We conclude three possible developmental procedures of the ant defense organ, domatia: pith cell division; programmed cell death; and secondary cell wall deposition.

Comparative transcriptomics analysis reveals a developmental process and the occurrence of cell death in the formation of domatia

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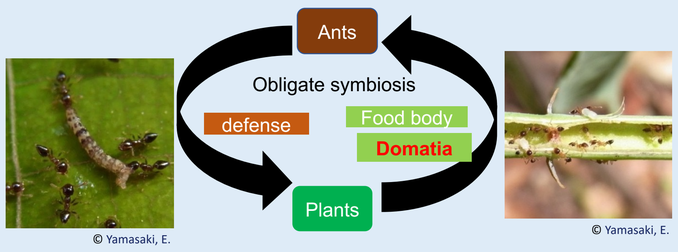
Intro



- The protective ant-plant interaction is very common in tropical region(1)
- Myrmecophytes use ants as anti-herbivore agents, and in turn provide the nesting sites (domatia)(2)
- Domatium is a modified structure from various plant organs and formed mainly spontaneously(3)
- Domatium is known as a key trait for the evolution of myrmecophytic ant-plant interaction, although it has rarely been studied at the developmental morphology and molecular level

Materials & Methods

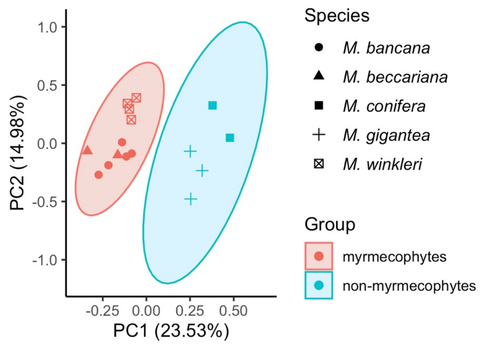
- Lambir Hills National Park, Sarawak, Malaysia.
- Well-known ant plants *Macaranga*(4).
  - Myrmecophytes: *M. winkleri, M. trachyphylla, M. bancana, M. beccariana*
  - Non-myrmecophytes: *M. gigantea, M. conifera*

RNA-seq analysis

- Quality control
- Mapping, STAR
- Counting, FeatherCount
- Differentially expressed genes, edgeR
- Gene ontology analysis, topGO

SUSHI(5)

1 Comparison of ant defense strategies points out that the programmed cell death occurs in the myrmecophytes species



Principal component analysis of the five *Macaranga* species.

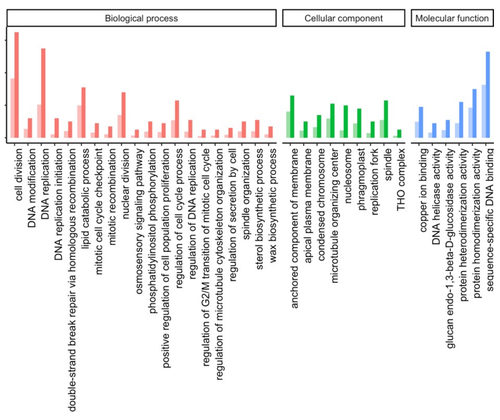
Cell death related GO terms enriched in myrmecophytes when comparing to non-myrmecophytes.

GO.ID	Term	Significant/annotated genes (n)	p-value
GO:0043067	regulation of programmed cell death	11/129	0.01087
GO:0010941	regulation of cell death	12/154	0.01566
GO:0042981	regulation of apoptotic process	8/85	0.01623
GO:0043065	positive regulation of apoptotic process	4/28	0.02118
GO:0043068	positive regulation of programmed cell death	5/46	0.03077

Key differentially expressed genes related to cell death comparing myrmecophytes to non-myrmecophytes.

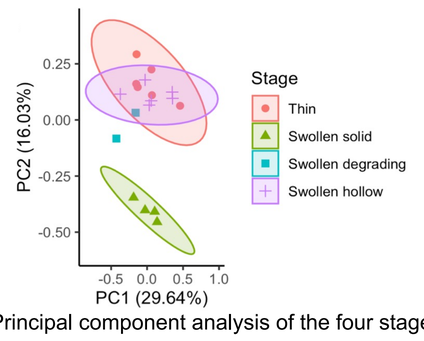
MCID	logFC	FDR	AGI code	Symbol	Description
MwinG010400	3.27	0.0021	AT5G47910	ATRBOHD	Respiratory Burst Oxidase Homologue D
MwinG021820	0.74	0.0022	AT4G02220	NA	Programmed Cell Death 2 C-terminal Domain-containing Protein
MwinG024620	1.02	0.0056	AT5G51290	ACD5	Accelerated Cell Death 5
MwinG342560	-3.86	0.0112	AT5G47120	ATBI-1	Arabidopsis Bax Inhibitor 1
MwinG025290	1.73	0.0189	AT1G02170	ATMC1	Arabidopsis Thaliana Metacaspase 1
MwinG014730	-1.45	0.0349	AT1G08860	BON3	Negative regulator of cell death

Extra Tables & Figures



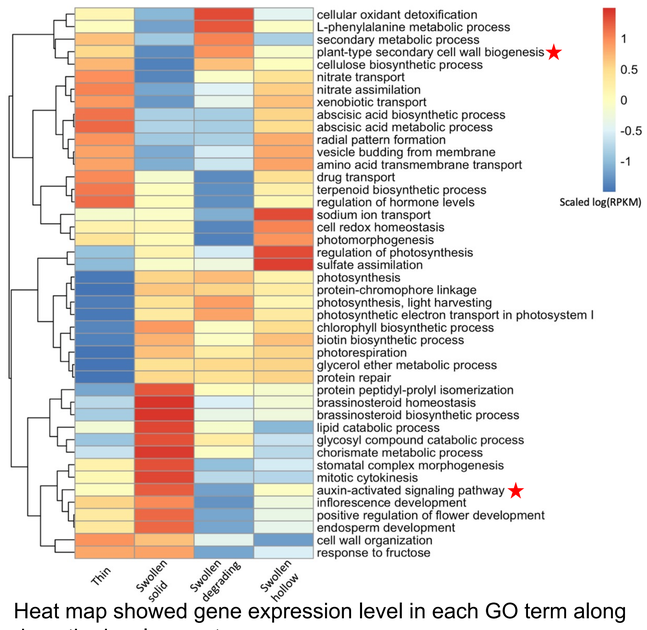
Enriched significant GO terms in three categories.

2 Comparison of domatia developmental stages indicates the other two possible procedures besides the cell death

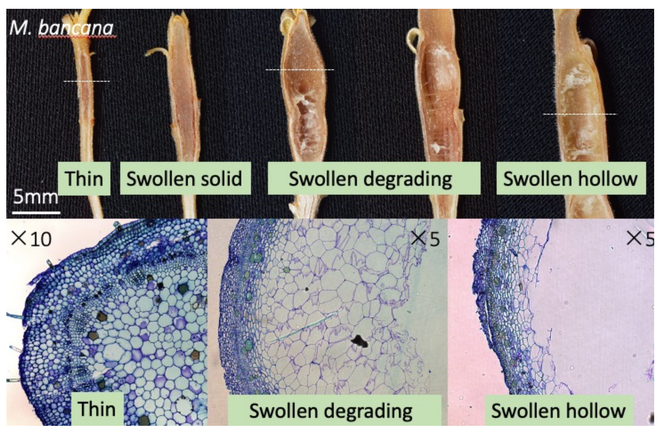


Principal component analysis of the four stages.

- Auxin signaling was enriched in swollen solid stage, while secondary cell wall was down-regulated. This suggested the pith cell growing with thin cell wall.
- The secondary cell wall was up-regulated again in the following swollen degrading and hollow stage, which indicated a final physical function growth.



Heat map showed gene expression level in each GO term along domatia development.

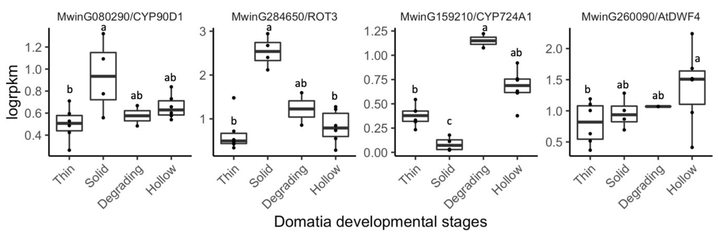


Longitudinal and transverse sections of four different developmental stages of the stem domatia.

Primary cell wall formation up-regulated & secondary cell wall formation down-regulated in the swollen solid stage.

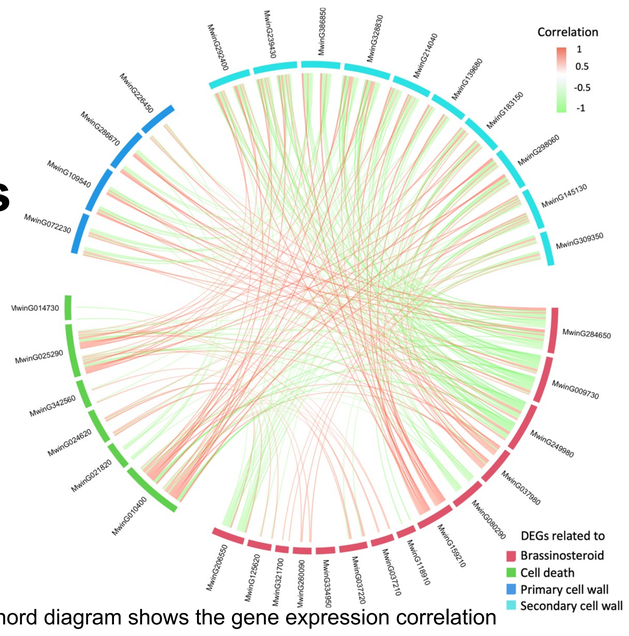
MCID	logFC	FDR	AGI code	Symbol	Description	Cell wall
MwinG072230	0.956	2.85.E-04	AT2G03220	ATFT1	Arabidopsis thaliana fucosyltransferase 1	PCW
MwinG109540	1.69	4.70.E-05	AT4G31590	ATCSLC05	cellulose-synthase like C5	PCW
MwinG286670	1.501	1.15.E-04	AT4G39350	ATCESA2	cellulose synthase A2	PCW
MwinG226450	1.09	2.56.E-04	AT5G05170	ATCESA3	cellulose synthase 3	PCW
MwinG292400	-2.71	1.50.E-05	AT2G40890	CYP98A3	cytochrome P450, family 98, subfamily A, polypeptide 3	SCW
MwinG239430	-2.373	1.59.E-05	AT5G48930	HCT	hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase	SCW
MwinG386850	-3.624	2.46.E-05	AT1G17950	ATMYB52	MYB domain protein 52	SCW
MwinG328830	-4.606	6.43.E-05	AT4G36220	CYP84A1	cytochrome P450 84A1	SCW
MwinG214040	-2.936	9.75.E-05	AT1G51680	4CL.1	4-coumarate:CoA ligase 1	SCW
MwinG139680	-1.862	1.52.E-04	AT1G15950	ATCCR1	cinnamoyl CoA reductase 1	SCW
MwinG298060	-6.424	2.28.E-04	AT5G60020	ATLAC17	Laccase 17	SCW
MwinG145130	-3.261	2.50.E-04	AT4G18780	ATCESA8	cellulose synthase 8	SCW
MwinG183150	-2.759	2.76.E-04	AT4G34050	CCoAOMT1	caffeoyl coenzyme A O-methyltransferase 1	SCW
MwinG309350	1.773	9.02.E-05	AT5G64530	ANAC104	Arabidopsis NAC domain containing protein 104	SCW

3 Brassinosteroid is a key hormone that regulates cell death & secondary cell wall deposition



Key BR related DEGs expression along domatia four developmental stages.

Key genes encoding BR enzymes were highly expressed in different stages, and showed high correlation with other procedures' gene expression.
These results indicate that BR is an important hormone regulating the whole domatia formation procedures.



Chord diagram shows the gene expression correlation

Discussion

- Cell death happens in the domatia formation of the myrmecophyte species.
- Three possible main procedures in the domatia formation:
  - **Stem expansion to loosen pith cells**
  - **Programmed cell death to form the cavity**
  - **Secondary cell wall deposition for the physical structure**
- BRs play a key role, and regulate cell death first, then secondary cell wall. This showed a conserved mechanism with the classical cell death model of tracheary elements formation.

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(1) Chomicki, G.; Renner, S. S. Phylogenetics and Molecular Clocks Reveal the Repeated Evolution of Ant-Plants after the Late Miocene in Africa and the Early Miocene in Australasia and the Neotropics. *New Phytologist* **2015**, *207* (2), 411–424. https://doi.org/10.1111/nph.13271.
(2) Buckley, R. C. Ant-Plant Interactions: A World Review. In *Ant-plant interactions in Australia*; Buckley, R. C., Ed.; Geobotany; Springer Netherlands: Dordrecht, 1982; pp 111–141. https://doi.org/10.1007/978-94-009-7994-9_11.
(3) Chanam, J., & Borges, R. M. (2017). Cauline domatia of the ant-plant Humboldtia brunonis (Fabaceae). Flora, 236–237, 58–66. https://doi.org/10.1016/j.flora.2017.09.005
(4) Roubik, D., Sakai, S., & Hamid, A. A. (Eds.). (2005). Pollination Ecology and the Rain Forest: Sarawak Studies. Springer-Verlag. https://www.springer.com/gp/book/9780387213095
(5) Hatakeyama, M., Opitz, L., Russo, G., Qi, W., Schlapbach, R., & Rehrauer, H. (2016). SUSHI: An exquisite recipe for fully documented, reproducible and reusable NGS data analysis. BMC Bioinformatics, 17(1), 228. https://doi.org/10.1186/s12859-016-1104-8

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