MSc Program

Leafy head development of Chinese cabbage

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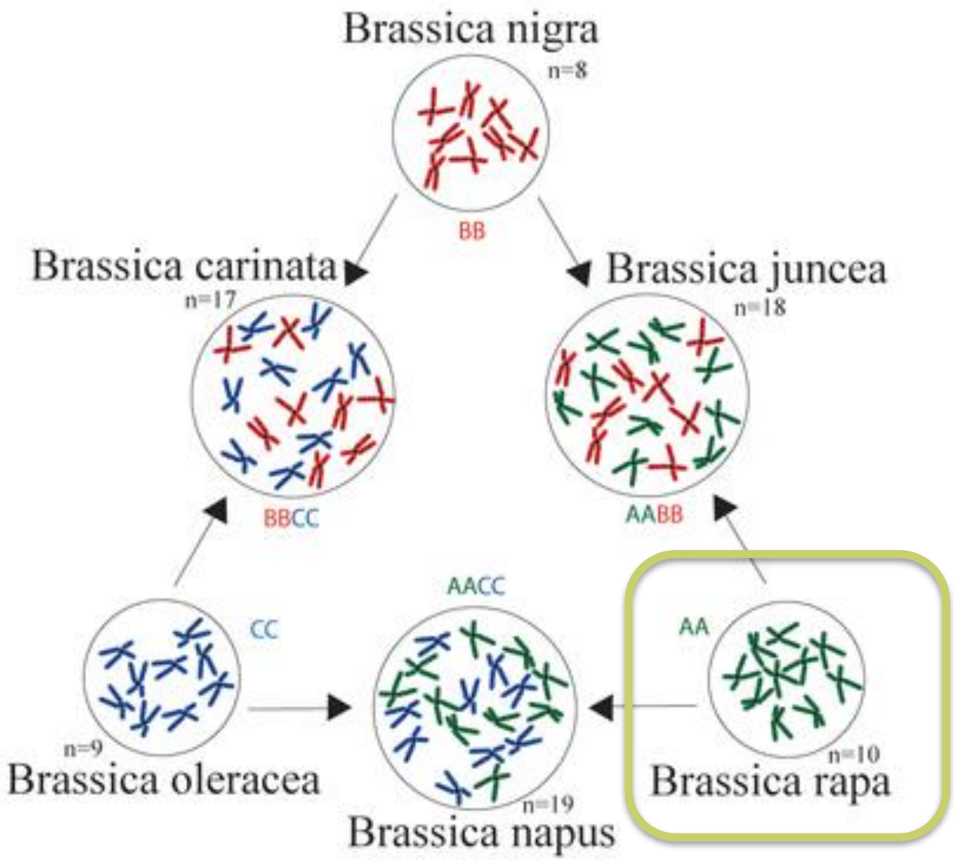
June 19, 2019

Brassicaceae



https://link.springer.com/chapter/10.1007/978-3-662-47901-8_1

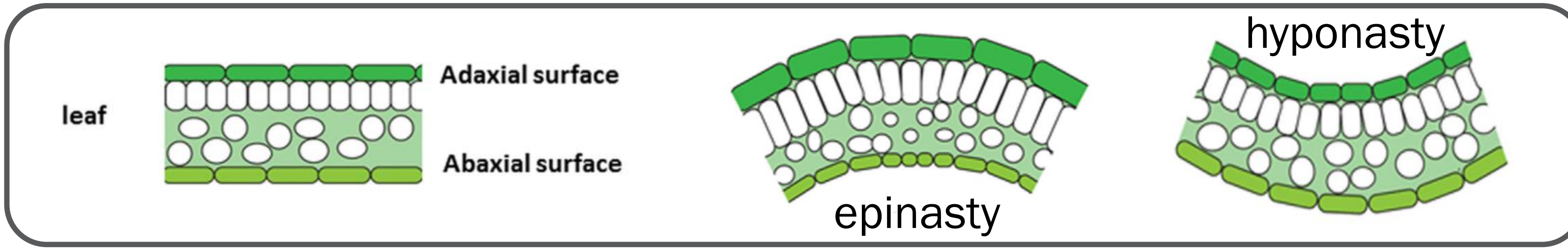
Heading

ssp. *pekinensis*

Non-heading

ssp. *chinensis*

Leaf development in *A. thaliana*



The diagram of the leaf curvature at the cellular level, based on the figure by L. Sandalio (Sandalio et al., 2016)

Ad/Ab polarity pathways

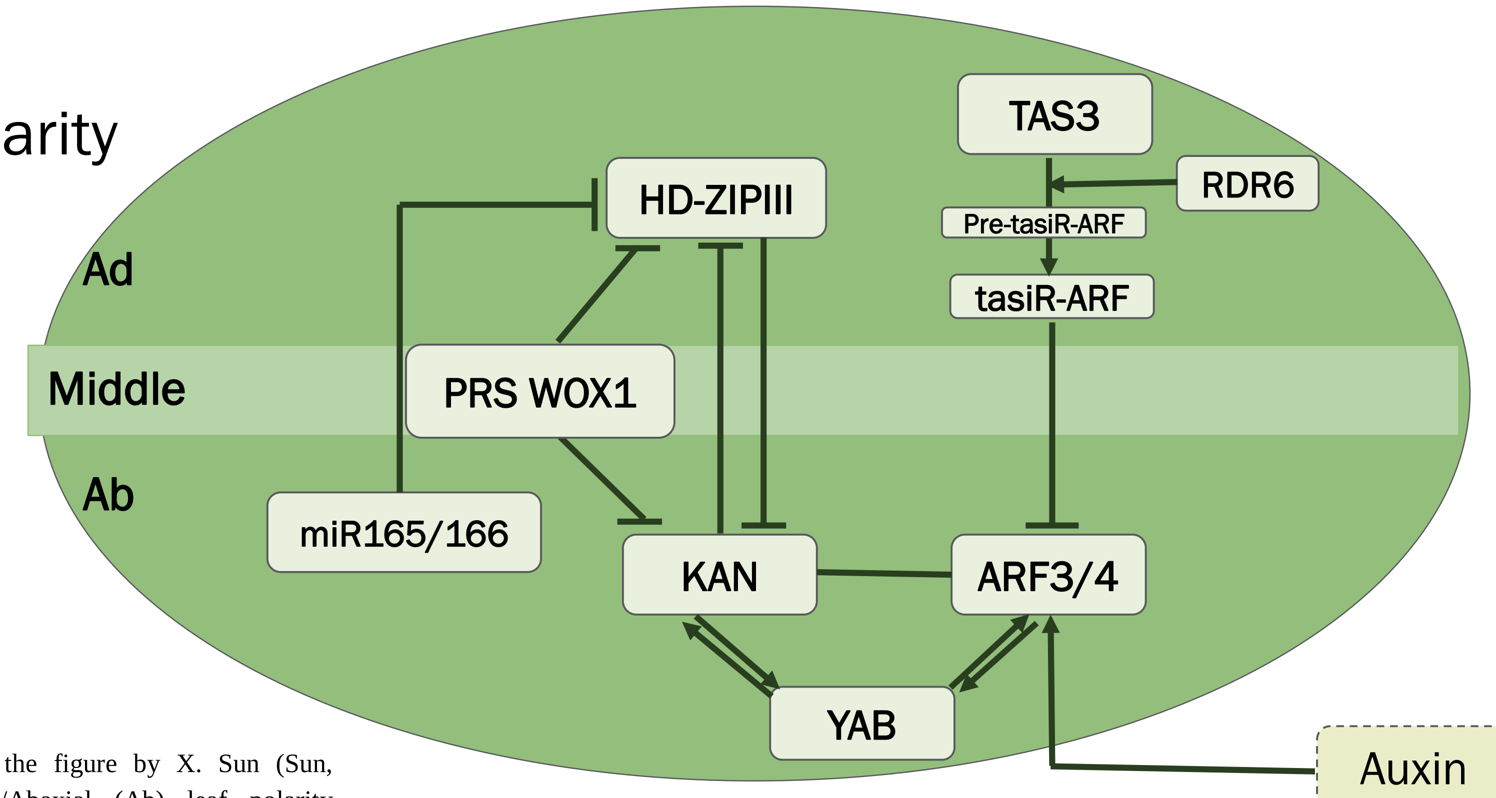


Figure adapted from the figure by X. Sun (Sun, 2018). Adaxial (Ad)/Abaxial (Ab) leaf polarity pathway in *A. thaliana*.

BrARF3.1

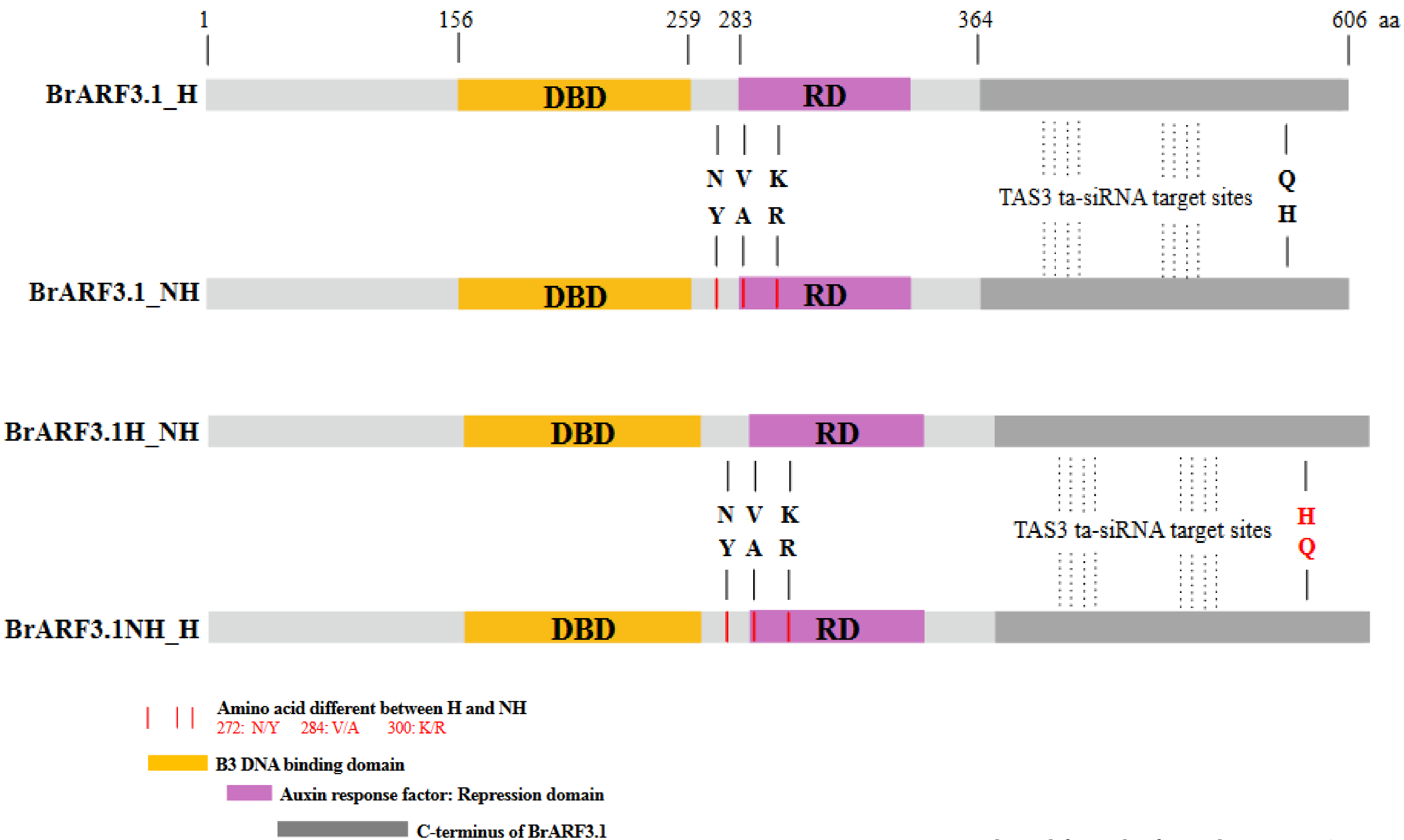
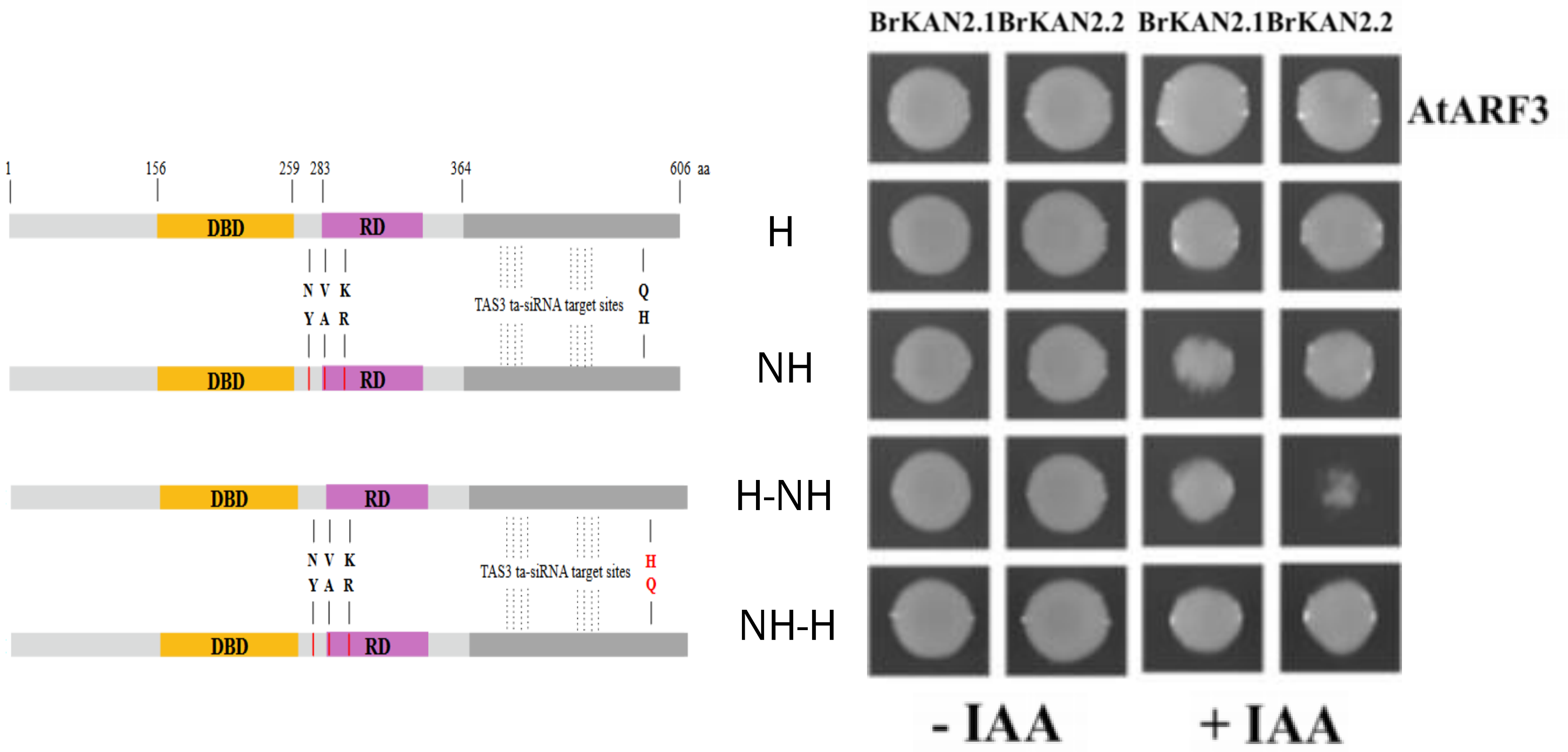


Figure adapted from the figure by X. Sun (Sun, 2018).

BrARF3.1
protein-protein interactions with BrKAN2



The figures are taken from Sun, 2018.

The aim of the work

- to understand better the role of ARF3.1 in head formation on morphological, cellular and molecular levels

Tasks

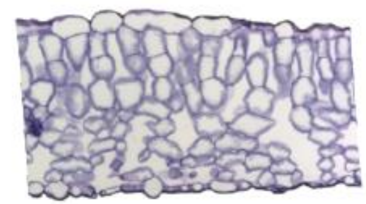
Plant material preparation

A. thaliana with 35S:BrARF3.1

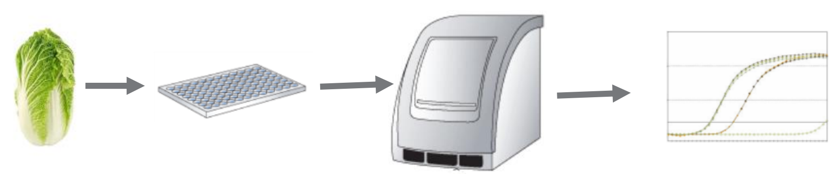
Transformed *B. rapa* with 35S:BrARF3.1 (Pak choi and Chinese cabbage)

Phenotyping of the plants

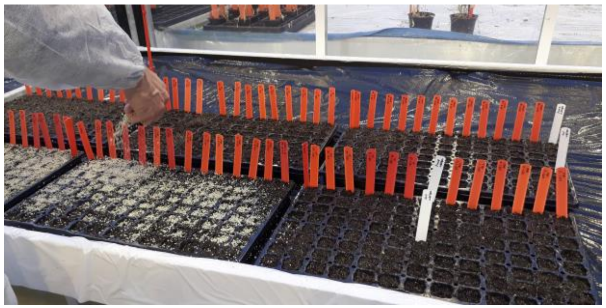
Cellular observation



Gene expression and ta-si RNA analysis

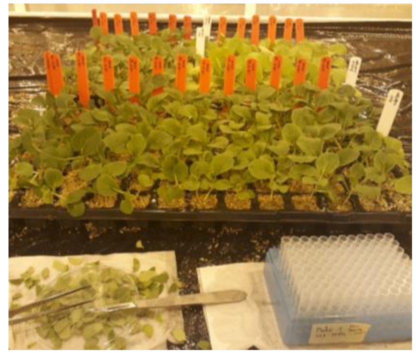


Plant material preparation



Sowing the seeds

A. thaliana: 192 plants
B. rapa: 588 plants



Plant material collection, DNA isolation for marker assisted selection

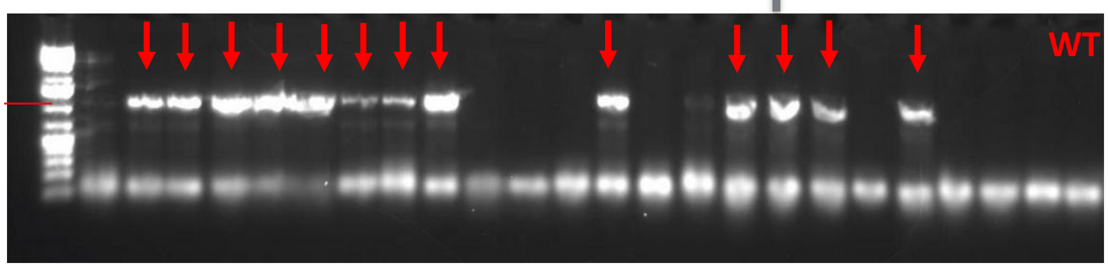
- *PCR*
- *Electrophoresis*

A. thaliana	
H: 6 (2 lines)	
NH: 6 (2 lines)	
H-NH: 9 (3 lines)	
NH-H: 9 (3 lines)	

B. rapa	
PC	**CC**
H: 2	H: 1
NH: 3	NH: 2
H-NH: 8	
NH-H: 5	

1) 35S:BrARF3.1
A. thaliana selection

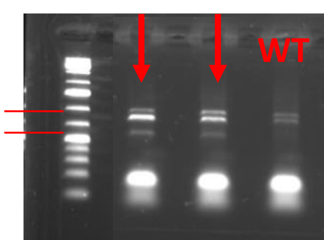
1700 bp



2) 35S:BrARF3.1 ***B. rapa*** (CC and PC) selection

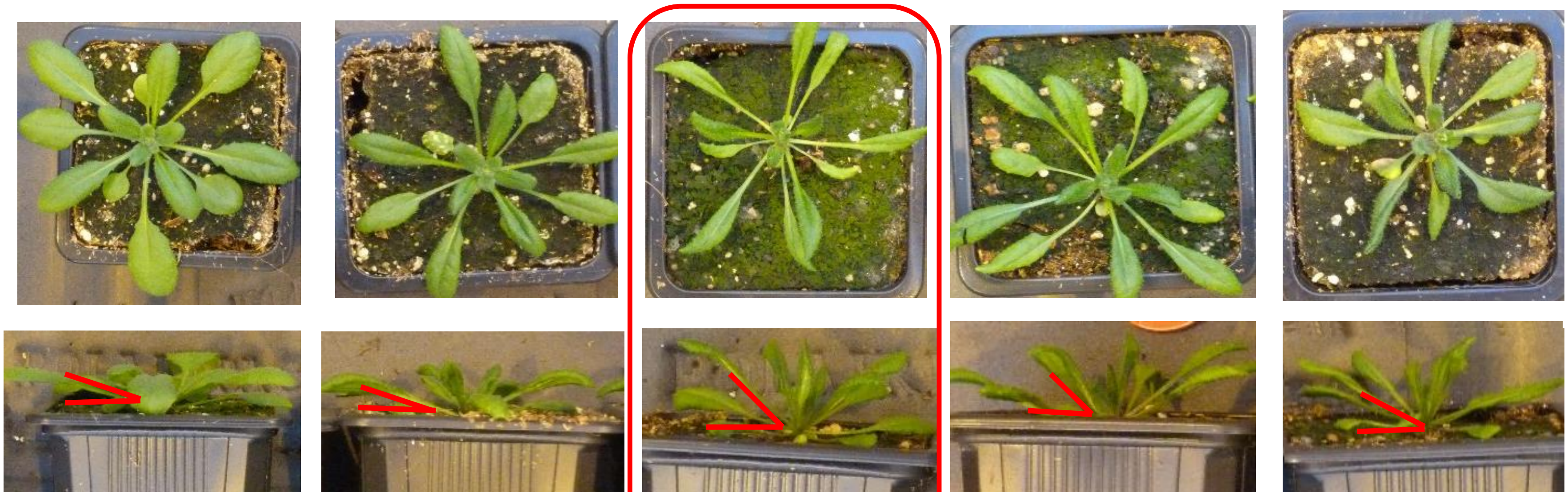
- primer selection
- marker-assisted selection of the plants

1000 bp
700 bp



Phenotyping *A. thaliana* with 35S:BrARF3.1

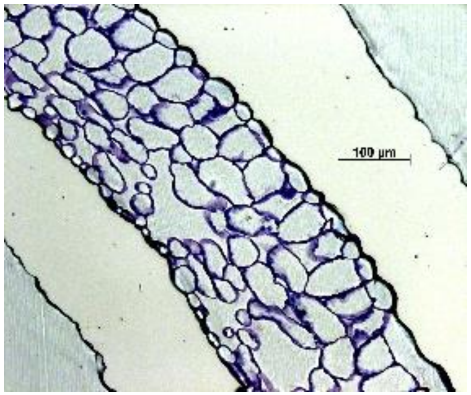
Construct type	Number of leaves	Leaf length	Leaf width	Leaf length/width index	Leaf area from the top view	Leaf curving area	Petiole length	Angle between petiole and surface
H	17	1,53	0,91	2,34	0,98	0,11	0,70	21,17
NH	14	1,45	0,23	3,79	0,48	0,25	0,87	36,73
H-NH	15	1,64	0,59	2,99	0,78	0,21	0,97	28,76
NH-H	15	1,71	0,93	2,58	1,13	0,21	0,91	25,49
WT	15	2,03	1,60	2,14	1,58	0,04	1,09	20,56
LSD	*2*	*0,36*	*0,41*	*0,51*	*0,42*	*0,11*	*0,36*	*15,7*



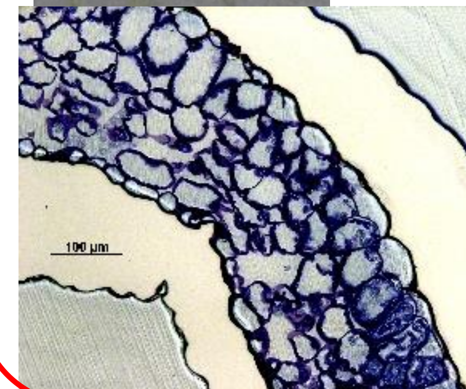
WT H NH H-NH NH-H

Cellular observation of *A. thaliana*

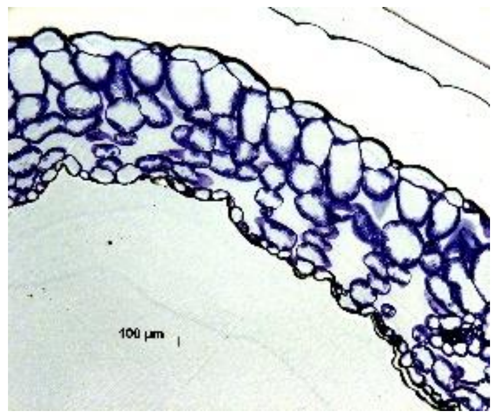
H



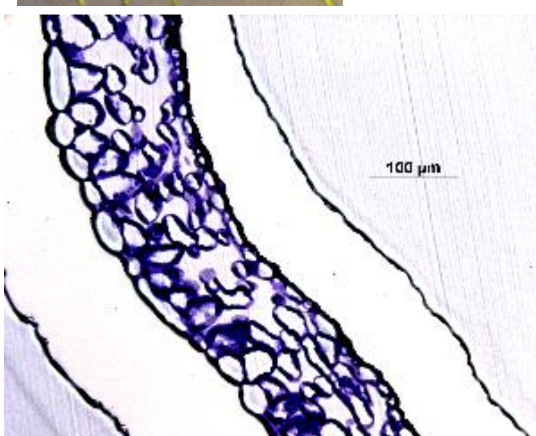
NH



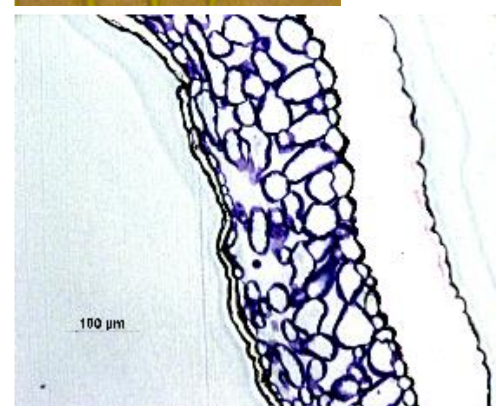
WT



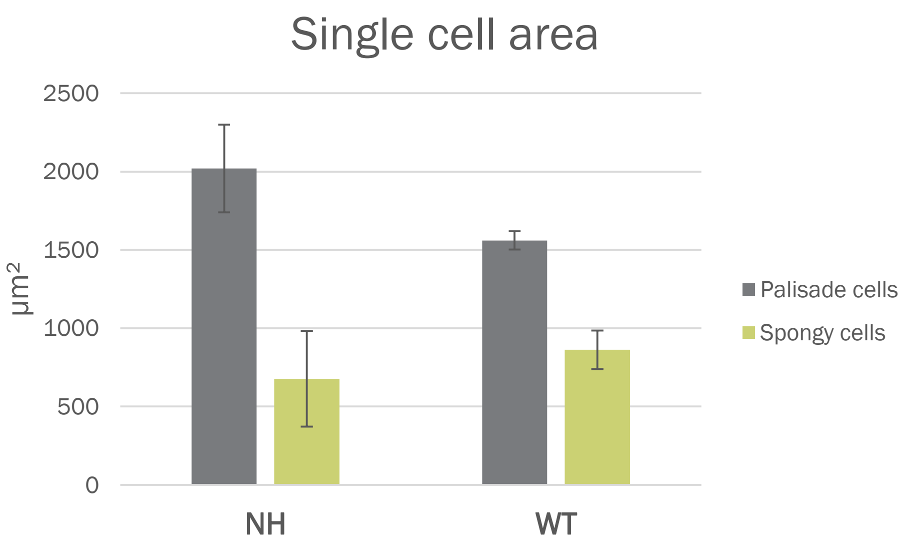
H-NH

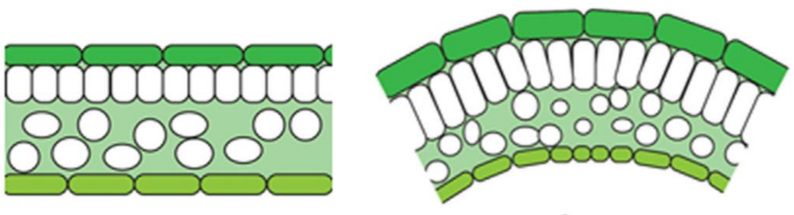


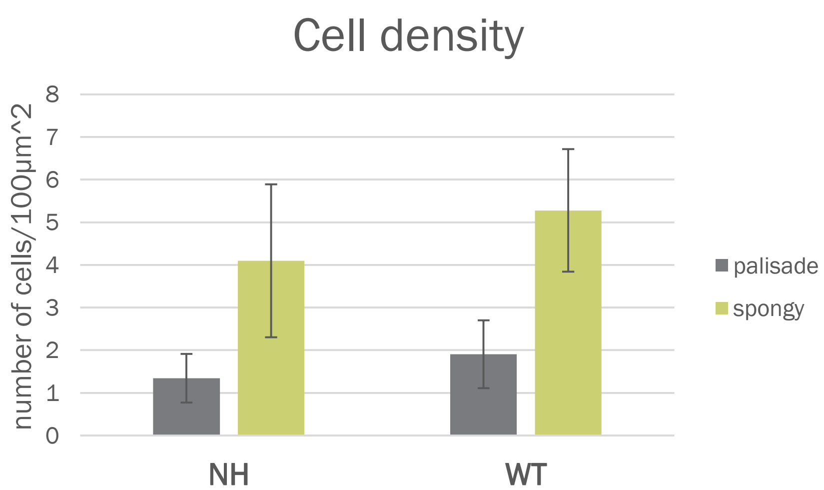
NH-H

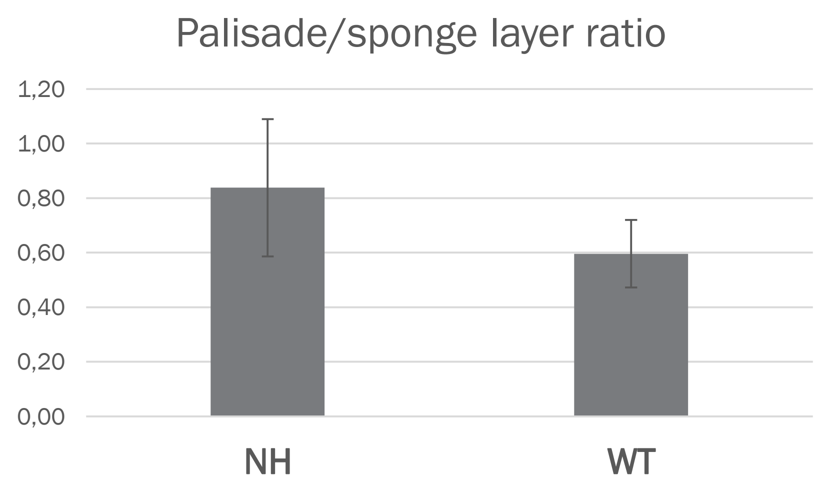


The expansion of palisade cells is the key factor for leaf epinasty of 35S:BrARF3.1 *A. thaliana* plants

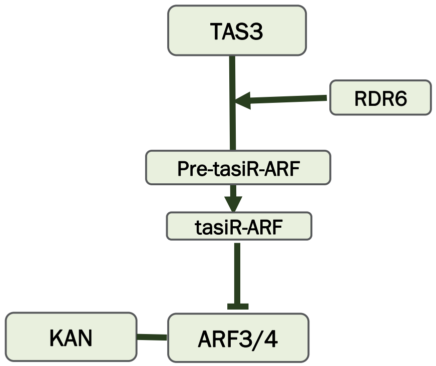


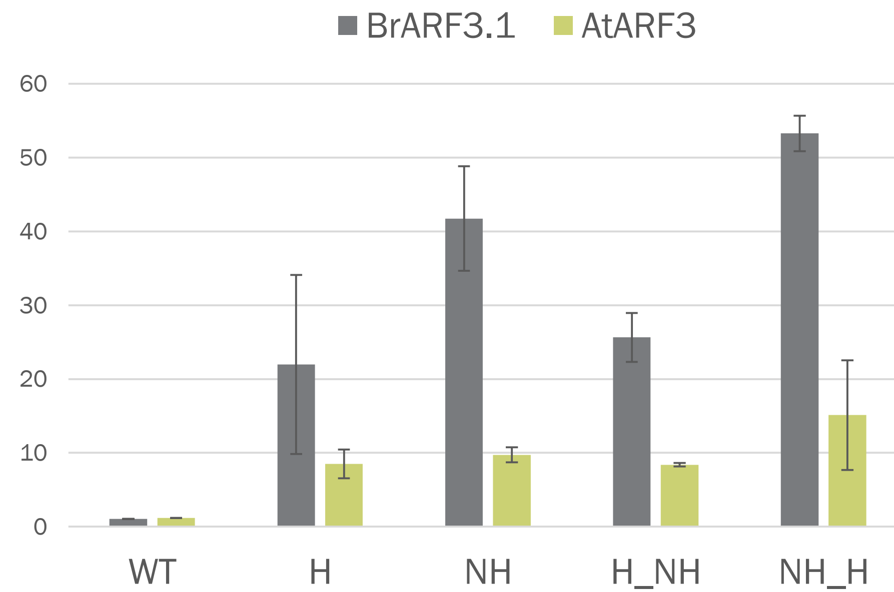


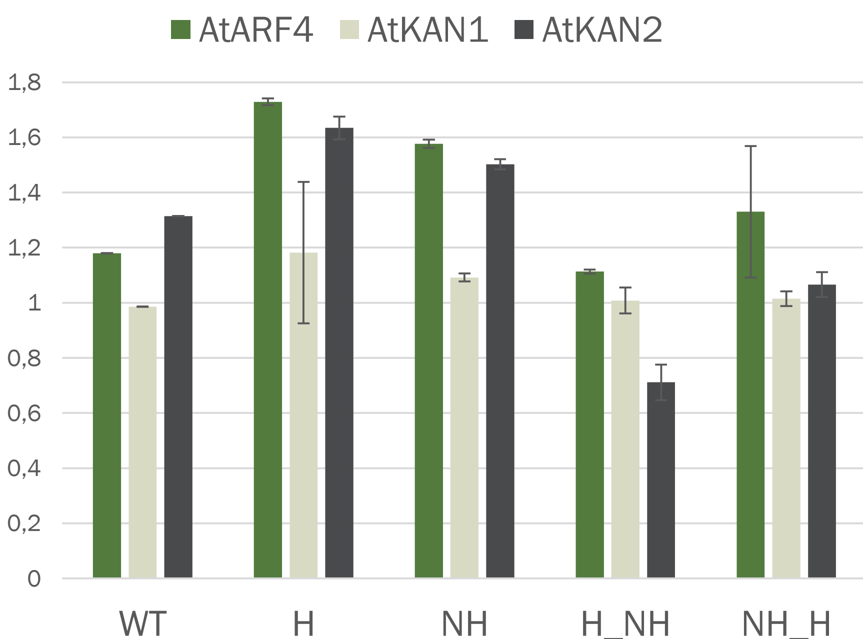


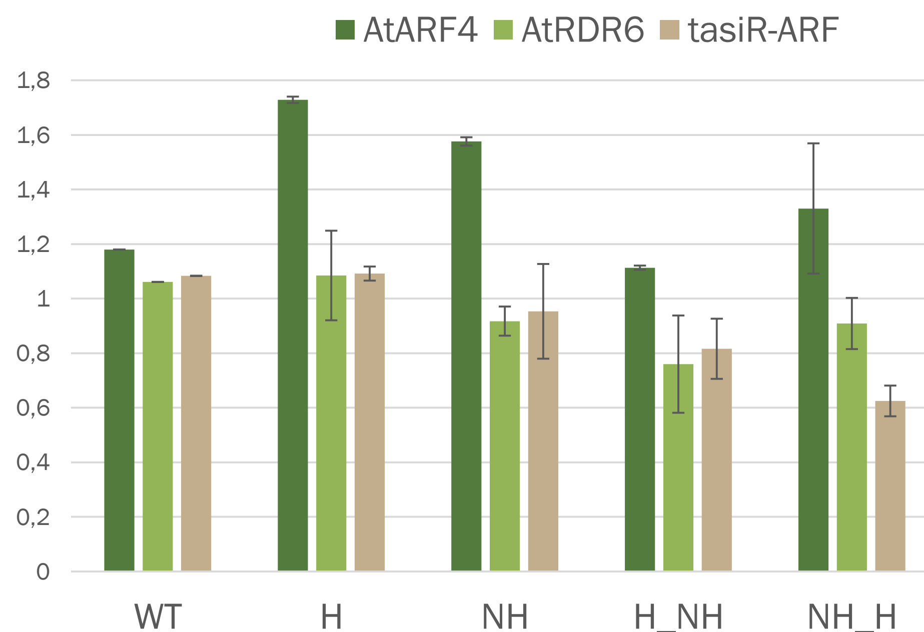


Gene expression analysis of *A. thaliana*
($2^{-\Delta\Delta Ct}$)

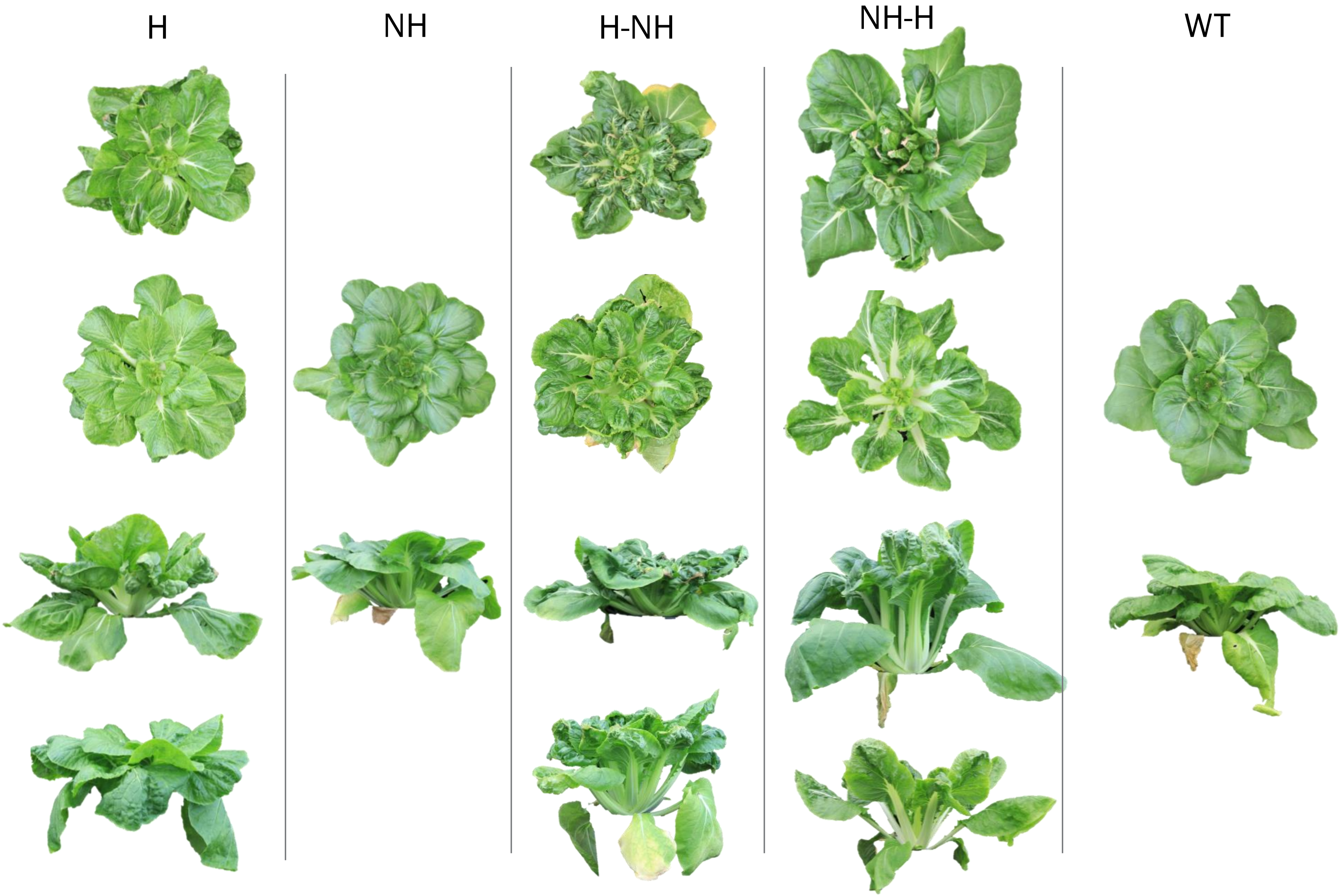




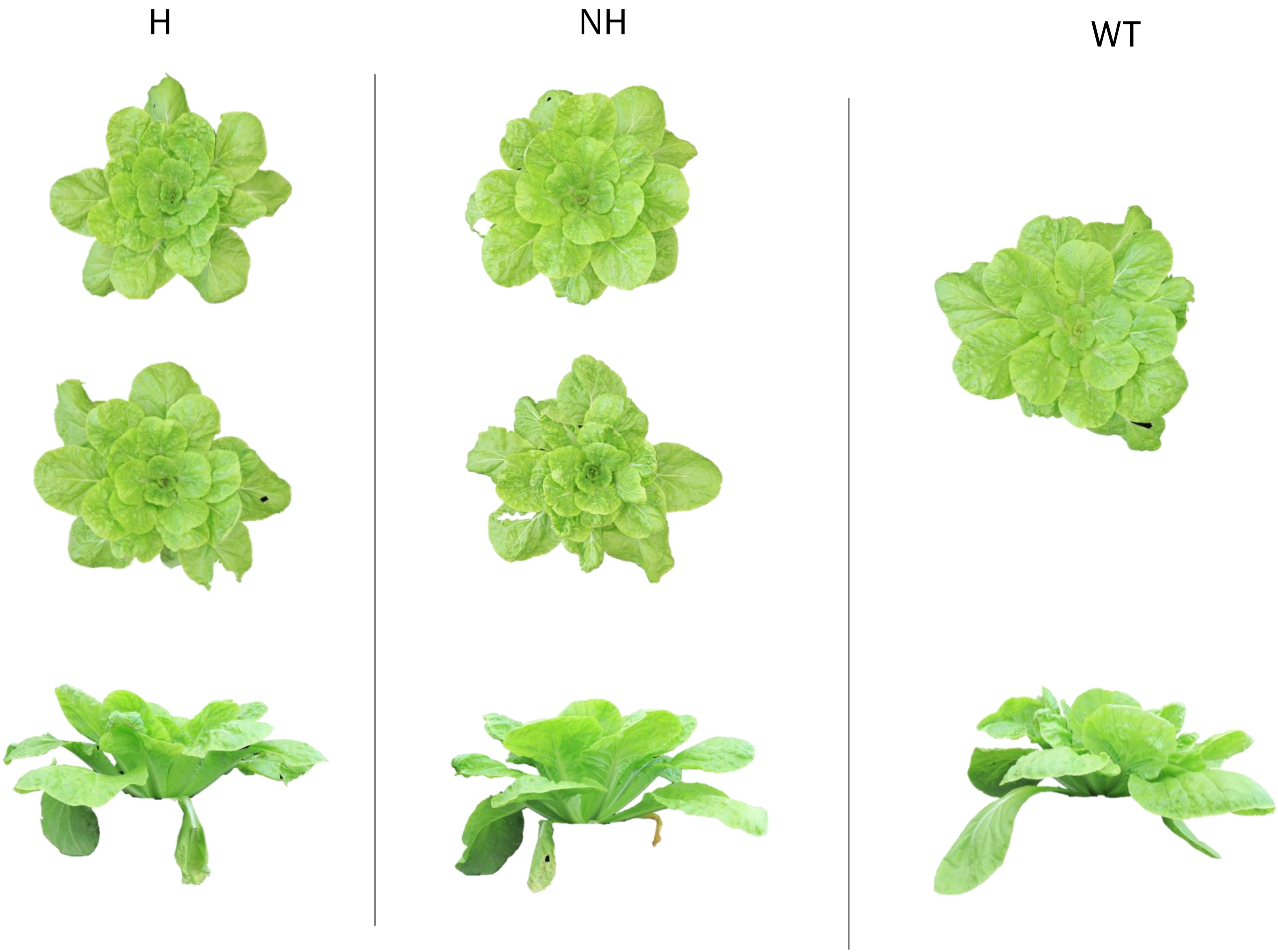




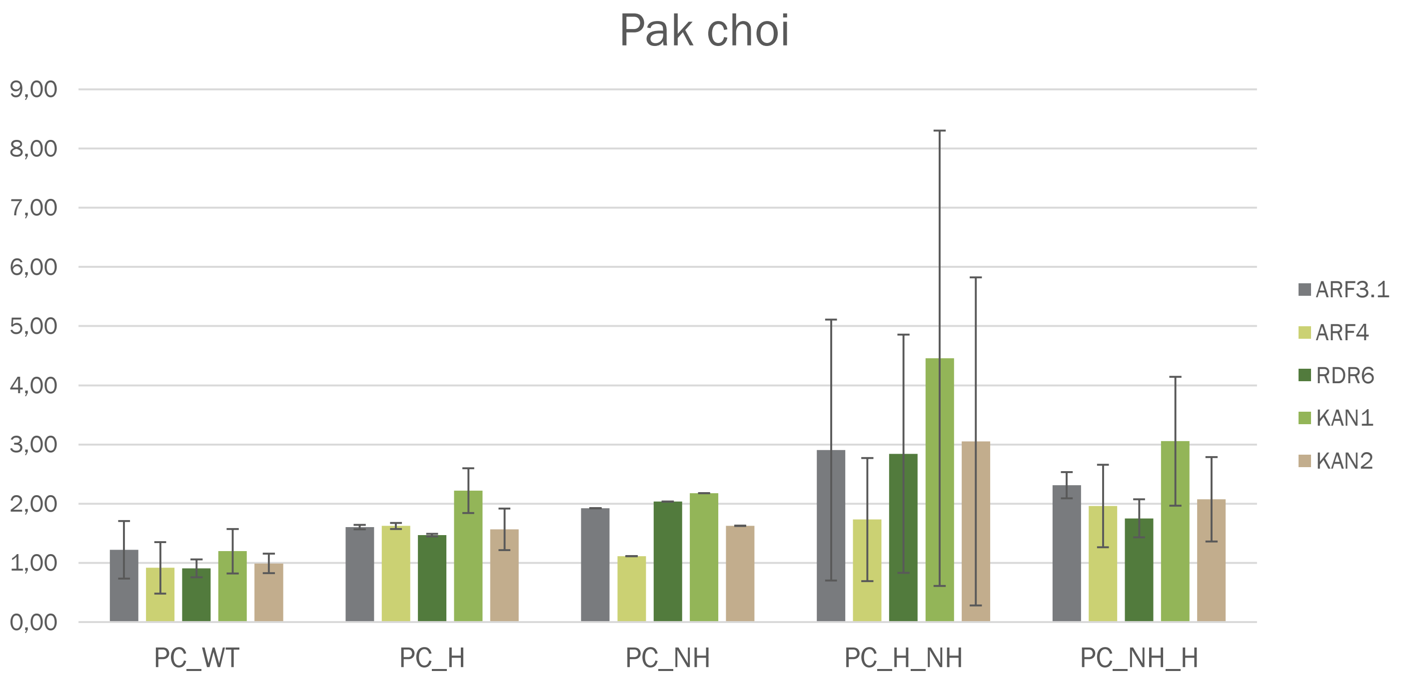
35S:BrARF3.1 Pak choi

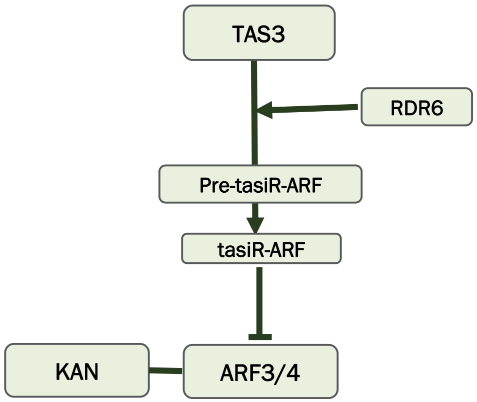


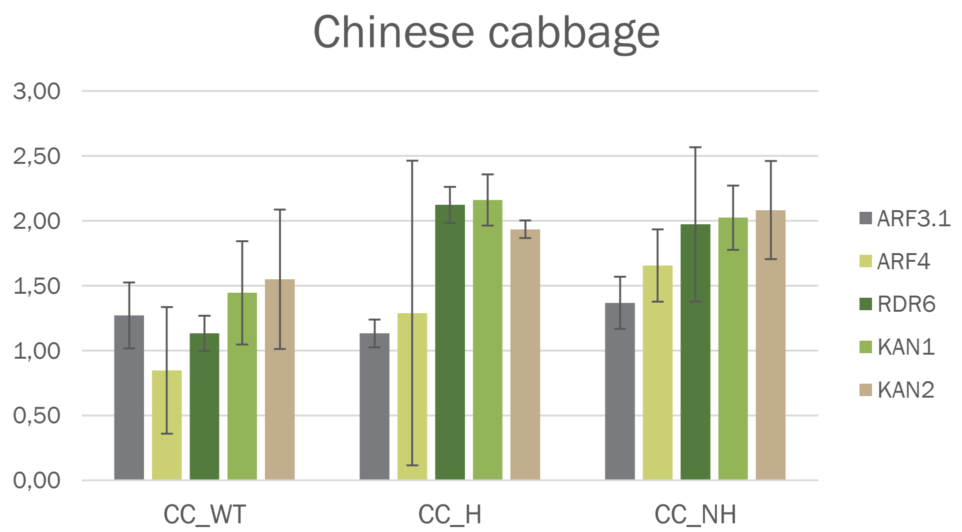
35S:BrARF3.1 Chinese cabbage



Gene expression analysis of 35S:BrARF3.1 *B. rapa* ($2^{-\Delta\Delta Ct}$)







Conclusions:

- *A. thaliana* and *B. rapa* plants carrying the 35S:BrARF3.1 constructs have been selected.
- 35S:BrARF3.1_NH mutants revealed the strongest phenotype in *A. thaliana.*
- The expansion of palisade cells is the key factor for leaf epinasty of 35S:BrARF3.1 *A. thaliana* plants.
- Pak choi revealed the greater phenotypic response to the 35S:BrARF3.1 inserted construct as compared to Chinese cabbage.
- The experiments with *A. thaliana* and *B. rapa* generally have shown the greater phenotypic response of heterologous expression system contrasted with the homologous one.

Acknowledgements

Konstantin Severinov

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Zihan Liu

Eugene Lysenko

1. What DNA markers were used for transgenic lines testing?

Gene	Forward	Reverse
For A. thaliana		
BrARF3.1	TGGTGATGCTGTGCTTTTCC	AAGAACTTCGGTGCAGGGA
AtARF3	CGCCTACTCAATAACCGATCATC	ACGGCCCACACCAAATGTT
AtARF4	CGCTTAAATCATTCCCGCAAT	ACTTGTTGGCTTGGTAAGCAAAG
AtKAN1	CCTTTCCACCAACAAACCTCTT	AACACCTCTTAGCCTTGGAGAA
AtKAN2	AAGGAACTAGATGGAAAGTGCTCAA	GCTTGTTCCCGAGATGCTTG
AtRDR6	ACGCCCTAATTTCCAGGCAAC	ACCATCAAATGTGGGGATGT
pre-tasiR-ARF	GAGATTATTGGATCCGCTGTGC	TGTGGAGATTAGCTCAGGAGGG
For B. rapa		
BrARF3.1	TGGTGATGCTGTGCTTTTCC	AAGAACTTCGGTGCAGGGA
BrARF4	ACCTGCATCTAACCTGAGCA	ACCATCATGTCGTCCTCACT
BrKAN1	GAGGATGCGTTGGAGGAGTA	GTTTGTCGGTTGTCTTCACTGT
BrKAN2	GAAGCAACGCCTAAATCAGTTCT	CTTTGTCGGTTGTCTTCACTGT
BrRDR6	GTAAGCATTGGTGGGTTTGG	ACCATCAAATGTGGGGATGT

2. What are replications in expression analysis experiments?

The following ***Arabidopsis*** samples were used:

- 6 samples with 35S:BrARF3.1_H overexpression,
- 5 samples with 35S:BrARF3.1_NH overexpression,
- 9 samples with 35S:BrARF3.1_H_NH overexpression,
- 7 samples with 35S:BrARF3.1_NH_H overexpression,
- 2 wild types (Col-0).

The following ***Brassica*** samples were used:

for **Pak choi**:

- 2 samples of 35S:ARF3.1_H overexpressors,
- 1 sample of 35S:ARF3.1_NH overexpressor,
- 8 samples of 35S:ARF3.1_H_NH overexpressors,
- 5 samples of 35S:ARF3.1_NH_H
- 3 wild type samples;

for **Chinese cabbage**:

- 2 samples of 35S:ARF3.1_H,
- 2 samples of 35S:ARF3.1_NH,
- 3 WT samples.

3. What specific leaves were taken for analysis?

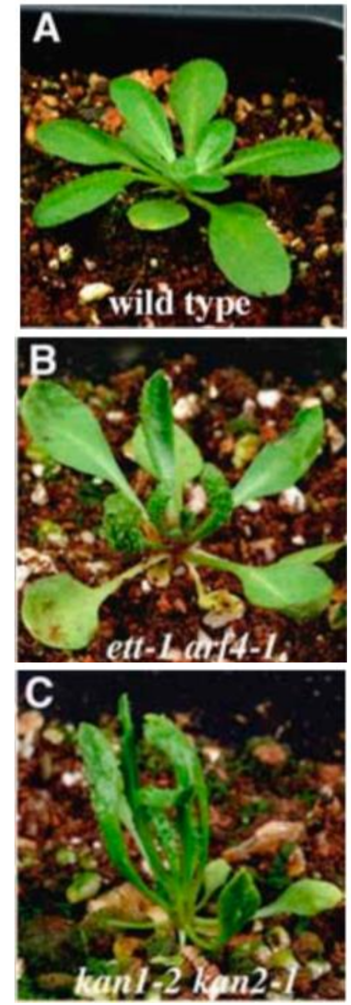
- The youngest *A. thaliana* leaves were collected from 3-weeks-old plants.

- The youngest *B. rapa* leaves were collected from 5-weeks-old plants.

The collected tissue was simultaneously frozen in liquid nitrogen and stored in -80˚. Before the isolation the leaf tissue was milled to powder.

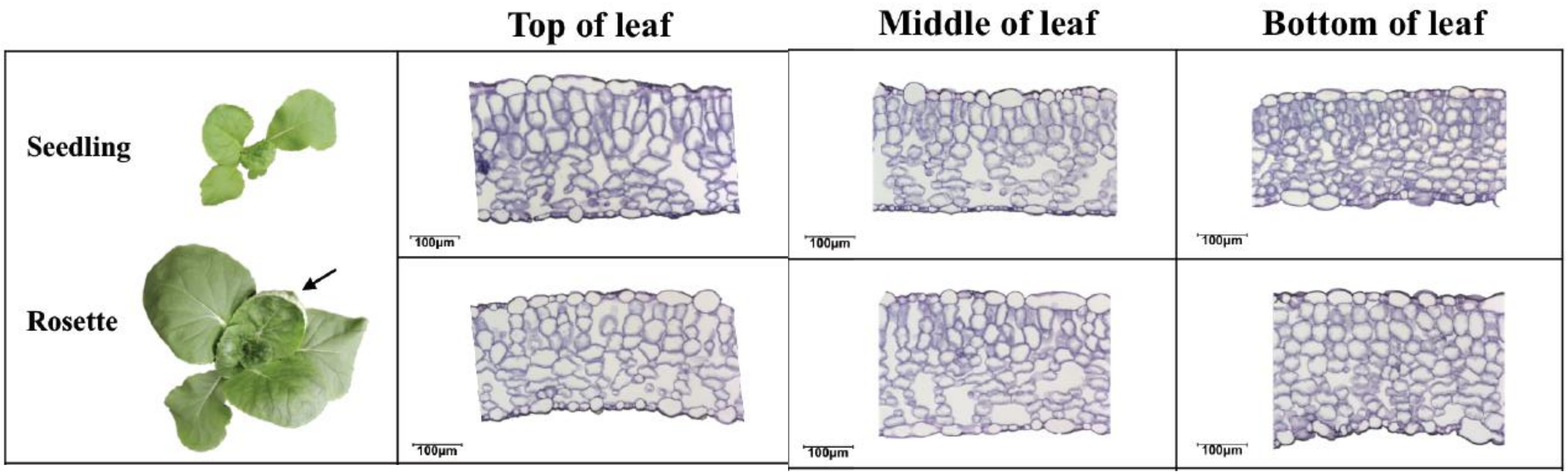
4. What are the similarities and differences in the phenotype of leaf abaxialization under expression of ARF3 and, for example, KANADI?

- The loss of ARF3 or ARF4 doesn't lead to the morphotype changes of leaves, while the *arf3-arf4* double mutants have narrow leaves with upward curling and abaxial overgrowth indicating that ARF3 and ARF4 act as sister-pair genes in leaf developmental process (Hunter et. Al., 2006)
- The ectopic expression of neither ARF3 nor ARF4 doesn't result in significant abaxialization phenotype (Pekker et al., 2005). But overexpression of ARF3, mutated in tasiR-RNA target sites revealed the downward curling leaves (Fahlgren et al., 2006).
- Ectopic expression of either of the KANADI genes in leaf primordia results in dramatic transformation of adaxial cell types into abaxial ones, failure of lateral blade expansion (Eshed et al., 2001).
- *kan1-kan2* double mutants have reduced blade expansion and form ectopic leaf-like outgrowths on the abaxial blade surface (Eshed et al., 2001)
- *kan1-kan2-kan3* triple mutants have almost no blade expansion and produce nearly cylindrical, adaxialized leaves with radialized stem vasculature (Eshed et al., 2004).



Pekker, I. (2005). *Auxin Response Factors Mediate Arabidopsis Organ Asymmetry via Modulation of KANADI Activity. THE PLANT CELL ONLINE, 17(11), 2899–2910.* doi:10.1105/tpc.105.034876

5. In the Sun 2018 the abaxialization gradient in Chinese cabbage leaves was shown. At the base of the leaf the palisade form of the parenchyma was absent. Has a similar gradient been observed in the leaves of transgenic *Arabidopsis*?



The figures are taken from Sun, 2018.