



THE AUSTRALIAN NATIONAL UNIVERSITY



Defining the molecular pathways that result in phi thickening formation & mechanical strengthening of the *Brassica* root

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Acknowledgements

Students

Make Aleamotu'a now Tonga National University
Aliaa Idris now Universiti Malaysia Terengganu

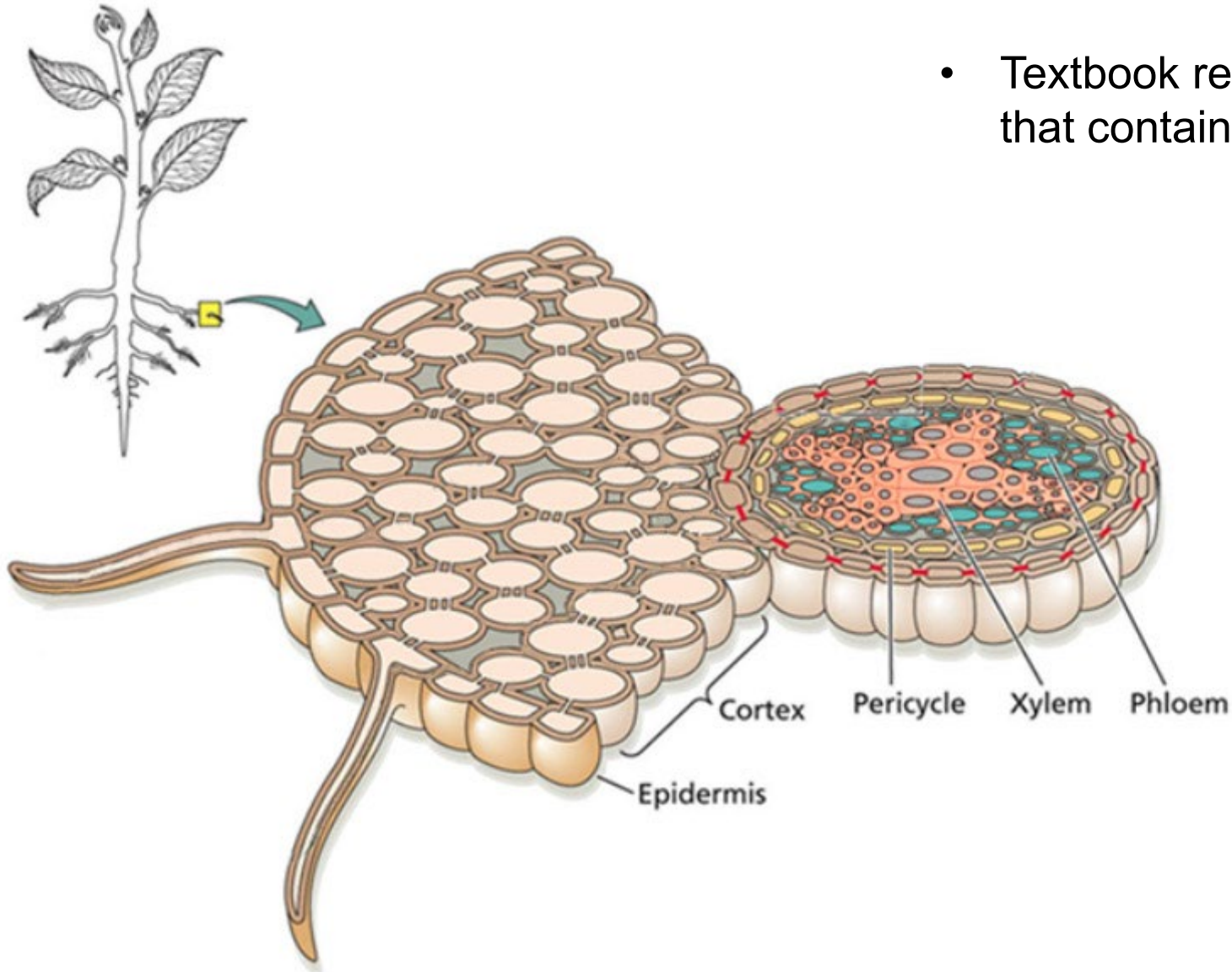
Colleagues

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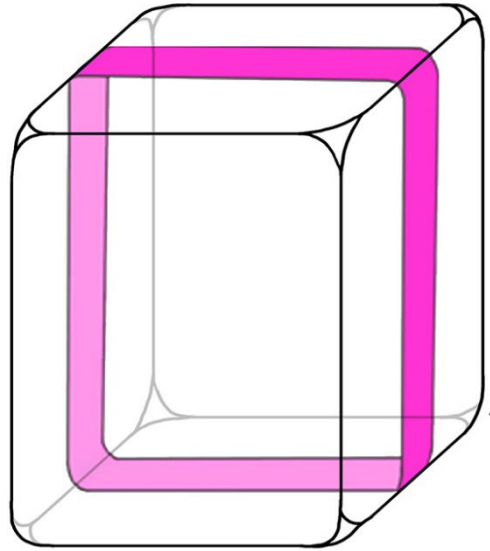


Phi Thickening Development in Roots

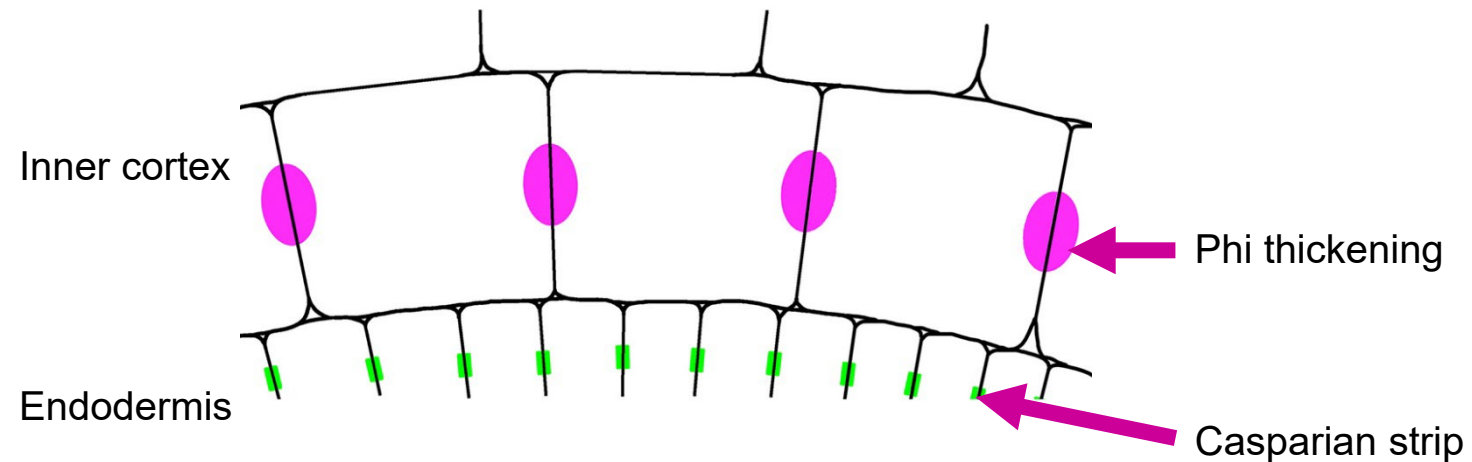
- Textbook representations of the root show a cortex that contains cells with only primary cell walls.



Phi Thickening Development in Roots



- Textbook representations of the root show a cortex that contains cells with only primary cell walls.
- **Phi thickenings** are bands of lignified secondary cell wall that encircle cells within the root cortex.
- These thickenings often occur in the **inner cortex**.
- They are structurally different to the **Casparian strip** found in the endodermis.



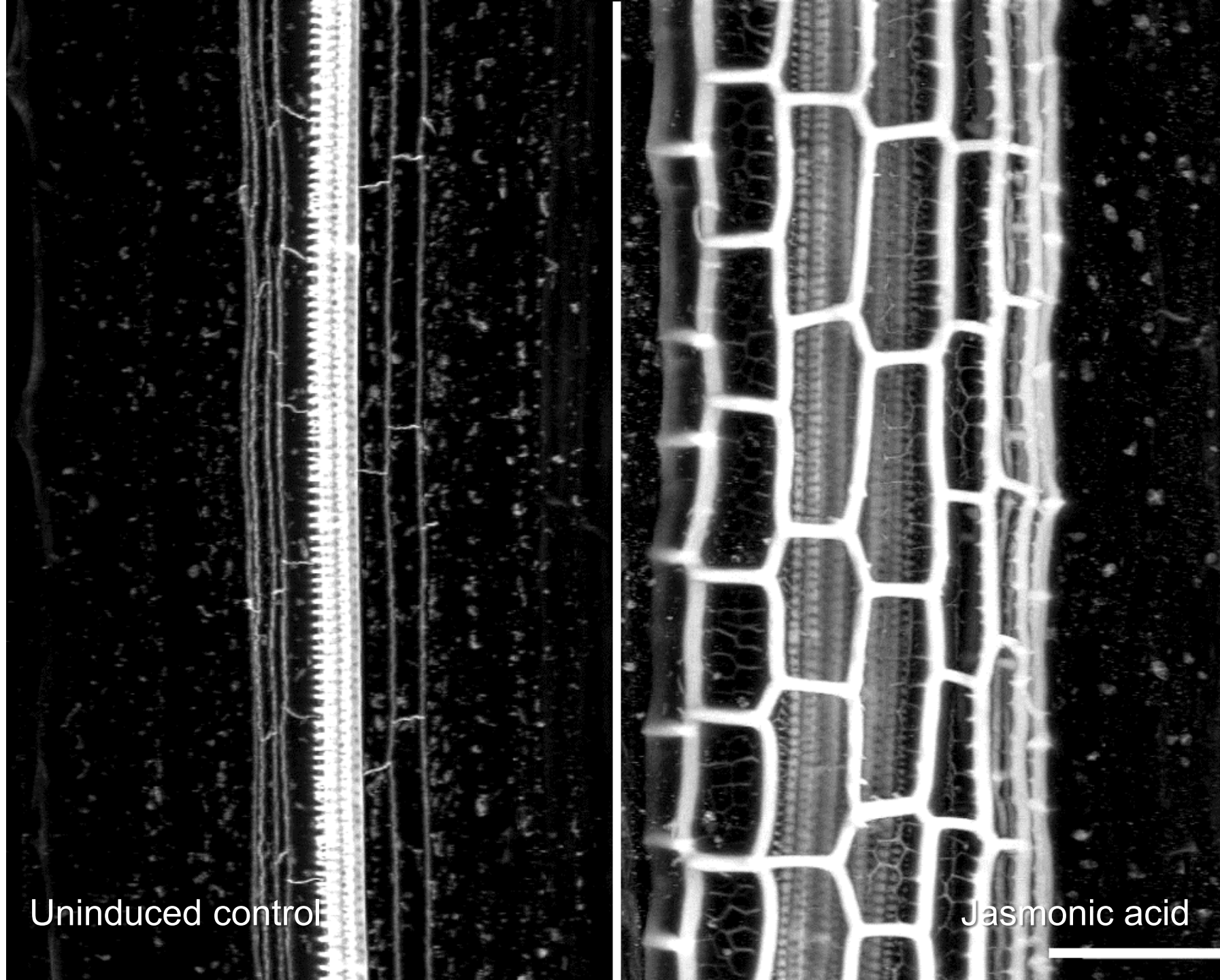
Structure

Brassica phi thickenings

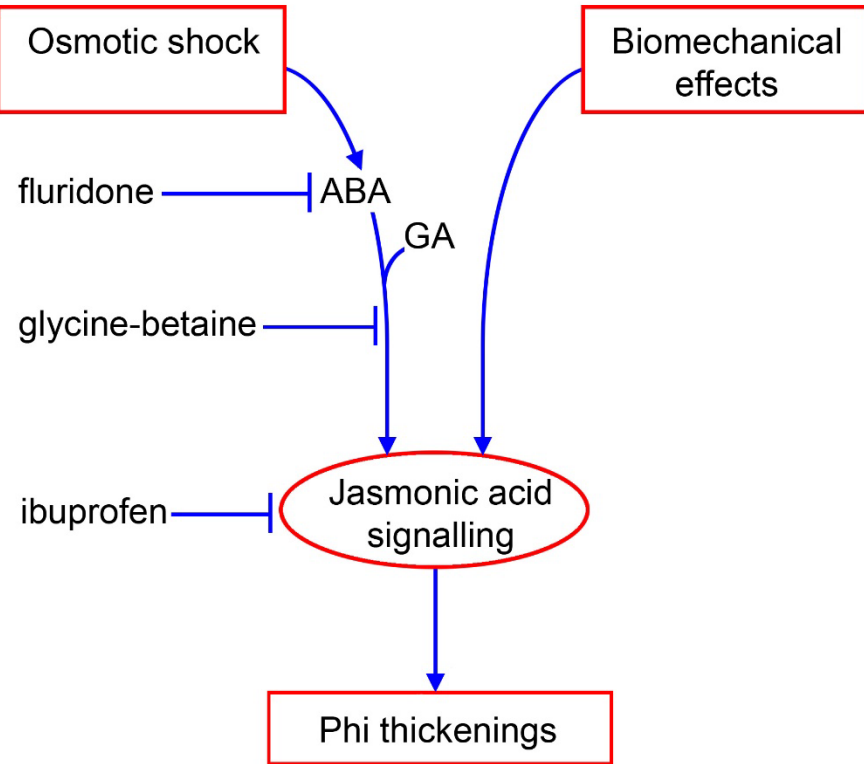
- Lignin stained with basic fuchsin & imaged by confocal microscopy.
- Image reconstruction in ImageJ.
- Control roots have a lignified Casparian strip.
- Induced phi thickenings encircle the stele.

Uninduced control

Jasmonic acid

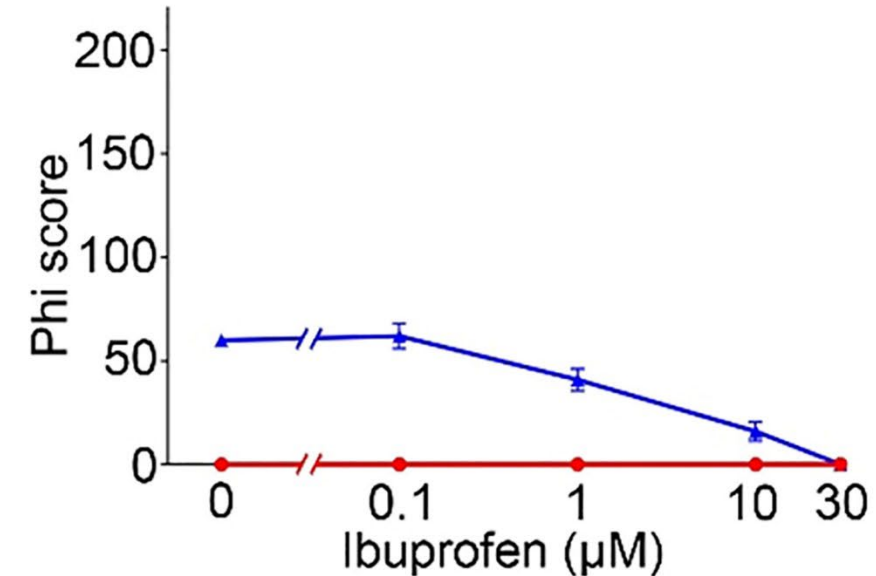
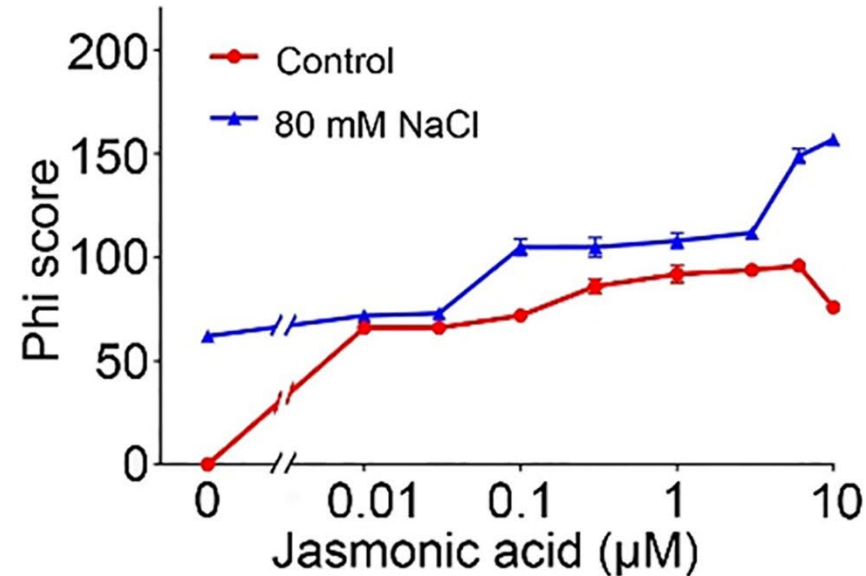
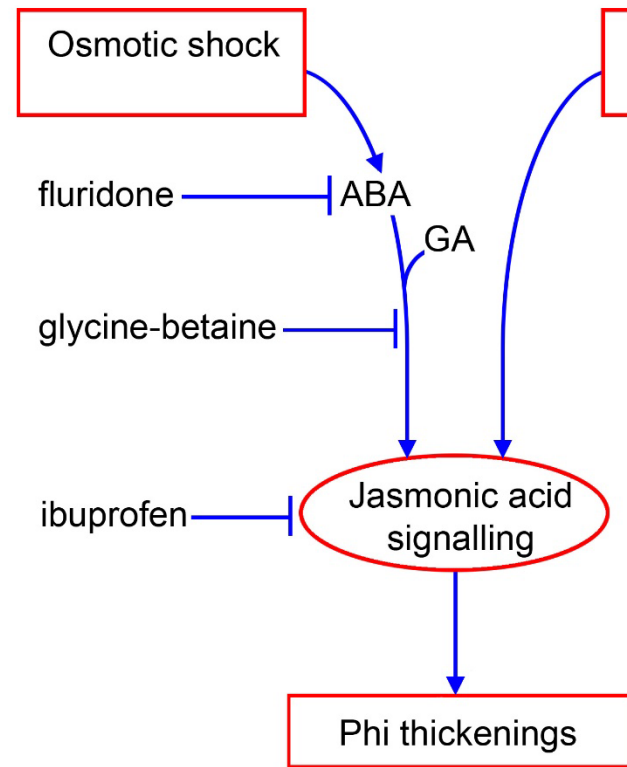


Jasmonic Acid Mediates Phi Thickening Development



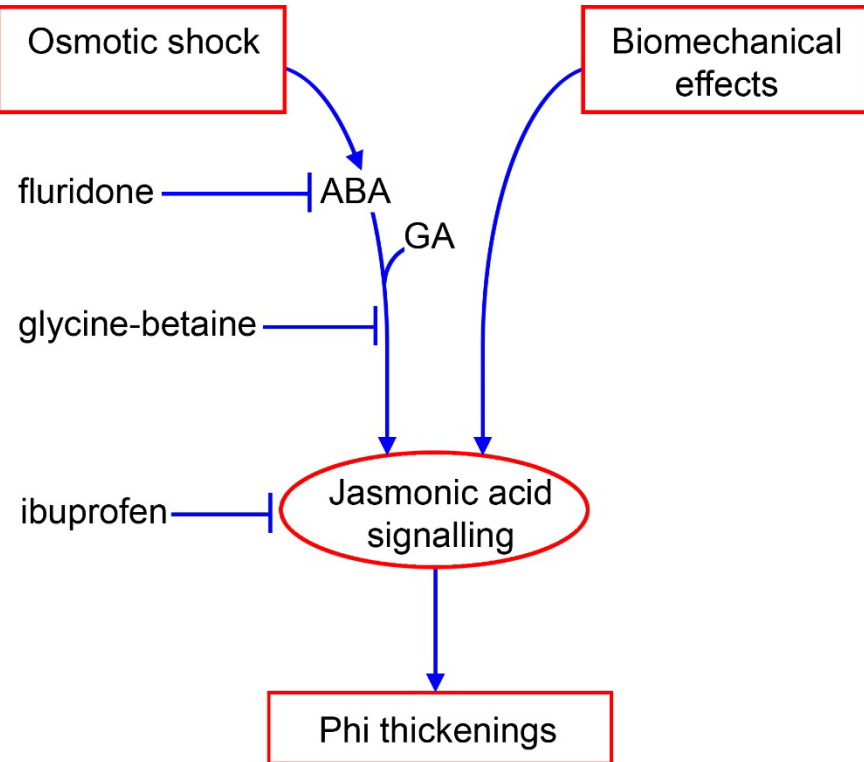
- Jasmonic acid (JA) is central to our model of phi thickening induction.

Jasmonic Acid Mediates Phi Thickening Development



- Jasmonic acid (JA) is central to our model of phi thickening induction.
- JA induces thickenings under non-inducing conditions while the JA-antagonist ibuprofen blocks thickening formation in inducing conditions.

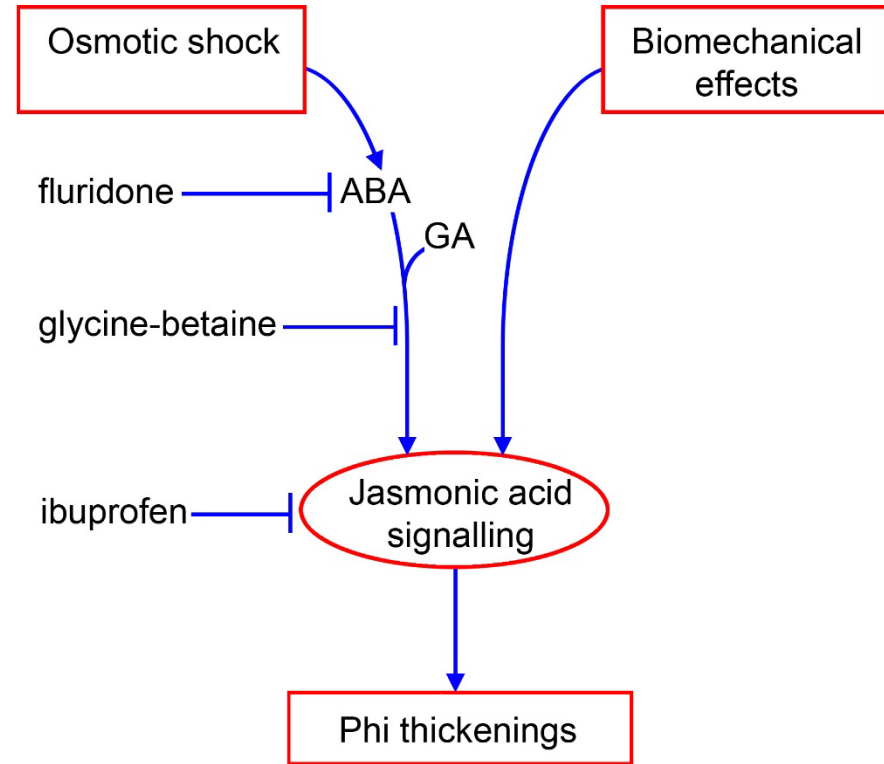
Identifying Phi Thickening Molecular Pathways



To investigate molecular pathways, we utilised

- Breeding populations
- GWAS

Identifying Phi Thickening Molecular Pathways

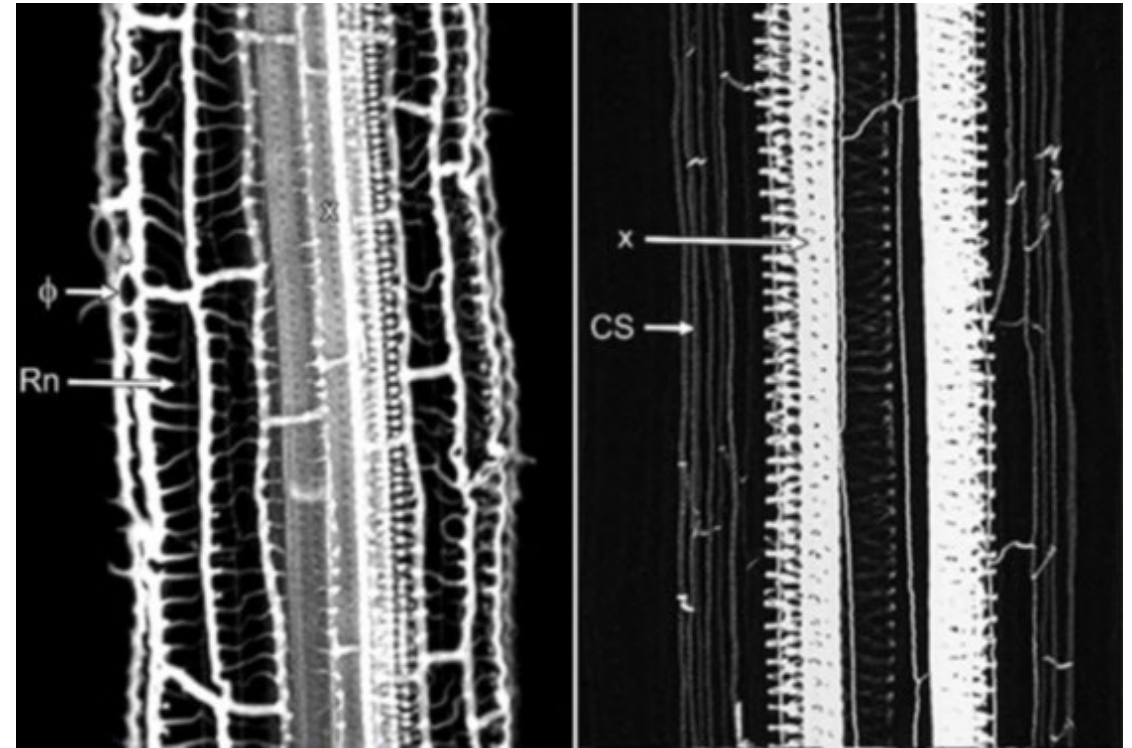
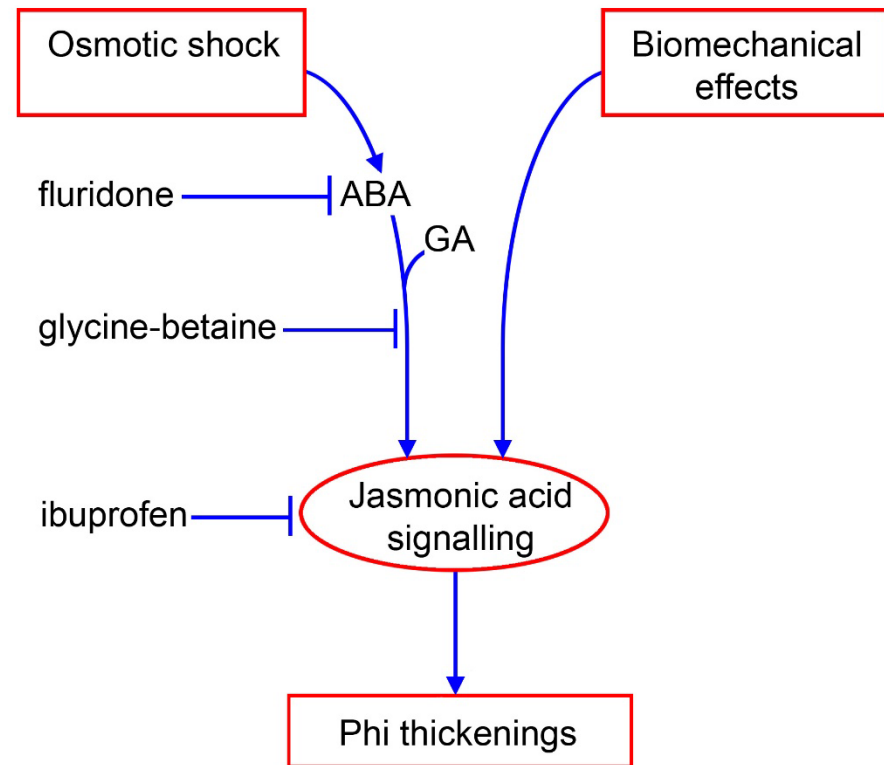


To investigate molecular pathways, we utilised

- Breeding populations
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		Phi thickenings (0 to 5 scale)	
	Line	Control	1 μ M JA
<i>B. rapa</i>	A	0	0
	B	0	5
<i>B. napus</i>	A	0	0.6
	B	0	4.1
<i>B. carinata</i>	A	0	0
	B	0	5

Mapping QTLs in *Brassica rapa*

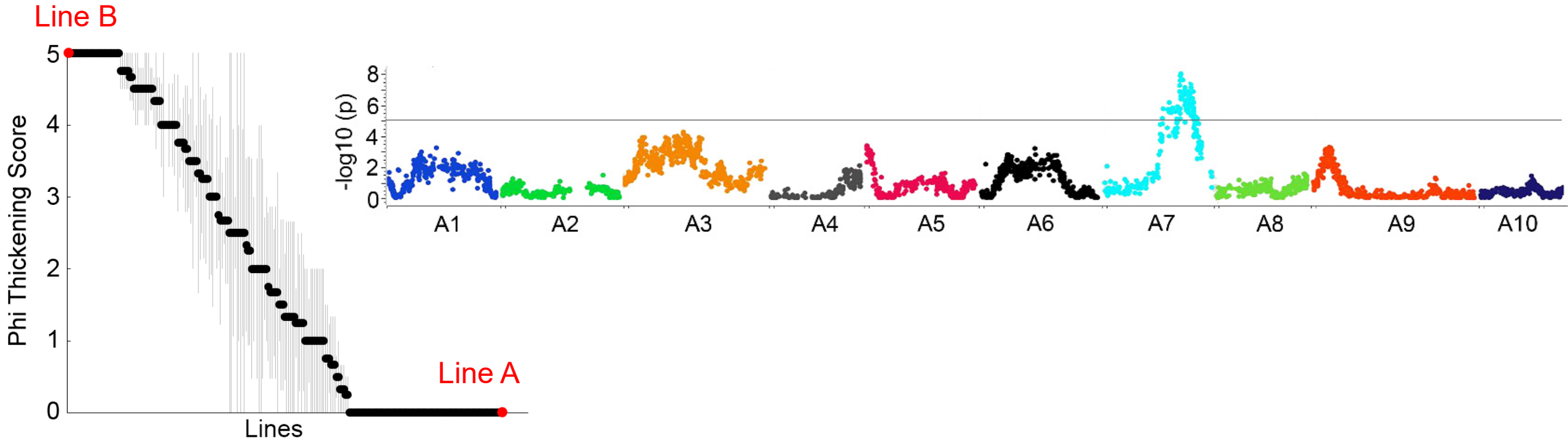


Line B - JA

Line A - JA

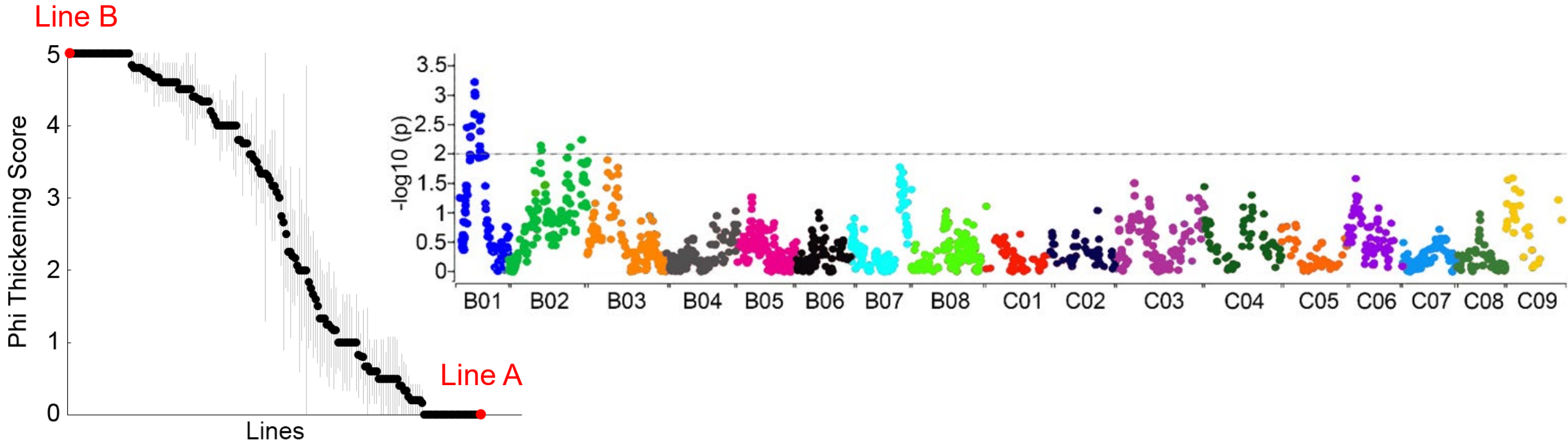
- Line B induces thickenings, but line A never does.

Mapping QTLs in *Brassica rapa*



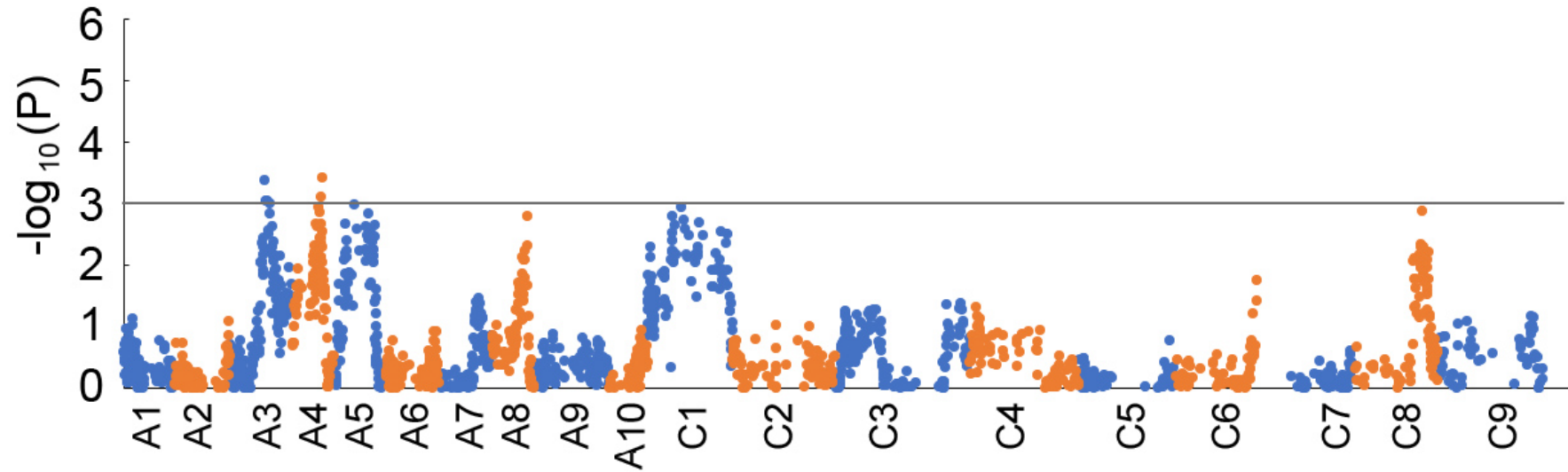
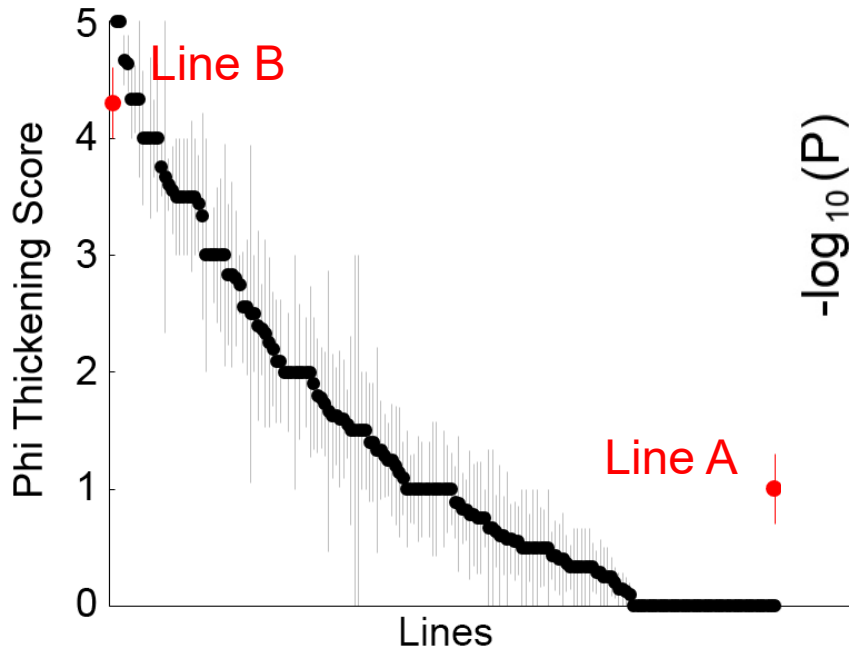
- 235 F2 progeny from the A x B cross were screened.
- A single QTL was mapped to chromosome A7.
- Several candidate genes were identified, but have not been confirmed.

Mapping QTLs in *Brassica carinata*



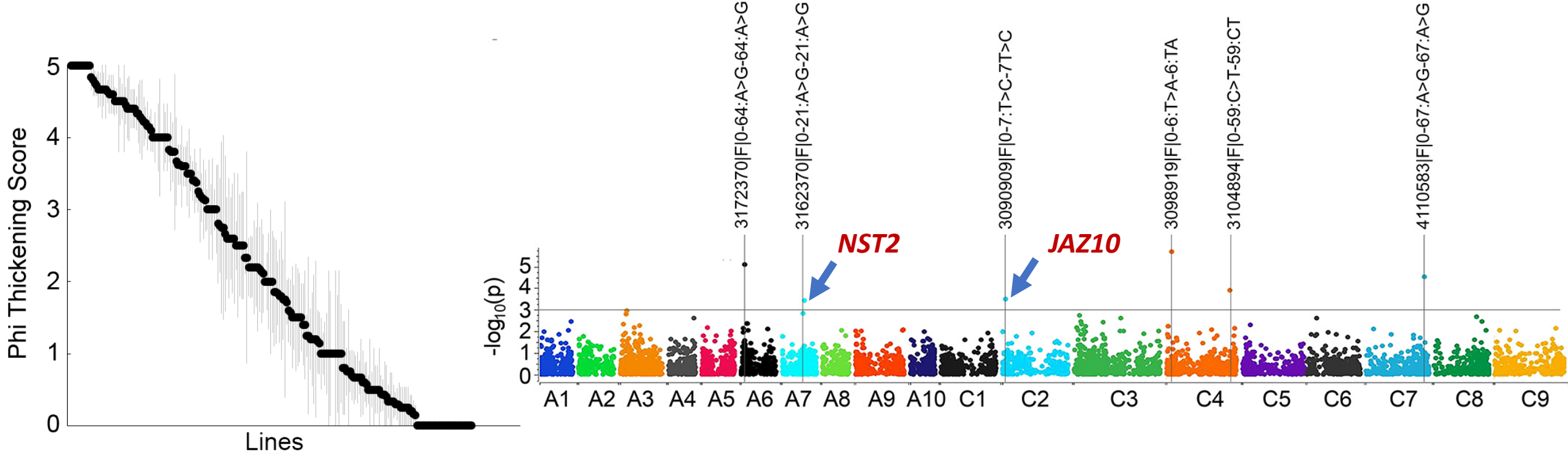
- 181 double haploid progeny from the A x B cross were screened.
- Multiple QTLs are located on chromosomes B1 and B2.
- Candidate genes have not yet been identified.

Mapping QTLs in *Brassica napus*



- 178 of ~240 double haploid progeny from the A x B cross have been screened so far.
- Mapping suggests possible QTLs on multiple chromosomes.
- Candidate genes have not yet been identified.

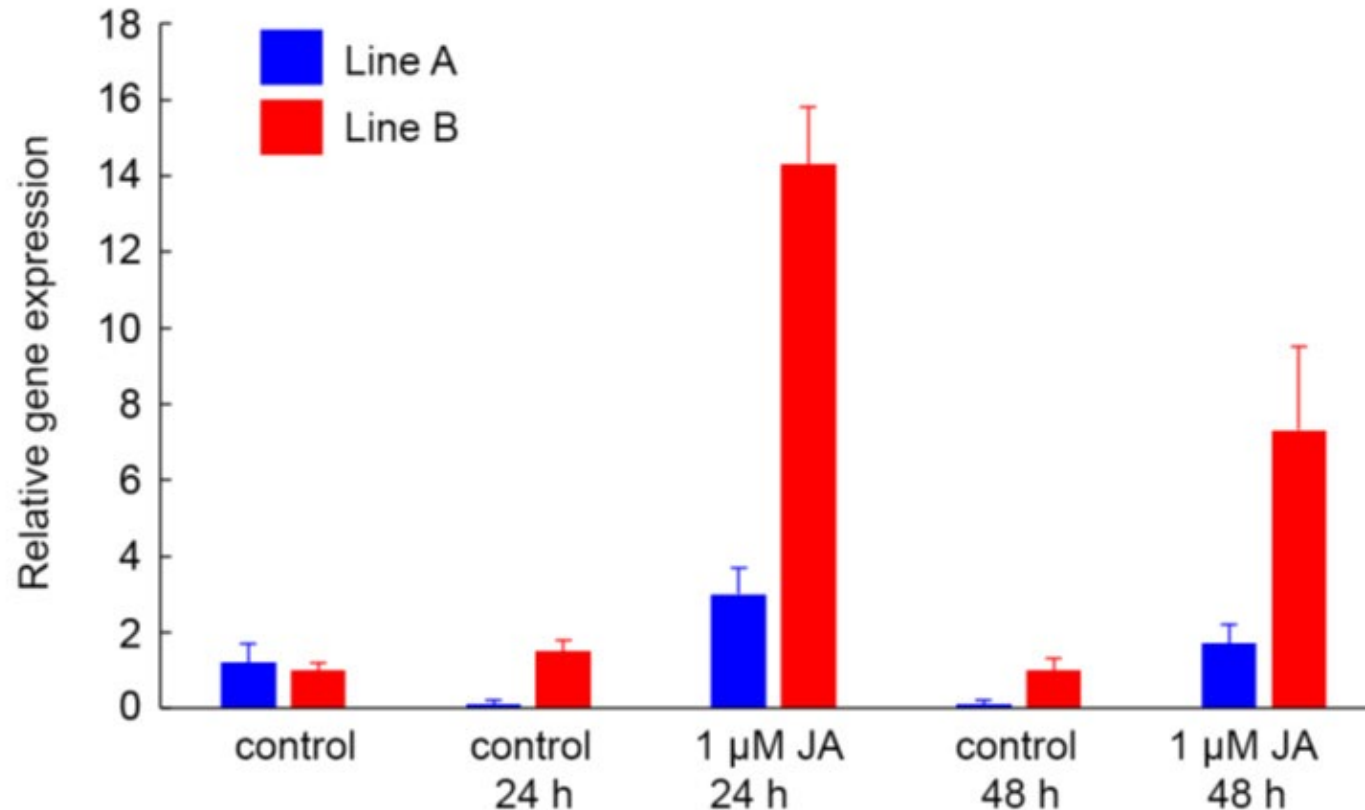
GWAS Analysis of Phi Thickening development in *B. napus*



- 221 lines from the BnASSYST collection were screened.
- 6 QTLs associated with thickening induction.
- These are different to QTLs identified from the *B. napus* breeding population.
- Candidate genes identified and tested.

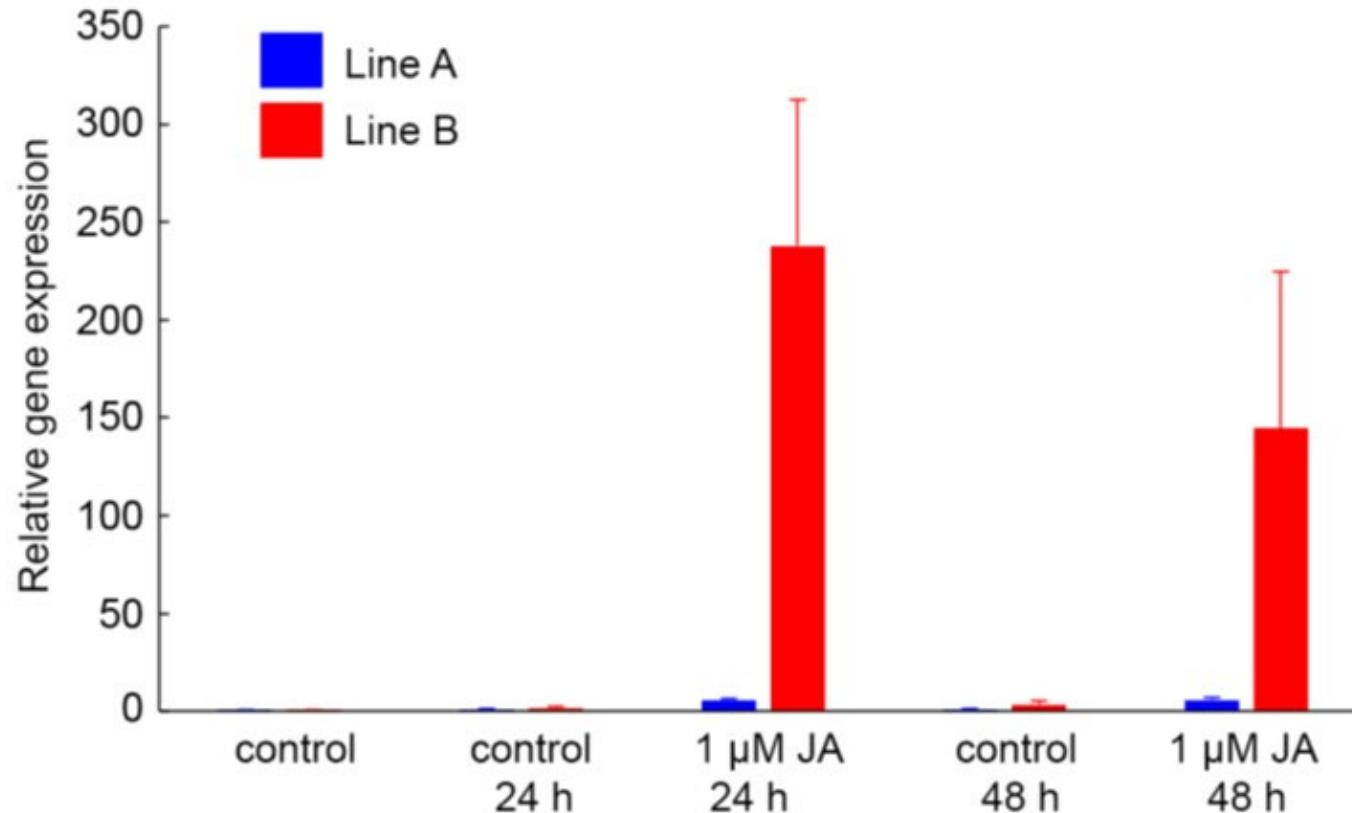
RT-PCR of candidate genes in *Brassica napus*

- On chromosome A07, an NST2 homologue lies only 38 kb from the significant SNP marker.
- *NST2* expression increases significantly in Line B on addition of JA but not in Line A.
- NST2 is a NAC transcription factor that drives secondary cell wall synthesis in *Arabidopsis* xylem.



RT-PCR of candidate genes in *Brassica napus*

- On chromosome C02, a JAZ10 homologue lies only 66 kb from the significant SNP marker
- *JAZ10* levels increase significantly in Line B on addition of JA but not in Line A.
- JAZ10 acts as a repressor of jasmonate signalling.



Conclusions

1. Multiple QTLs linked to phi thickening development are present in different *Brassica* species.
2. The molecular pathways that lead to phi thickening development are now being revealed, with NST2 and JAZ10 strong candidates for functional roles in thickening formation.

Further Questions

1. Do phi thickenings contribute to the mechanical stabilisation or strengthening of the root?
2. Are the variations in JA sensitivity seen with phi thickening induction important to other JA-dependent pathways such as pathogen responses?