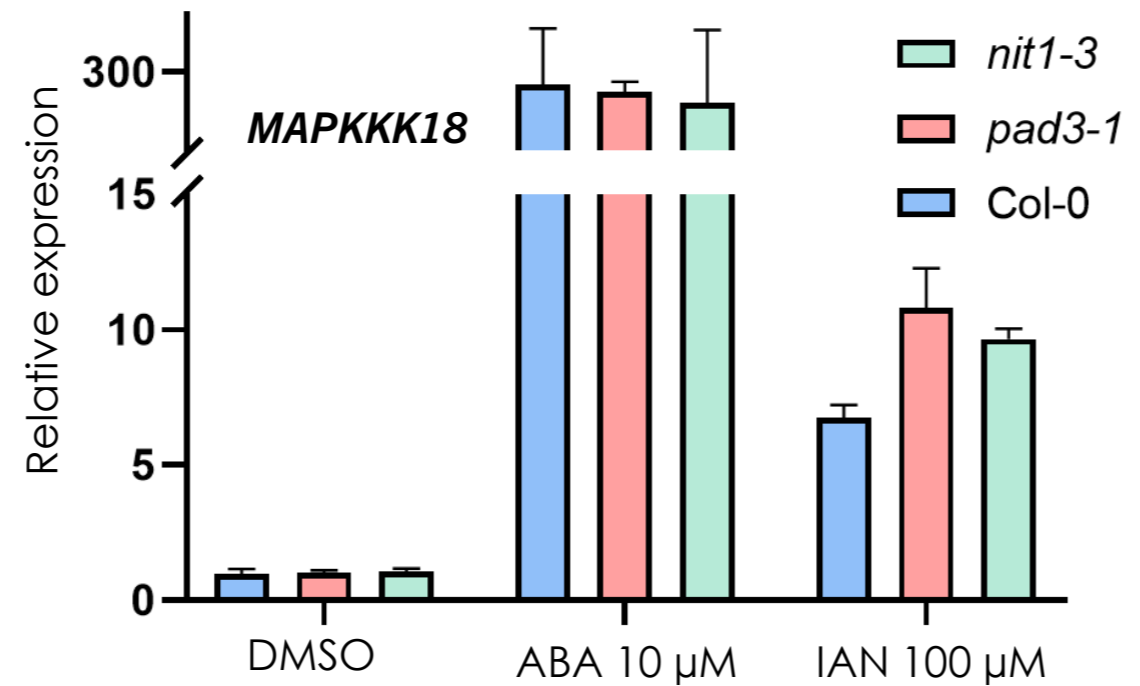


### B Discard false positives

We have found several hits, including **indole-3-acetonitrile (IAN)**, which caused the greatest activation of the reporter.

Indole compounds comparison

Compound	Chemical Structure	Fluorescence Image
DMSO		
ABA		
indole-3-acetonitrile (IAN)		
4-chloroindole		
indole-3-acetic acid (IAA)		
indole-3-acetamide (IAM)		
camalexin		



Upon verification of differential expression of stress genes by qPCR, we chose the novel compound "**MGB02**" to be further investigated.

### C Hit expansion

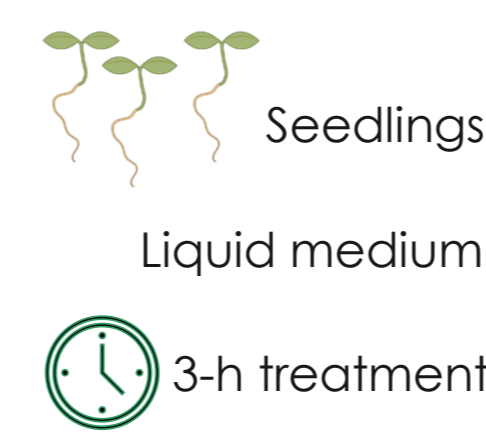
Similarity search



SwissSimilarity

## 2 Characterization of the activity of identified molecules

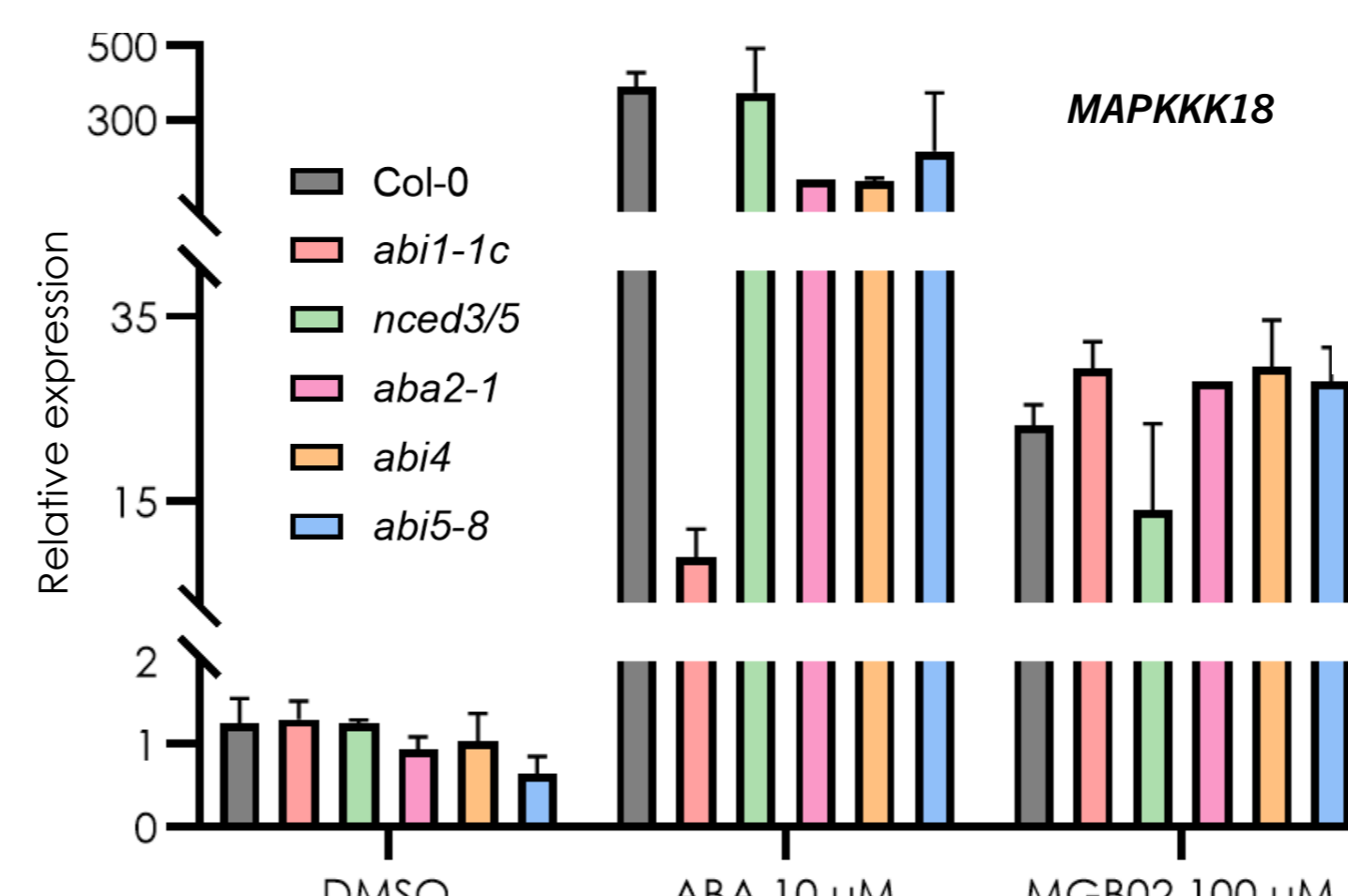
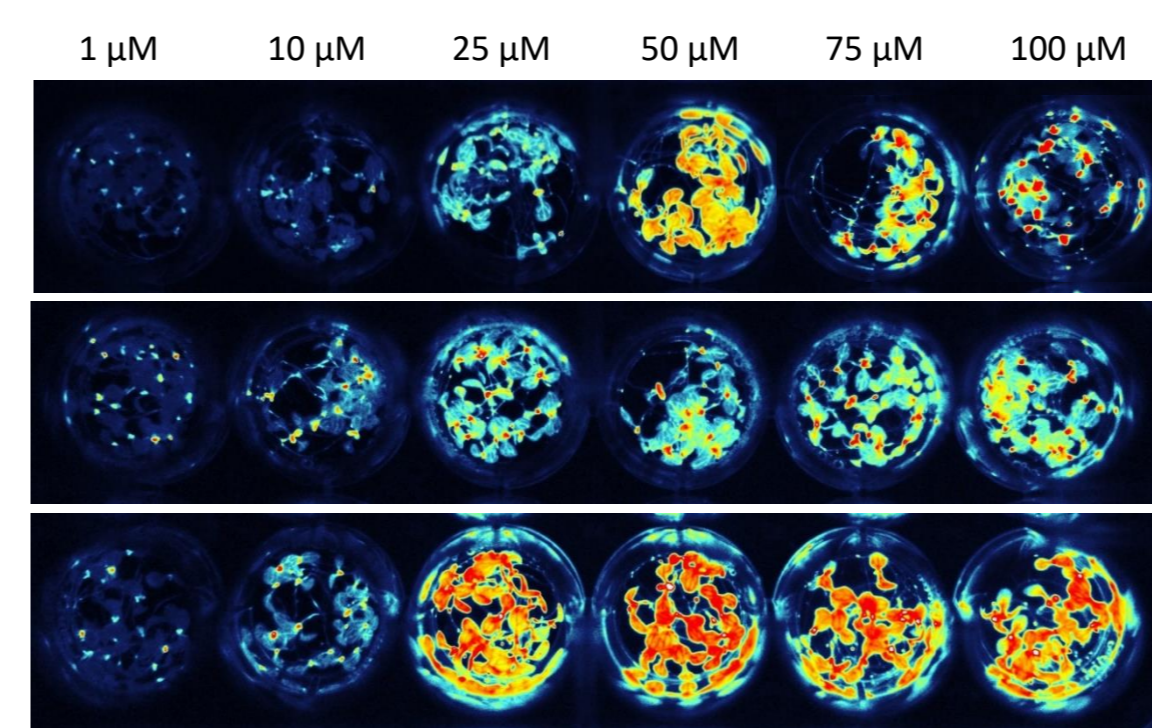
### RNA-Seq



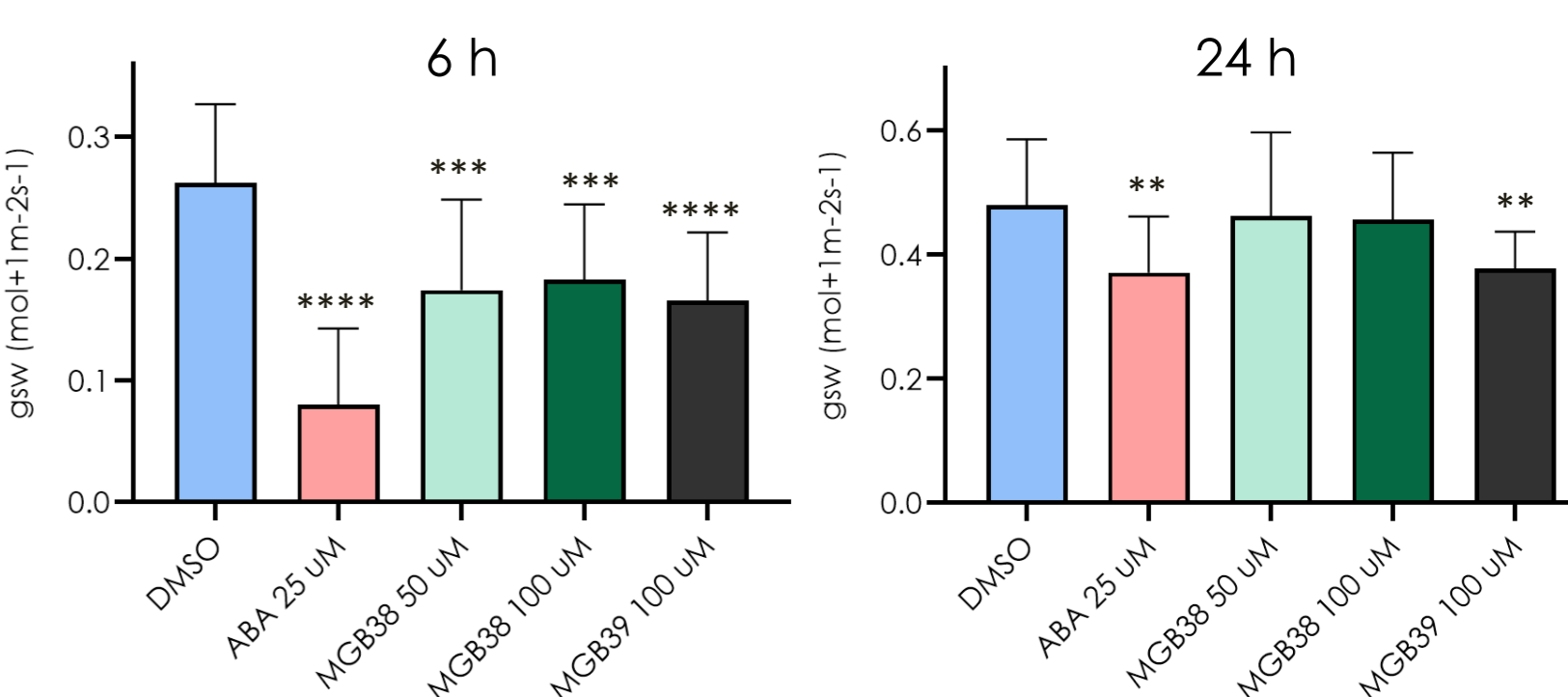
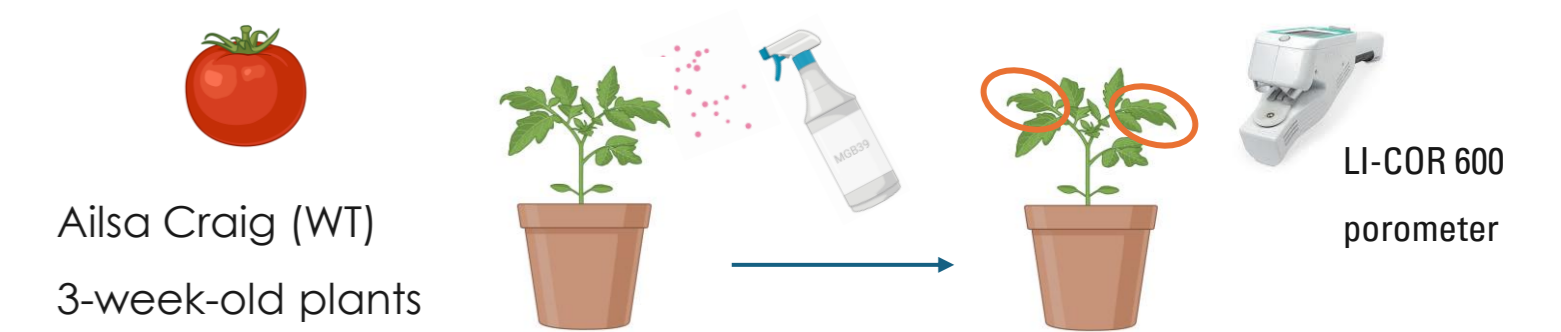
Genes upregulated by **MGB02** and **also** by **ABA** include genes involved in **ABA synthesis**

Gene	Fold change
<i>ABA1</i>	2.20
<i>NCED3</i>	7.11
<i>SDR4</i>	2.62

### Dose-response luciferase assays



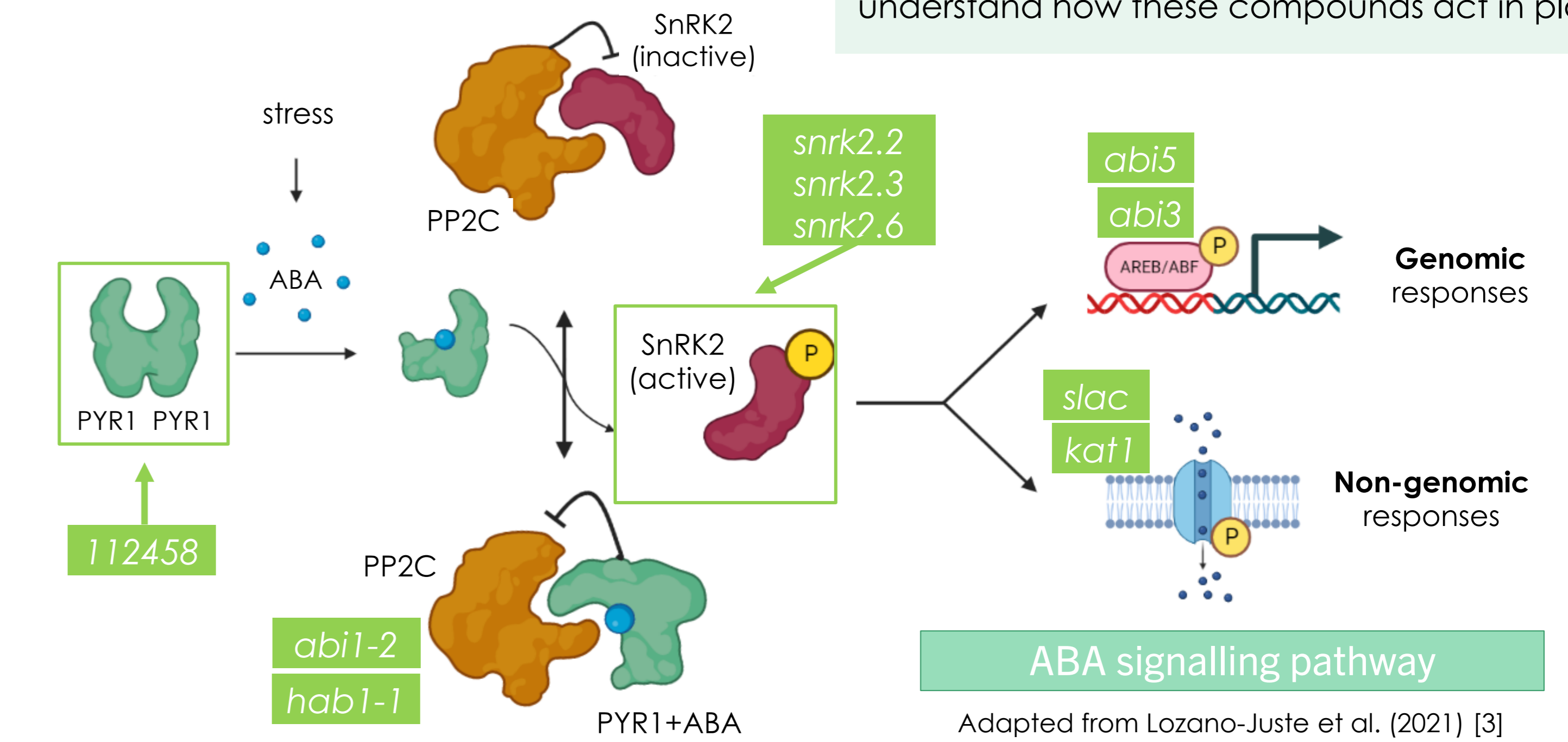
### Stomatal conductance



### Ongoing and future experiments

**MGB38** has shown greater activity than **MGB02**. Stomatal conductance measurements in tomato indicate **possible physiological effects**.

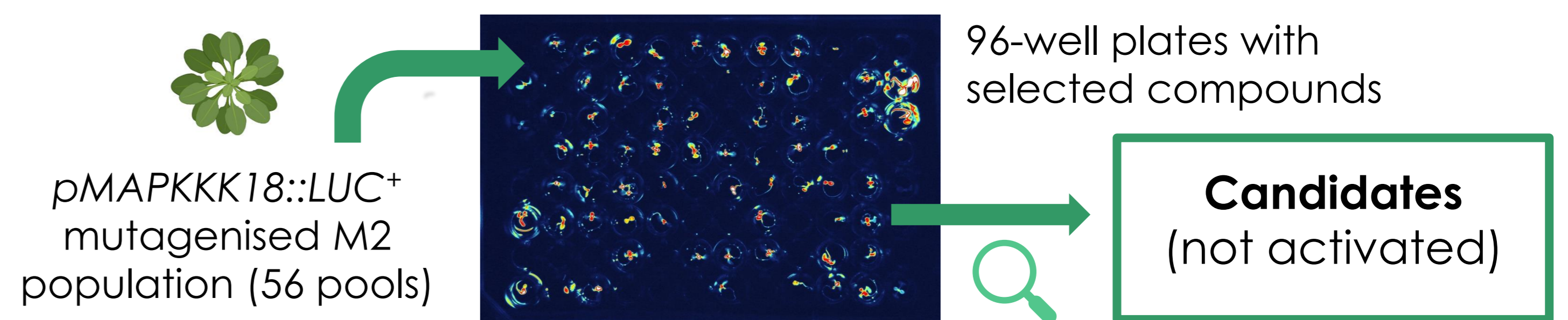
**Expression analyses**, together with **germination** and **root growth** assays, in ABA-synthesis and ABA-signaling mutants, will help to better understand how these compounds act in plants.



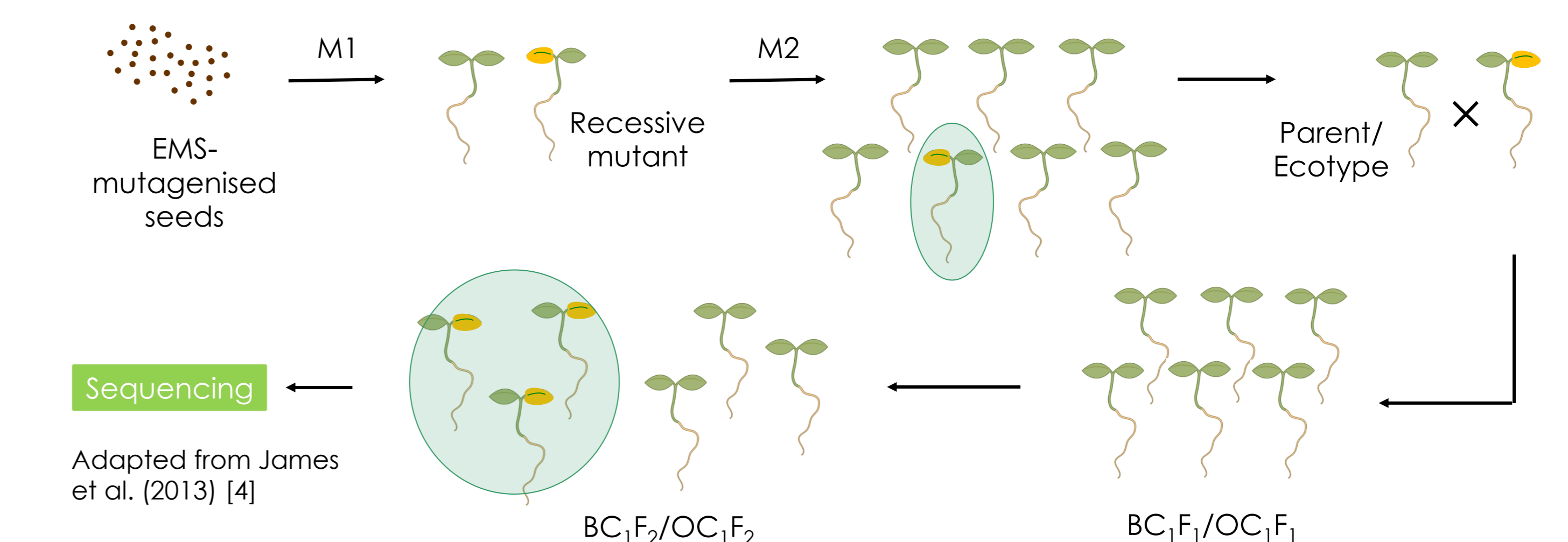
## 3 Identification of molecular targets of the selected compounds

A genetic screening is being performed to **identify the molecular targets** of the most promising compound by **mapping-by-sequencing**.

### Forward chemical genetic screening



### Mapping by sequencing



References [1] Vaidya et al. 2019. *Nature* 366(6464) [2] García-Maquílón et al. 2021. *Methods Mol Biol* 2213, 113-121 [3] Lozano-Juste et al. 2021. *Methods Mol Biol* 2213, 99-111 [4] James et al. 2013. *Genome Biology* 14

