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# Hormonal Responses to *Plasmodiophora brassicae* Infection in *Brassica napus* Cultivars Differing in Their Pathogen Resistance

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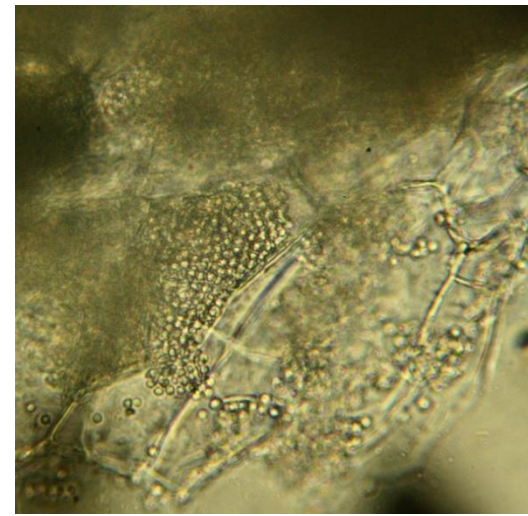
# Clubroot

- Obligate biotroph *Plasmodiophora brassicae* - cause clubroot disease in 3700 members of *Brassicaceae*, including oilseed rape (canola)
- Chemical treatments (e.g. sodium N methyldithiocarbamate) have had limited effects
- Cultivation of (at least partially) resistant cultivars - the only effective solution
- The understanding of mechanisms of efficient plant defence - an important prerequisite for the breeding of new resistant cultivars



# Clubroot and Phytohormones

- Infection development requires modulation of host metabolism, including hormone pools
- **Gall formation** - associated with the stimulation of cell division - *cytokinin* and *auxin* activity
- **Cytokinin (CK):**
  - enhance the sink strength
  - elevation in galls important for the attraction of nutrients to infected roots
  - P. brassicae* convert adenine to *trans*-zeatin and its riboside → pathogen is able to form CKs at least in the limited amount
- **Auxin (IAA, indole-3-acetic acid):**
  - elevation of content as well as stimulation of auxin-inducible genes in *A. thaliana* → morphological changes in the roots including cell elongation



# Clubroot and Phytohormones

- The pathogen imposes alteration of the levels of other hormones

- ***Abscisic acid (ABA)***:

hormone associated with abiotic stress responses

found elevated in the host plant at the later stages of *P. brassicae* infection

at least partially due to the restriction of water transport by roots and the resulting water deficit in above-ground parts

- ***Salicylic acid (SA)***:

the key hormone in the defence against biotroph infection

Its level was found to be strongly enhanced by *P. brassicae* infection in resistant ecotype of *A. thaliana*

- ***Jasmonic acid (JA)***

Elevation of biosynthesis and JA-responsive gene expression may raise a question whether this effect is a part of the host plant defence or a part of the pathogen strategy to overcome plant defence by suppression of the SA pathway





# Aims and Goals

- Hypothesis: the pathogen responses - differ among different species or even cultivars
- Compared two cultivars of *Brassica napus* :
  - SY Alister*** - the more resistant cultivar
  - Hornet** - the sensitive cultivar
- Characterize responses associated with enhanced resistance to *P. brassicae*
- Determine the responses at the:
  - levels of hormones (CK, auxin, SA, JA and ABA)
  - expression of hormone-related genes
- The effect of *P. brassicae* infection - followed in roots and galls and also in leaves - taking into account the communication between roots and shoots

SY Alister



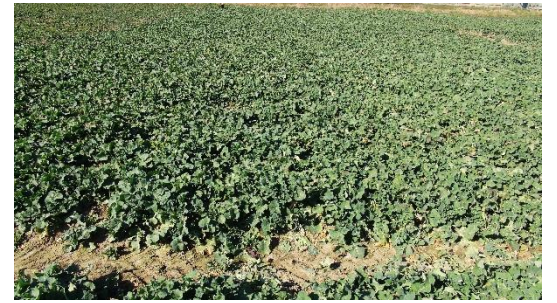
Hornet



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SY Alister



Hornet





# Material and Methods

- Biological materials and pathogen inoculation
  - ✓ susceptible cultivar Hornet
  - ✓ the clubroot resistant cultivar SY Alister
  - ✓ *Plasmodiophora brassicae* pathotype 6 (according to Williams, 1966)
- Disease assessment (Strelkov et al., 2006)

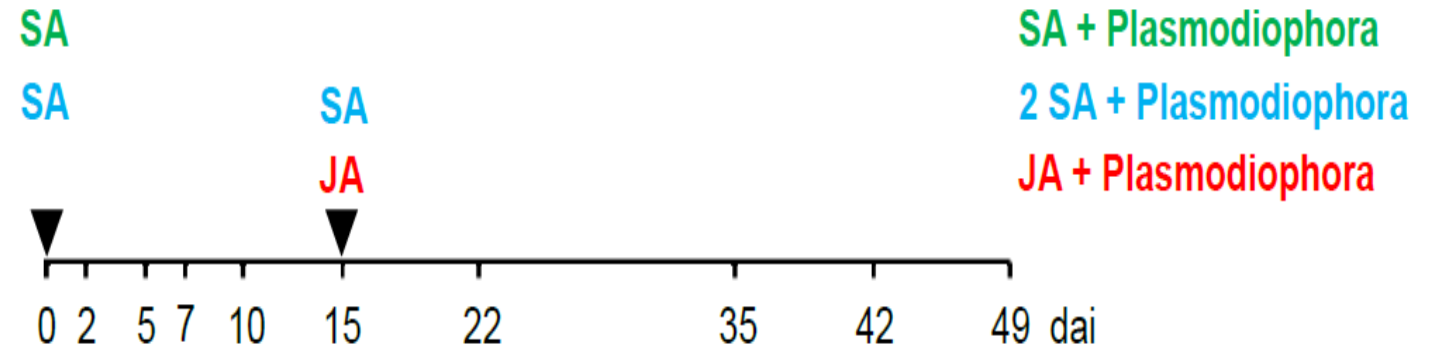
$$DI(\%) = \frac{\sum(n \times 0 + n \times 1 + n \times 2 + n \times 3)}{N \times 3} \times 100$$

0–3 scale: 0 – no galling, 1 – a few small galls, 2 - moderate galling on the main and lateral roots, and 3 – severe galling, the root was totally deformed.



# Material and Methods

- Experimental design:



- Control
- Plants inoculated by *P. brassicae*
- Inoculated plants treated once by foliar spray with 1 mM SA at the day of inoculation
- Inoculated plants treated by foliar spray with 1 mM SA at the day of inoculation and 15 dai (the beginning of gall formation)
- inoculated plants treated with 1 mM JA 15 dai.

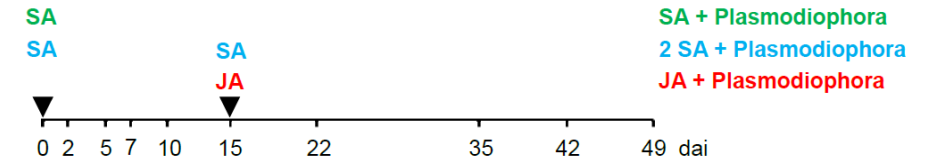


# Material and Methods

- Phytohormone analyses - analysed using HPLC (High Performance Liquid Chromatography)
- Quantitative RT-PCR – RNA isolation, reverse transcription, qPCR
- Statistic analysis - two-sample Student's t-test with the software PAST 3.01.

# Results: Plasmodiophora brassicae infection responses differ between cultivars and hormone treatments

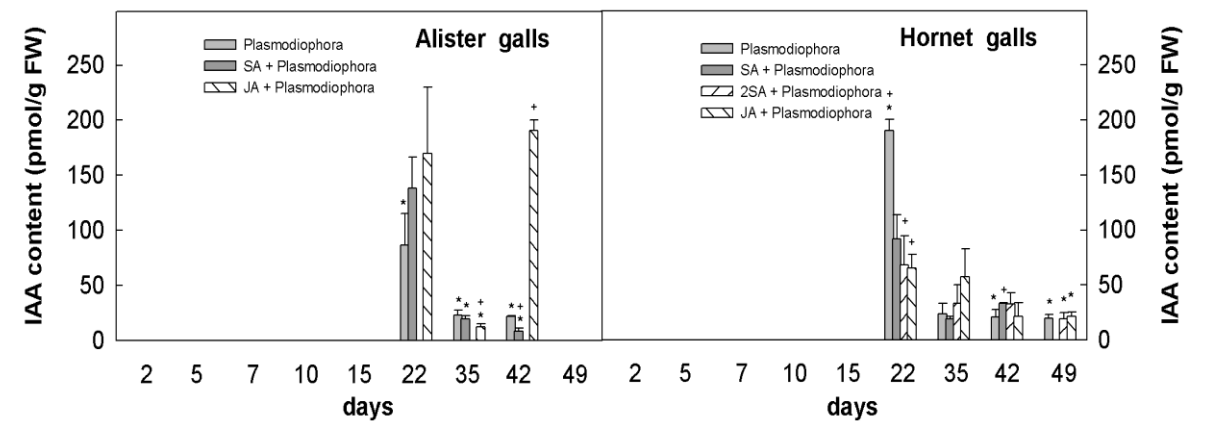
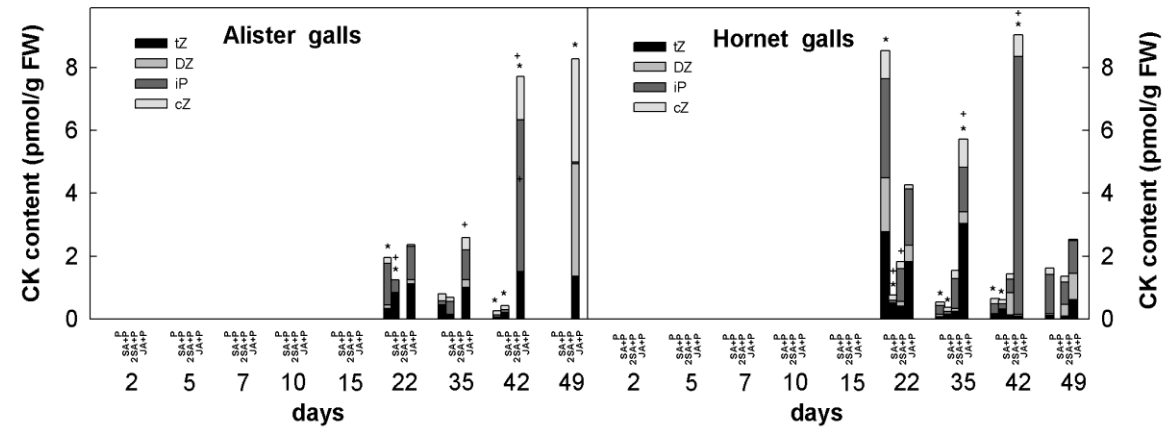
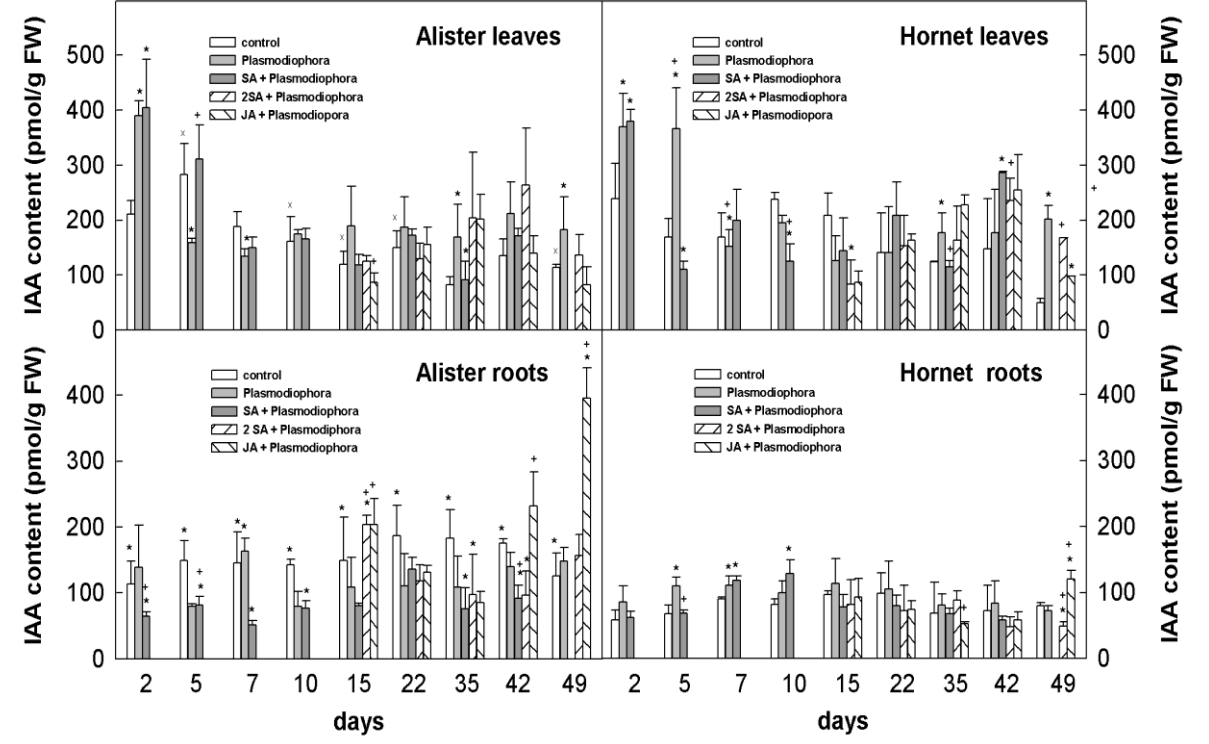
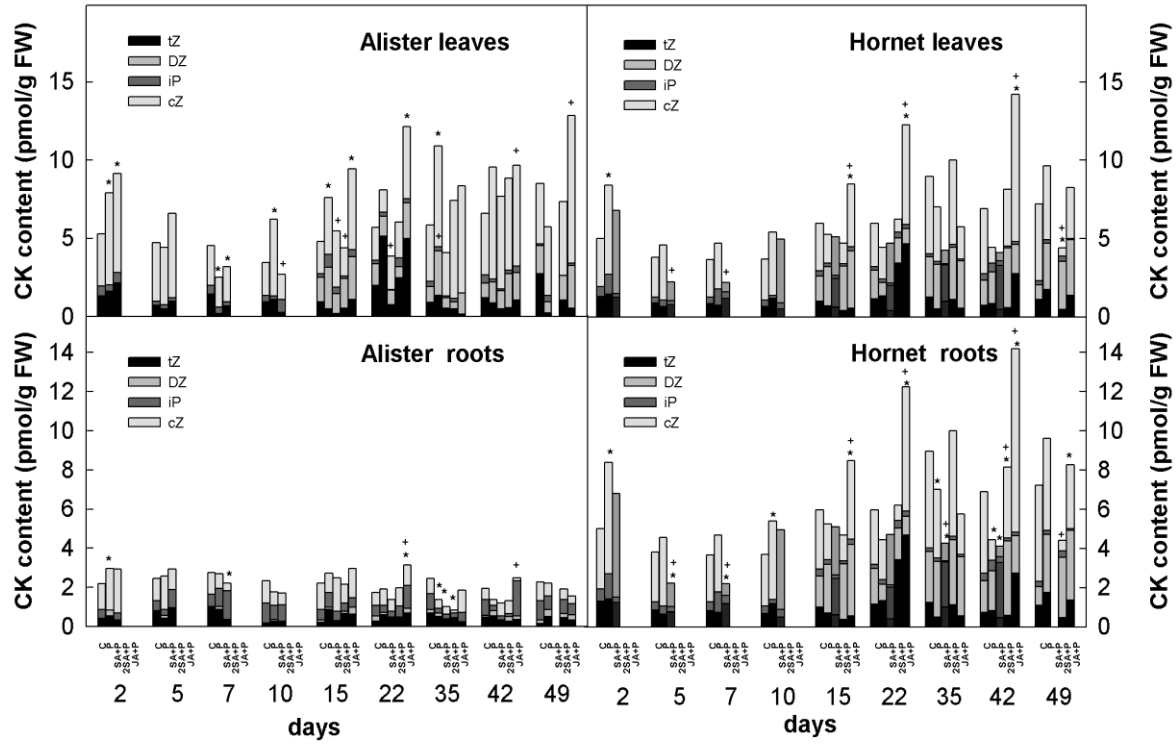
dai	Disease Index (%) variant	Hornet		Alister	
		2016	2017	2016	2017
10	Plasmodiophora	0	0	0	0
	SA + Plasmodiophora	0		0	
	2 SA + Plasmodiophora	0	0	0	0
	JA + Plasmodiophora		0		0
15	Plasmodiophora	0	0	0	0
	SA + Plasmodiophora	0		0	
	2 SA + Plasmodiophora	0	19.0	0	0
	JA + Plasmodiophora		55.6		0
22	Plasmodiophora	100.0	40	6.7	6.7
	SA + Plasmodiophora	46.7		6.7	
	2 SA + Plasmodiophora	40.0	53.3	0	0
	JA + Plasmodiophora		60.0		13.3
35	Plasmodiophora	100.0	33.3	6.7	0
	SA + Plasmodiophora	53.3		13.3	
	2 SA + Plasmodiophora	46.7	60.0	0	0
	JA + Plasmodiophora		83.3		0
42	Plasmodiophora	100.0	53.3	40.0	0
	SA + Plasmodiophora	86.7		6.7	
	2 SA + Plasmodiophora	60.0	58.3	0	0
	JA + Plasmodiophora		88.9		3.7
49	Plasmodiophora		100		0
	SA + Plasmodiophora				
	2 SA + Plasmodiophora		66.7		0
	JA + Plasmodiophora		75.0		0



# Results: Growth promoting hormones

Cytokinins

Auxins

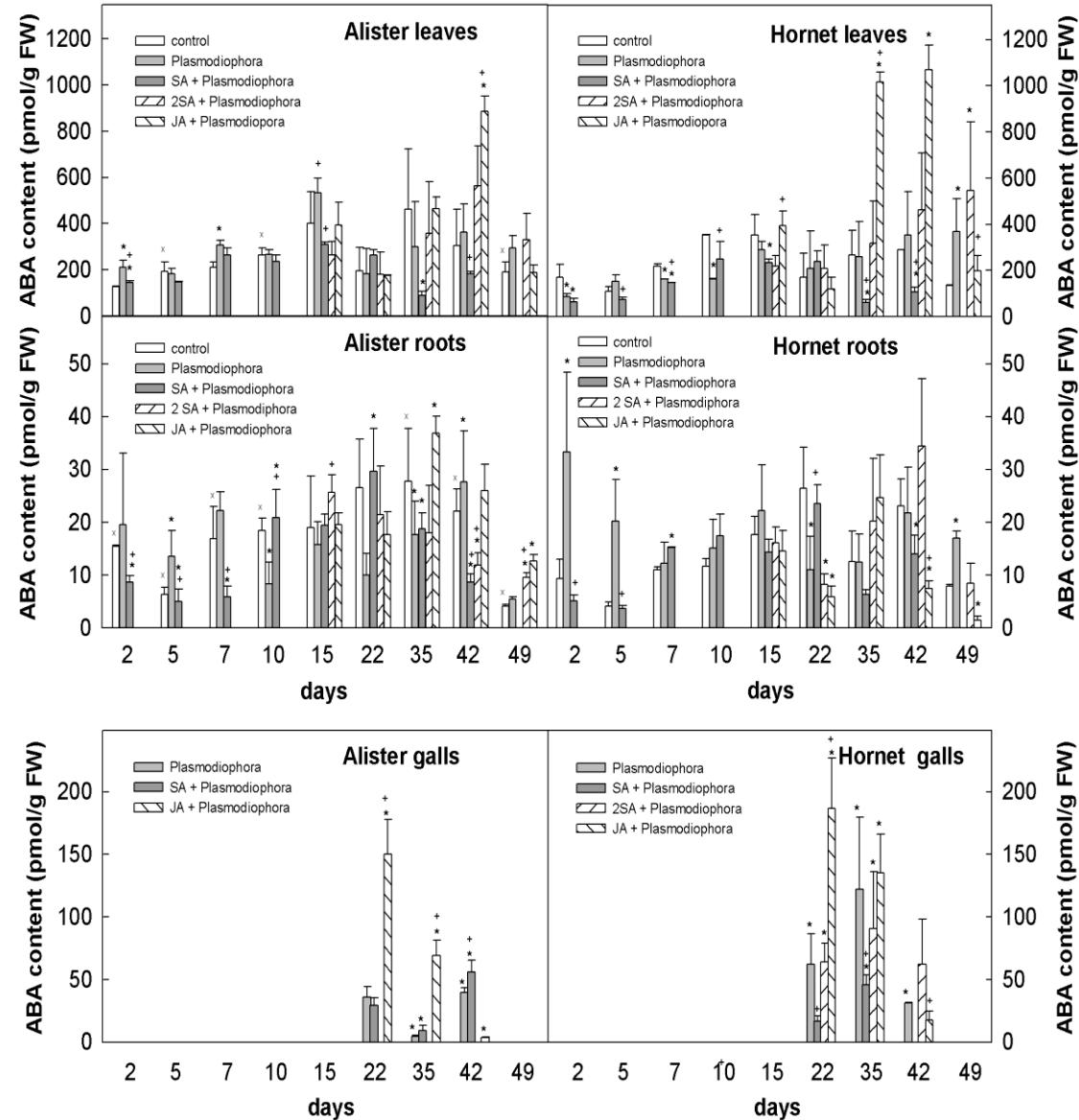






# Results: Stress hormone

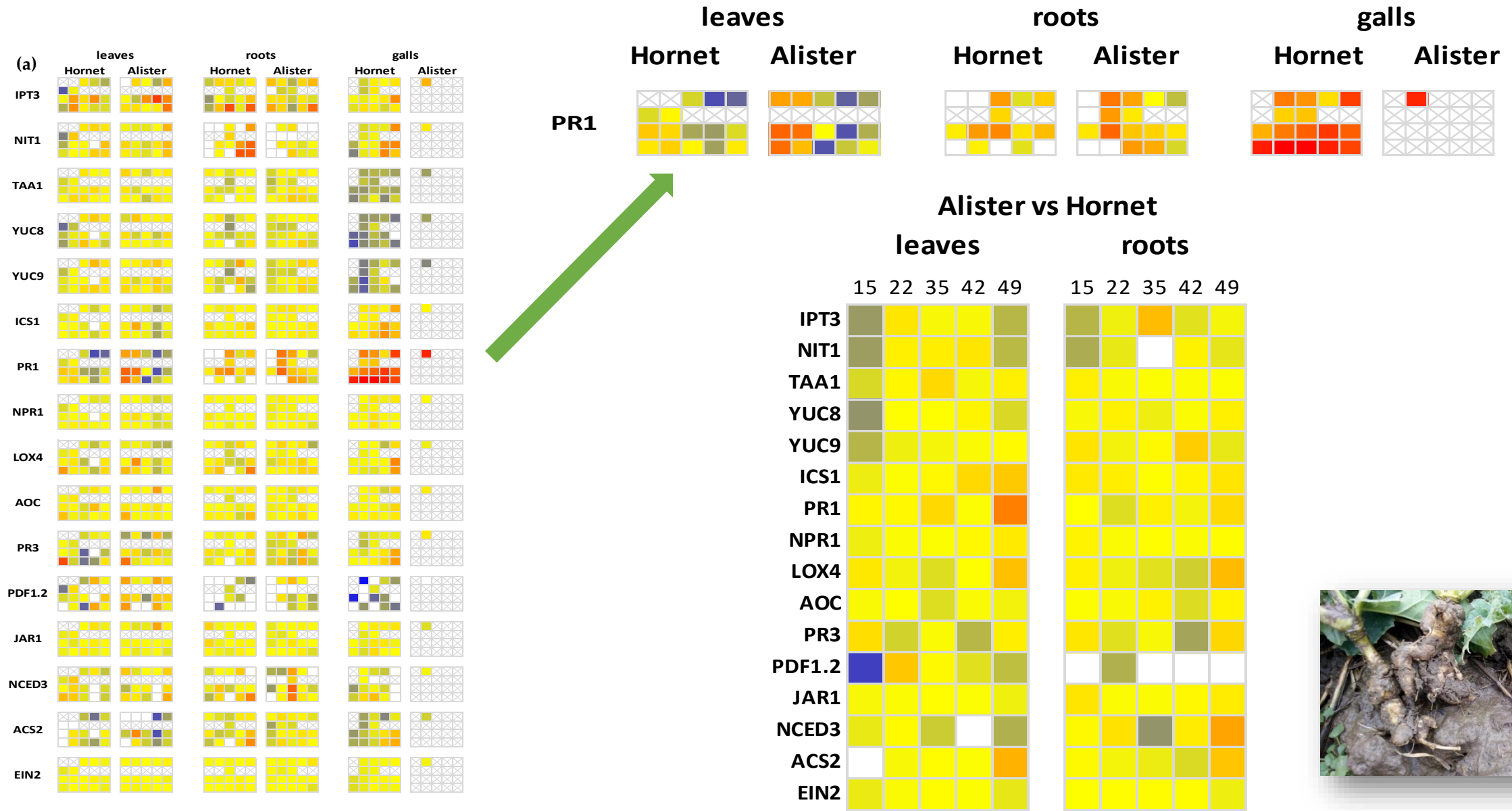
## Abscisic acid







# Results: Hormone-related gene expression



# Summary: the *Plasmodiophora brassicae* impact on *Brassica napus* plants during infection progression



DAI	Early response (2-7 d)	Gall formation (10-22 d)	Late response (35-49 d)
Leaves			
Roots			
Galls			

# Conclusion

- Comparison of hormonal pools revealed rather minor differences between Alister and Hornet
- **BUT resistant cultivar is able to increase levels of salicylic acid** during the infection in roots and leaves
- Differences might reflect variance among cultivars - it seems that it is not possible to distinguish the resistant ones by screening of non-infected plants
- ***P. brassicae* infection** associated with early **elevation of CKs and auxin** in leaves and roots of both cultivars
- In Alister, the CK content in roots was down-regulated quite early
- **Galls** of both cultivars had **at the beginning** of their formation **high levels of CKs and IAA** (together with SA and JA)
- **SA increased during later stages of infection**, but to a somewhat earlier time point in Alister
- The SA marker **gene *PR1*** exhibited the **highest expression around the beginning of gall formation** in leaves, roots and especially in galls
- **JA level was higher in Hornet** than in Alister during the whole experiment
- Difference between the activation of defence hormone pathways in resistant and sensitive cultivar is in accordance with the impact of SA and JA applications – **SA further suppressed gall formation in Alister**, while **JA mildly promoted gall formation in both cultivars**



# Acknowledgements:



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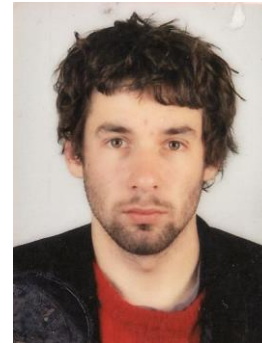
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