RT-qPCR analysis of putative mutants of the *Arabidopsis* karrikin signal transduction pathway as a Course-based Undergraduate Research Experience

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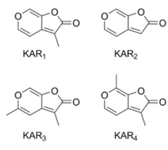
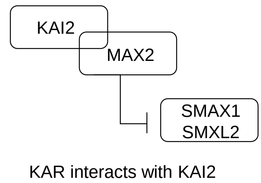
- 13 students in an upper division molecular genetics class
- Each student evaluates a set of putative mutants from ongoing EMS screen
- Quantifying transcriptional markers of karrikin signaling in seedling development

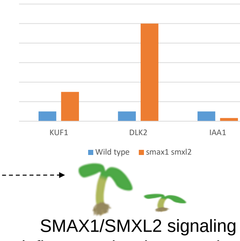
Karrikin signaling in Arabidopsis



Karrikins (KAR) are produced during combustion and are present in smoke.
(Flematti et al., 2004)

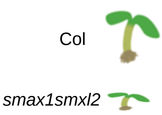


KAR interacts with KAI2 receptor, likely promotes SMAX1/SMXL2 degradation via MAX2 F-box protein.
(Morffy et al., 2016)

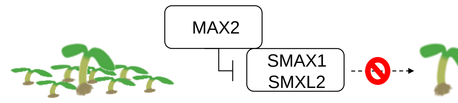


SMAX1/SMXL2 signaling influences developmental and transcriptional changes in red-light-grown seedlings.
(Stanga et al., 2016)

Mutant screen for signaling components before semester begins



In red-light-grown seedlings, *smax1smxl2* mutants have a short stature



EMS mutagenesis of *smax1smxl2* might suppress short hypocotyls

Putative suppressors might represent KAR signaling components downstream of SMAX1/SMXL2

Weekly 3-hour group lab activities

Genotyping

Week 1
- Plating seeds for genotyping

Week 2
- Measure hypocotyl length
- Isolate seedling DNA – spin column

Week 3
- PCR

Week 4
- Gel electrophoresis

Gene expression

Week 5
- Plating seeds for RNA

Week 6
- Isolate seedling RNA – spin column

Week 7
- RNA quality control – electrophoresis and absorbance
- cDNA synthesis
- qPCR primer design

Week 8
- qPCR – SYBR green

Week 9
- qPCR data analysis

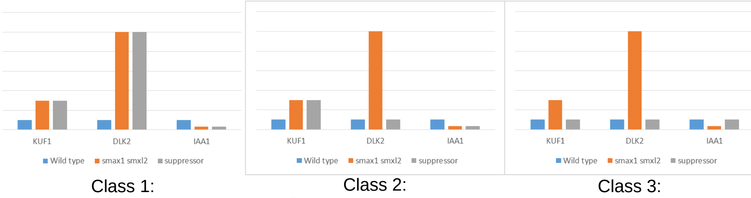
Student inquiry experiments

Remaining weeks:
- Troubleshooting genotyping
- Repeating gene expression
- Testing gene expression of plausible candidates
- Summarizing results in written report

Anticipated outcomes

Classify putative mutants based on suppressive effect on transcription of 3 KAR-responsive genes

Use classification system to rank candidates for future linkage mapping



Class 1:
No change in transcription profiles of 3 KAR-responsive genes

Class 2:
Subset of transcription responses suppressed

Class 3:
All 3 transcription responses suppressed

References

Flematti G, Ghisalberti E, Dixon K, Trengrove R. 2004. A compound from smoke that promotes seed germination. Science 305, p 977.

Morffy N, Faure L, Nelson DC. 2016. Smoke and hormone mirrors: Action and evolution of karrikin and strigolactone signaling. Trends in Genetics 32, p 176.

Stanga J, Morffy N, Nelson DC. 2016. Functional redundancy in the control of seedling growth by the karrikin signaling pathway. Planta 243, p 1397.