Assessment of induced allelopathy and differential gene expression in crop-weed co-culture with rye-pigweed model

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1. Introduction

- Induced allelopathy?

Objectives

- conditions where pigweed (*Amaranthus retroflexus*) growth is suppressed most by Rye (*Secale cereale*)
- Impact of seed sowing time
- Rye detects its neighbours at the earliest
- Explore the root uptake of benzoxazinoids (BXs)
- Differential gene expression of BXs genes

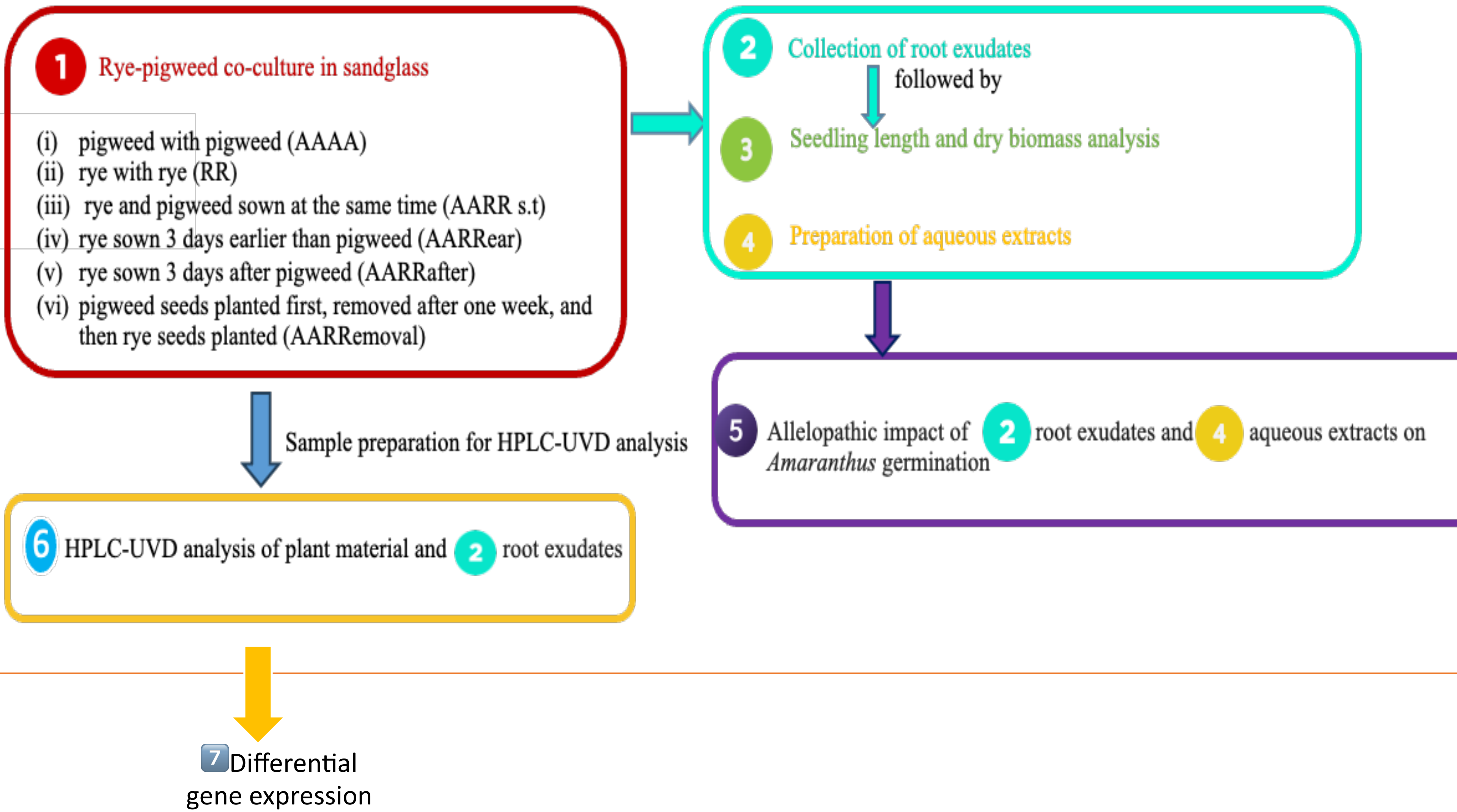
2. Experimental Design

3. Methodology

4. Results and Discussion

5. Conclusion

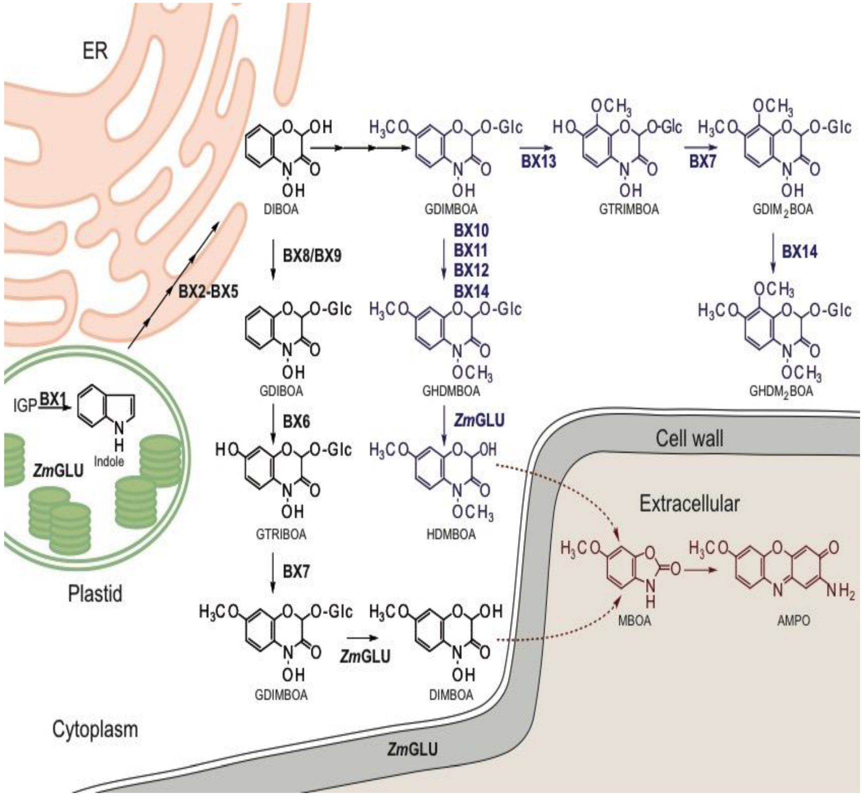
2. Experimental Design



Rye key genes for BXs production

chromosome 7R (*ScBx1-ScBx2*)
chromosome 5R (*ScBx3-ScBx5*)

Gene	Enzyme	Function	Cellular Localization
Bx1	indole-3-glycerol phosphate lyase	Converts indole-3 glycerol phosphate into indole	Chloroplast
Bx2-Bx5	cytochrome P450 oxygenases	Converts indole into DIBOA	Endoplasmi reticulum



2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA)
2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA)

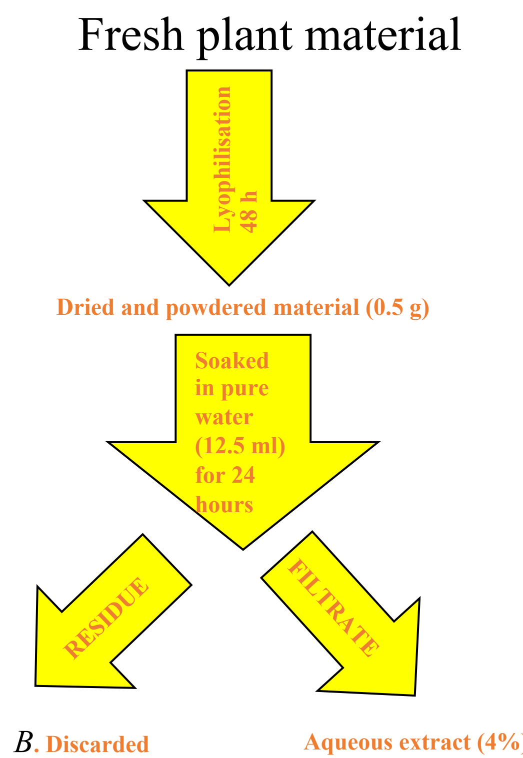
3. Methodology

Modalities	Types of aqueous Extracts	Types of Exudates
1. AAAA (Amaranthus growing with Amaranthus)	ET1	ED1
2. **RR** (Rye growing with Rye)	ET2	ED2
3. **AARR s.t** (Rye, Amaranthus were sowed at the same time)	ET3	ED3
4. **AARRear** (Rye sown 3 days earlier than Amaranthus)	ET4	ED4
5. **AARRafter** (Rye sown 3 days after Amaranthus)	ET5	ED5
6. **AARRemoval** (In a plastic tube, we start by planting Amaranthus seeds, remove them after one week, and then plant Rye seeds, with the day of Rye planting considered as day 1.)	ET6	ED6

*Blank (sand serves as blank or control); Sampling time: 2 and 4 weeks

Growth conditions in phytotron
12-hour day/night photoperiod; temperature 21 -18°C-day/night ; relative humidity 70%.

A. Customised device for root-exudate extraction



3. Methodology

Germination tests (Petridishes experiment):

Co-germination of rye and pigweed (5 seeds each). It will allow us to understand if rye detects its neighbour at the germination stage. To serve as a control, rye and pigweed are allowed to germinate in separate Petri dishes

Allelopathic impact (i) root exudates (ii) aqueous extracts on pigweed seeds.

3. Methodology

Benzoxazinoid extraction

freeze-dried plant material crushed ➡️ 25 mg in Eppendorf tubes + 1 mL of extraction solvent (methanol/water/formic acid; 50/50/1; v/v/v) and 4 glass beads (<1 mm).

Extraction time 1 h in a Heindolph Multireax Agitator set at 2000 rpm

Separation of solid phase in an Eppendorf MiniSpin centrifuge for 8 minutes at maximum rpm (13,400)

The supernatant is syringed and filtered in a 0.45 µm PTFE filter placed in a vial and finally stored at 4 °C.

HPLC analysis (Agilent 1200 HPLC System)
Agilent Poroshell C18 column

Solution A (methanol/water/ortho-phosphoric acid 85%; 10/90/0.1; v/v/v)

Solution B (methanol/ ortho-phosphoric acid 85%
100/0.1; v/v).

3. Methodology

Differential gene expression

Plant material

↓

RNA extraction

↓

Quality analysis by Nanodrop

↓

cDNA

↓

Quality analysis by Nanodrop

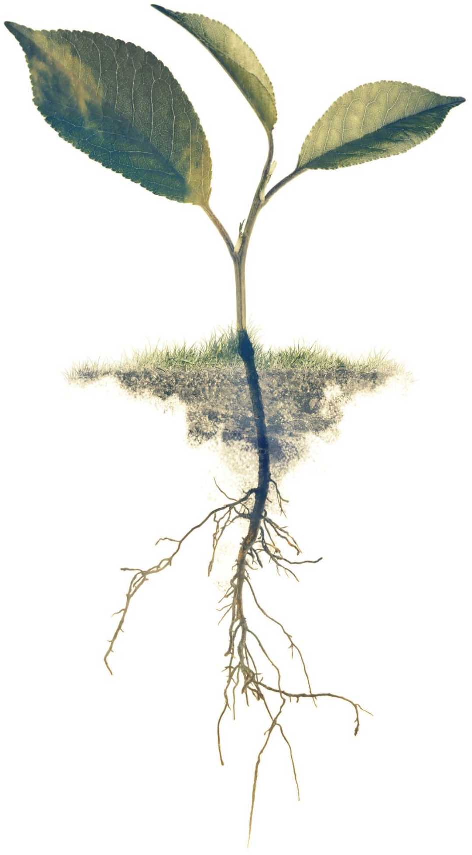
PCR

Gel electrophoresis

↓

qPCR

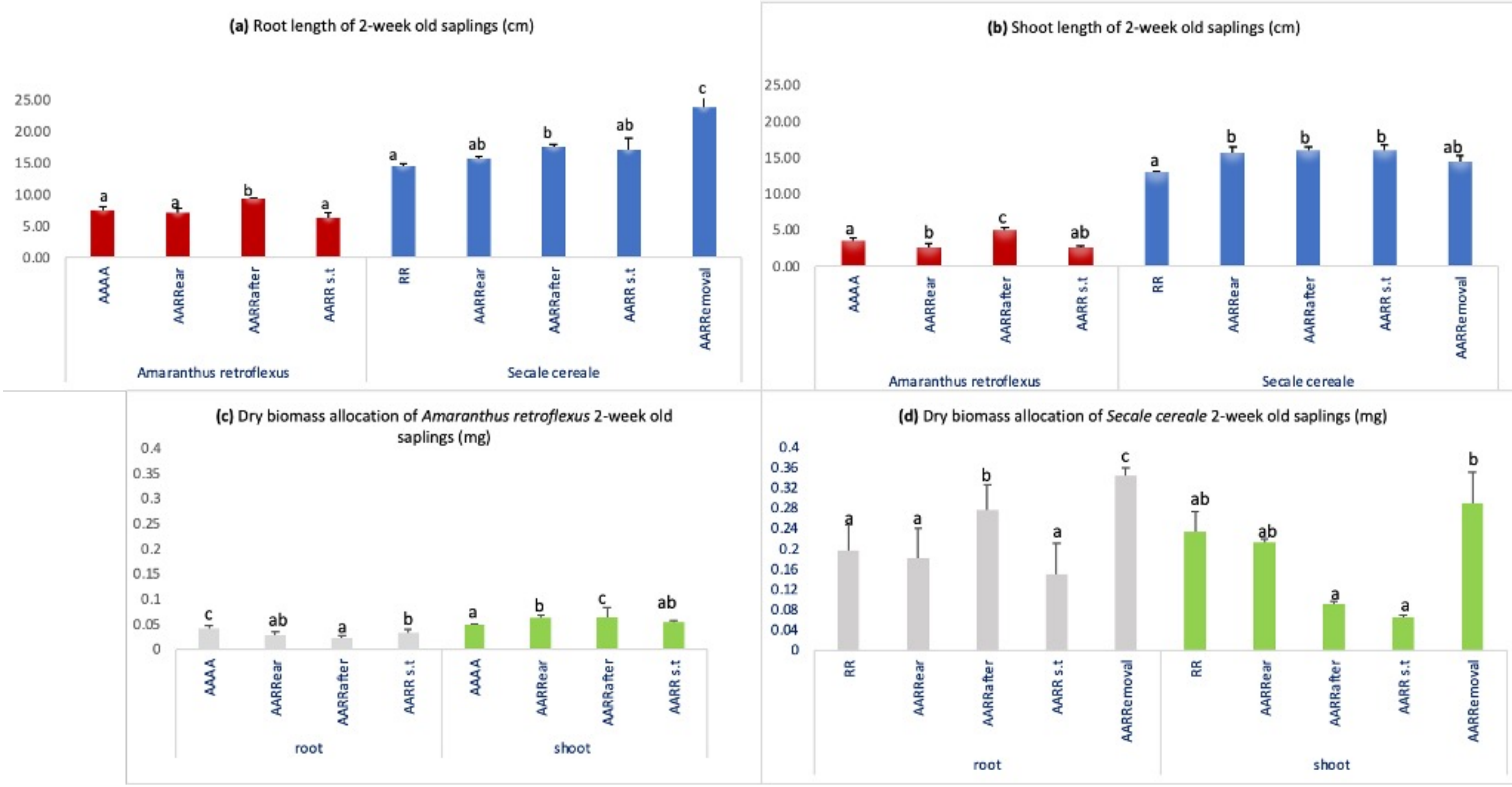
4. Results



- Growth parameters (root length, shoot length and dry biomass) separately* in all modalities after 2 and 4 weeks
- Germination indices of 2 and 4-week samples on exposure to exudates and extracts
- HPLC analysis of exudates and plant material (root and shoot separately* in all modalities) after 2 and 4 weeks. Standards used bezoxazinoids (DIBOA, DIMBOA and MBOA)

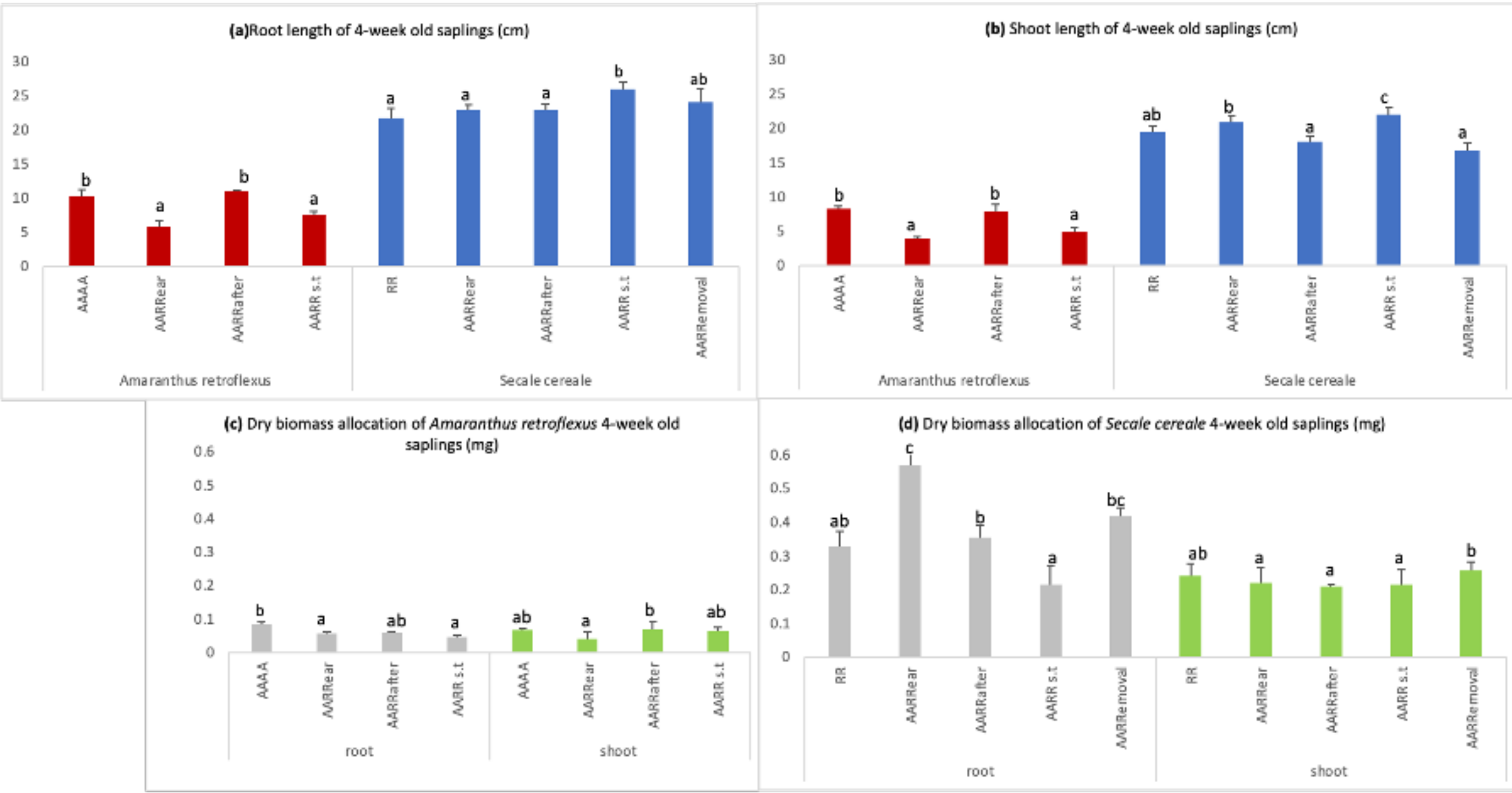
*for example in AARR modality, Amaranthus root and shoot are evaluated separately, so is Rye root and shoot

4.1. Growth parameters



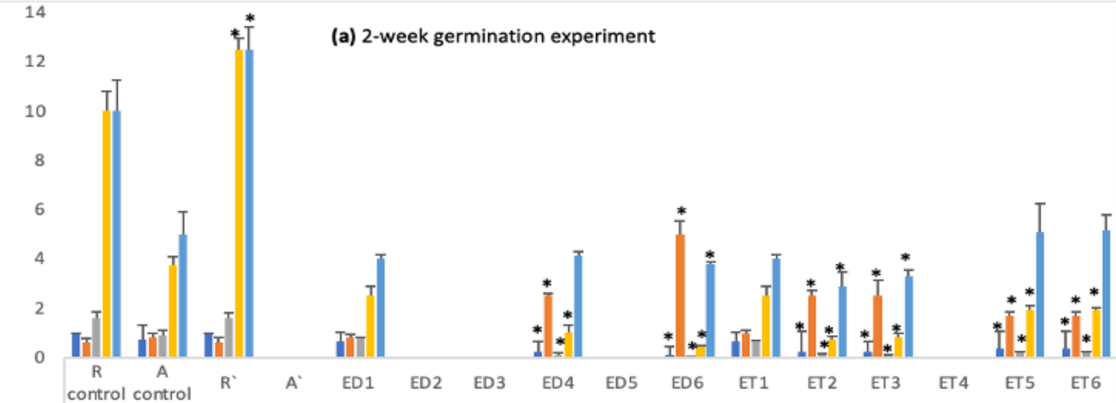
- AARRafter shows improved growth (both root and shoot) (Fig. a,b)
- Rye shows improved seedling length
- Reduction in dry biomass of Amaranthus root in all modalities. However, not in AARRafter.
- The trend is different in Rye. Rye in AARRemoval shows the highest shoot/root biomass.

4.1. Growth parameters

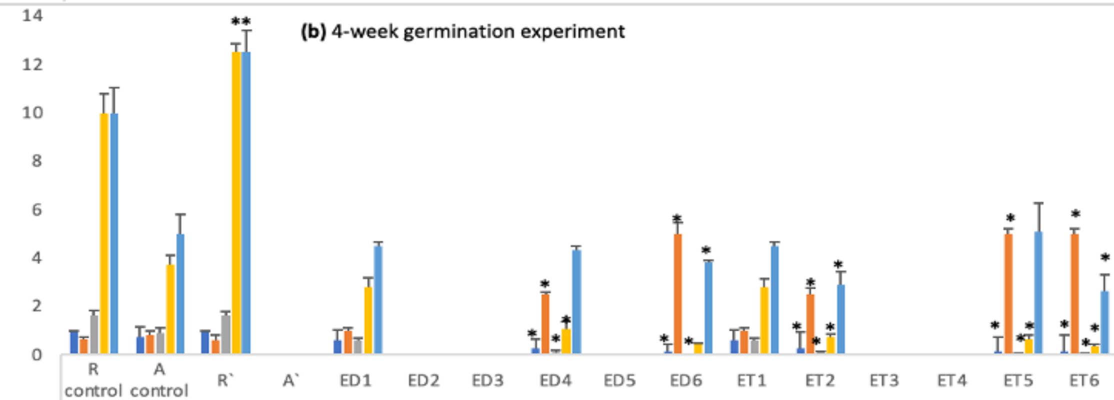


- ❖ Reduction in pigweed seedling length is observed in all modalities except AARRafter.
- ❖ Rye did not show any major changes in root length except AARR s.t showing improved growth (Fig. a).
- ❖ Reduction in dry biomass of pigweed root was observed in all modalities except AARRafter.
- ❖ AARRemoval (and control) shows the highest shoot biomass which coincides with our results of the 2-week-old experiment.

4.2 Germination indices of 2 and 4-week samples on exposure to exudates and extracts



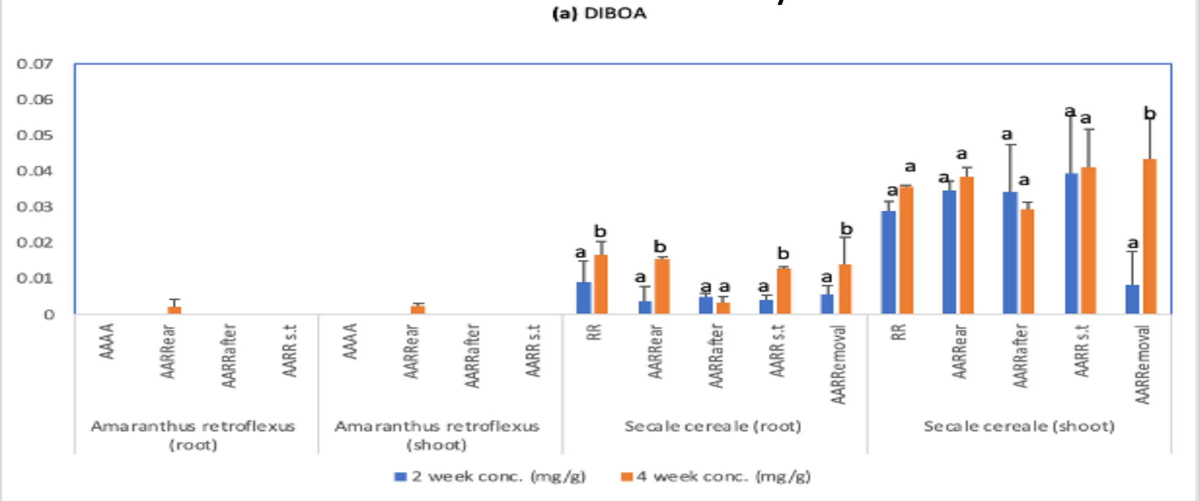
	R control	A control	R`	A`	ED1	ED2	ED3	ED4	ED5	ED6	ET1	ET2	ET3	ET4	ET5	ET6
Germination Rate (%) x 100	1	0.75	1	0	0.63	0	0	0.25	0	0.125	0.63	0.25	0.25	0	0.375	0.375
Mean Germination Time (in days)	0.63	0.83	0.63	0	0.83	0.00	0.00	2.50	0.00	5.00	1.00	2.50	2.50	0.00	1.67	1.67
Germination Index	1.60	0.90	1.60	0	0.76	0.00	0.00	0.10	0.00	0.03	0.63	0.10	0.10	0.00	0.23	0.23
Vigour Index	10.00	3.75	12.50	0	2.52	0.00	0.00	1.03	0.00	0.48	2.52	0.73	0.83	0.00	1.91	1.93
Seedling length (cm)	10.00	5.00	12.50	0	4.00	0.00	0.00	4.12	0.00	3.80	4.00	2.90	3.30	0.00	5.10	5.15

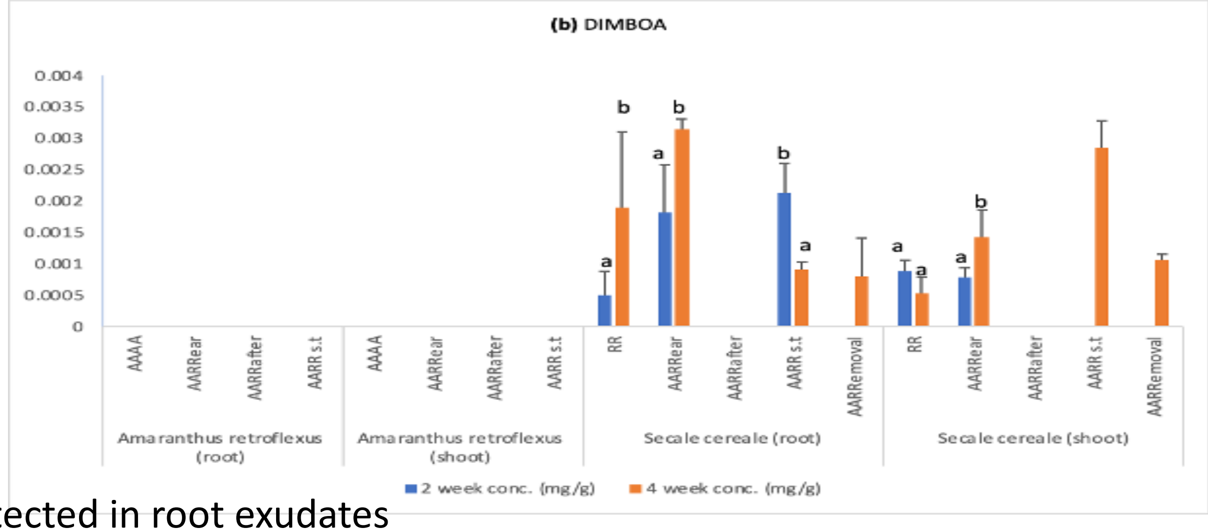


	R control	A control	R`	A`	ED1	ED2	ED3	ED4	ED5	ED6	ET1	ET2	ET3	ET4	ET5	ET6
Germination Rate (%) x 100	1	0.75	1	0	0.63	0	0	0.25	0	0.13	0.63	0.25	0.00	0.00	0.13	0.13
Mean Germination Time (in days)	0.63	0.83	0.63	0.00	1.00	0.00	0.00	2.50	0.00	5.00	1.00	2.50	0.00	0.00	5.00	5.00
Germination Index	1.6	0.90	1.60	0.00	0.63	0.00	0.00	0.10	0.00	0.03	0.63	0.10	0.00	0.00	0.03	0.03
Vigour Index	10	3.75	12.50	0.00	2.81	0.00	0.00	1.08	0.00	0.48	2.81	0.73	0.00	0.00	0.64	0.33
Seedling length (cm)	10	5	12.5	0	4.50	0.00	0.00	4.32	0.00	3.80	4.50	2.90	0	0	5.10	2.65

- In the co-germination test, rye shows a 100% GR both in control (R control) and in the presence of pigweed (R’) (Fig. a,b).
- Moreover, rye shows improved VI and SL in the presence of pigweed.
- However, pigweed which showed 75% GR in control (A control) did not germinate in the presence of rye (A’).

4.3. Benzoxazinoid analysis





- NO BXs detected in root exudates
- DIBOA (shown in Fig. a) was found in all 2-week and 4-week-old rye plants
- Significant increase in the DIBOA in an age-dependent manner.
- “AARRemoval” had the highest DIBOA.
- DIBOA was detected in some 4-week-old pigweed samples.

scientific reports

Article | Open access | Published: 07 May 2024

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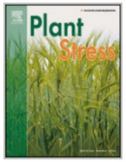
Scientific Reports **14**, Article number: 10446 (2024) | Cite this article

ELSEVIER

Plant Stress

Volume 13, September 2024, 100523



Phenolic profiling unravelling allelopathic encounters in agroecology

Waseem Mushtaq, Marie-Laure Fauconnier

Work in PROGRESS

Differential gene expression

Plant material

RNA extraction

Quality check by Nanodrop

cDNA

Quality check by Nanodrop

PCR
Gel electrophoresis

qPCR

allelopathy inducers?

Rutin -----------------C1
trans-ferulic acid--C2
caffeic acid---------C3
p-coumaric acid---C4
quercetin------------C5
kaempferol----------C6

Rye

5. Conclusion

- Germination experiments show rye's ability to germinate in the presence of pigweed, while pigweed exhibits reduced germination with rye.
- BX production in response to pigweed
- BX production in an age-dependent manner
- Allelochemicals uptake by neighbouring plants
- Results suggest that allelopathy in rye-pigweed co-cultures is influenced

  seed timing

  age-dependent dynamics

Thank You
And Acknowleged

Allelopathy Team

Prof. M. L. Fauconnier (Promoter)

Dr. Caroline de Clerck

Dr. Waseem Mushtaq

Jeremy Berdy

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