Ni hyperaccumulation in *Brassica juncea*: A whole plant vision

UAB
Biologia Ambiental
Research Project
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Background

Plants constitute a high nutritive source and therefore they are constantly attacked. As a consequence they have developed defence mechanisms through evolution such as spines (physical defence), associations with other organisms (symbiotic defence) and a great variety of secondary compounds (alkaloids, terpenes, etc.) which constitute the chemical defence. However, nowadays science is focusing on one of them: heavy metal hyperaccumulation.

What is an hyperaccumulator plant?

Hyperaccumulator plants take up heavy metals from the soil and store them at exceptionally high concentrations (>1000 µg metal/g) in aboveground organs (especially leaves) and show no symptoms of phytotoxicity although they exceed usual toxicity threshold.

Has hyperaccumulation any biological significance? Many explanations have been proposed but two of them are more reliable:

- "Elemental defence hypothesis" where hyperaccumulated metals can deter or kill "plant natural enemies" by direct toxicity.
- "Joint-effect hypothesis" where presence of both metal and natural plant defences could produce a synergic effect increasing plants defence responses.

HYPOTHESIS:

Ni hyperaccumulation can defend *Brassica juncea* from fungi and virus pathogens through both "Elemental defence" and "Joint-effect" hypotheses.

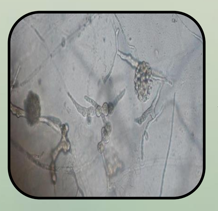
OBJECTIVES:

1. Ni effects on pathogen survival. Establish if *B. juncea* pathogens (*Leptosphaeria* maculans, *Pythium sp*, *CaMV* and *TuMV*) are susceptible to Ni by comparing their growth rates in media with different Ni concentrations.
2. Combination effects in defence. Establish if Ni presence in *B. juncea* tissues increases plant natural defence response by comparing leaf levels of Salicylic Acid, Jasmonic Acid and Ethylene from both infected and non-infected plants.
3. Pathogen specificity and defence. Establish if pathogen specificity confers more resistance to *B. juncea* defences by comparing results between specific and generalist pathogens.

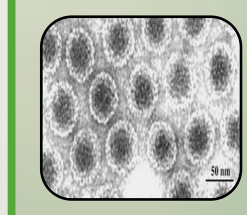
Biologic material

Brassica juncea (Brassicales)
A model species of mustard plant known by its Ni phytoextraction potential and because its oil can be used as a feedstock for biodiesel.

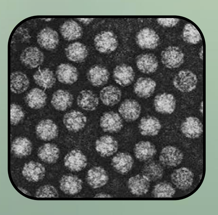
Leptosphaeria maculans (Pleosporales)
Causal agent of blackleg disease in *Brassica* crops.



Pythium sp (Pythiales)
A generalist plant pathogen that causes severe damages in agriculture.



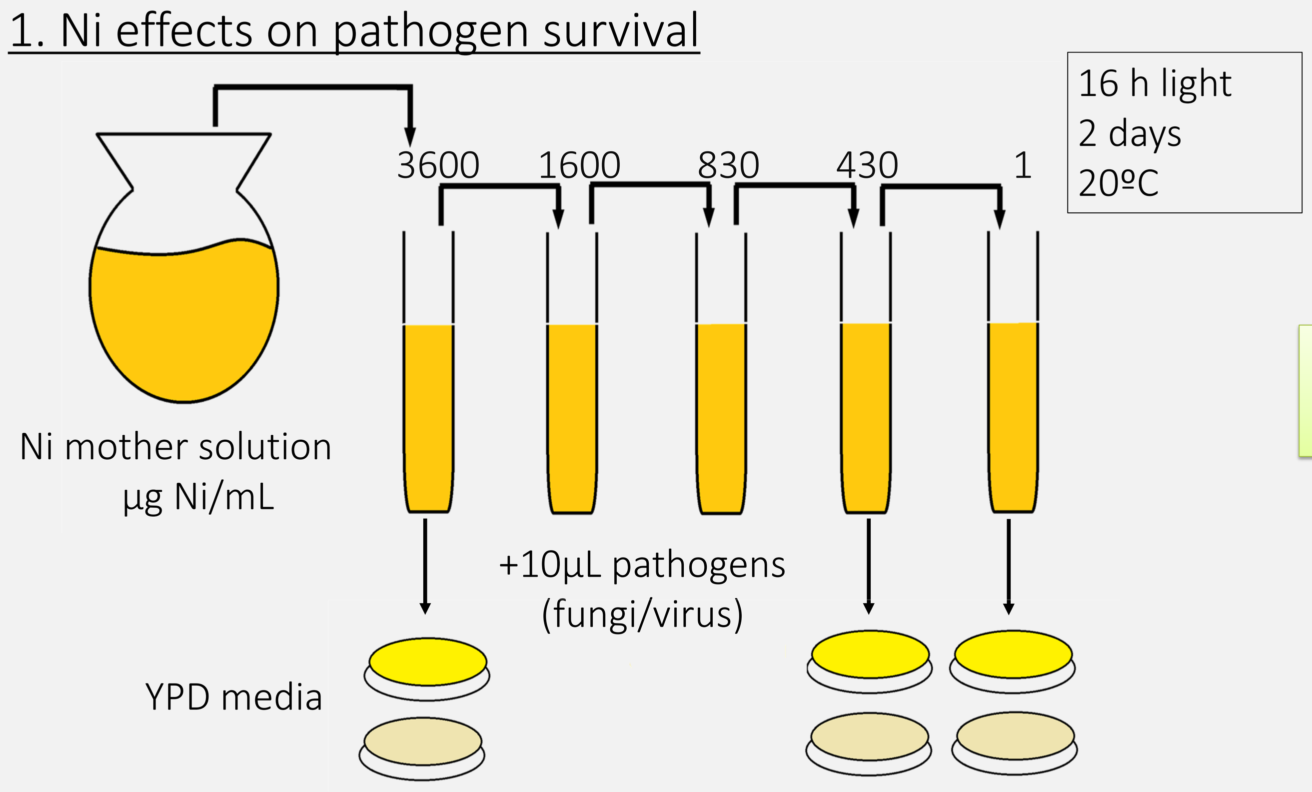
CaMV (Caulimoviridae)
A *Brassica* specific pathogen. Induces a variety of symptoms such as mosaic and necrotic lesions on leaf surfaces.



TuMV (Potyviridae).
A generalist plant virus. Produces chlorotic local lesions and puckering in their hosts.

Materials & Methods

1. Ni effects on pathogen survival

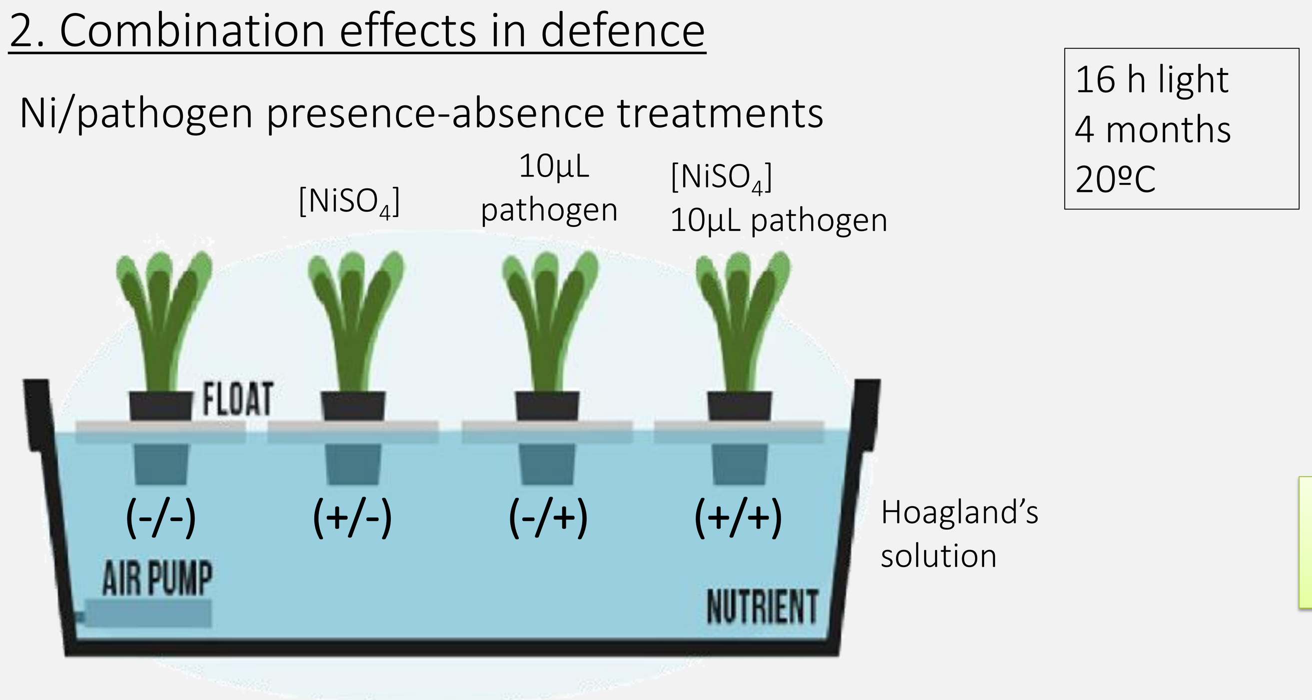


Growth rate + EC_{50}

Leaf extract added to virus Petri capsules (yellow)

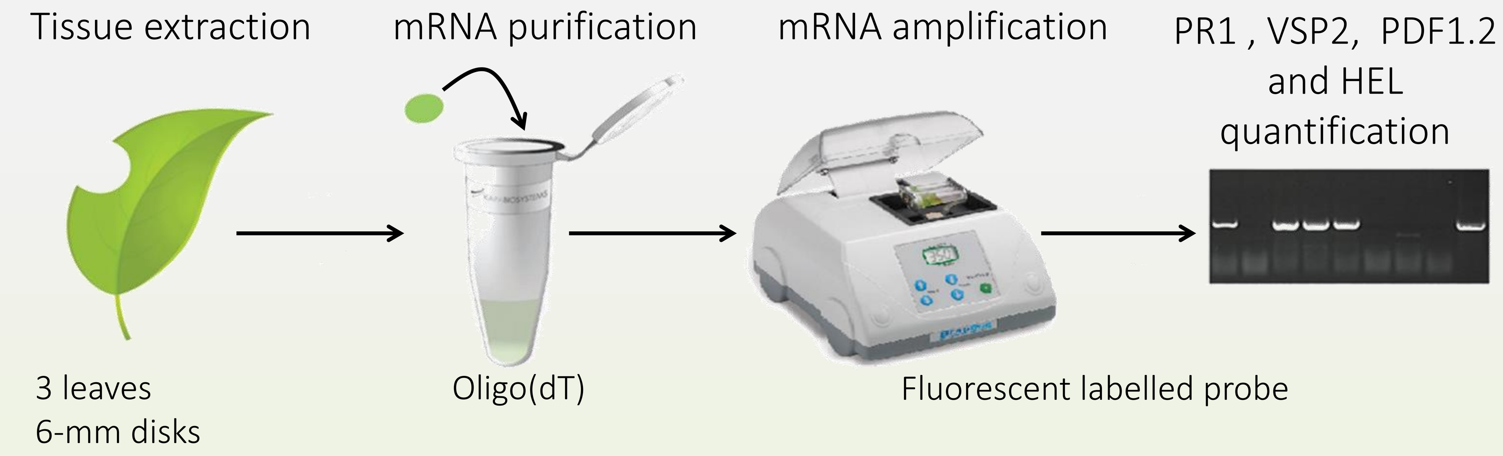
2. Combination effects in defence

Ni/pathogen presence-absence treatments

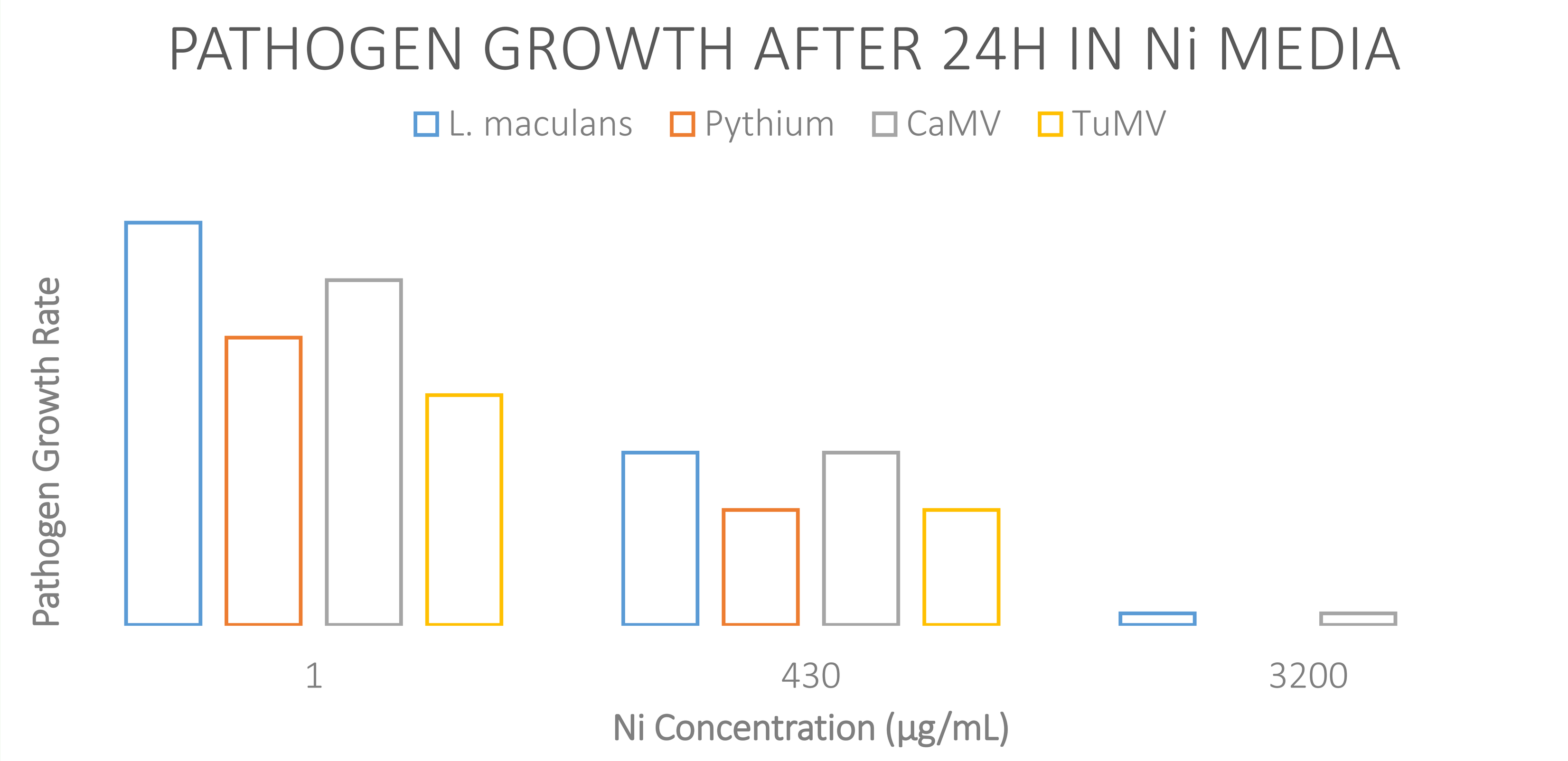


[mRNA] in tissues

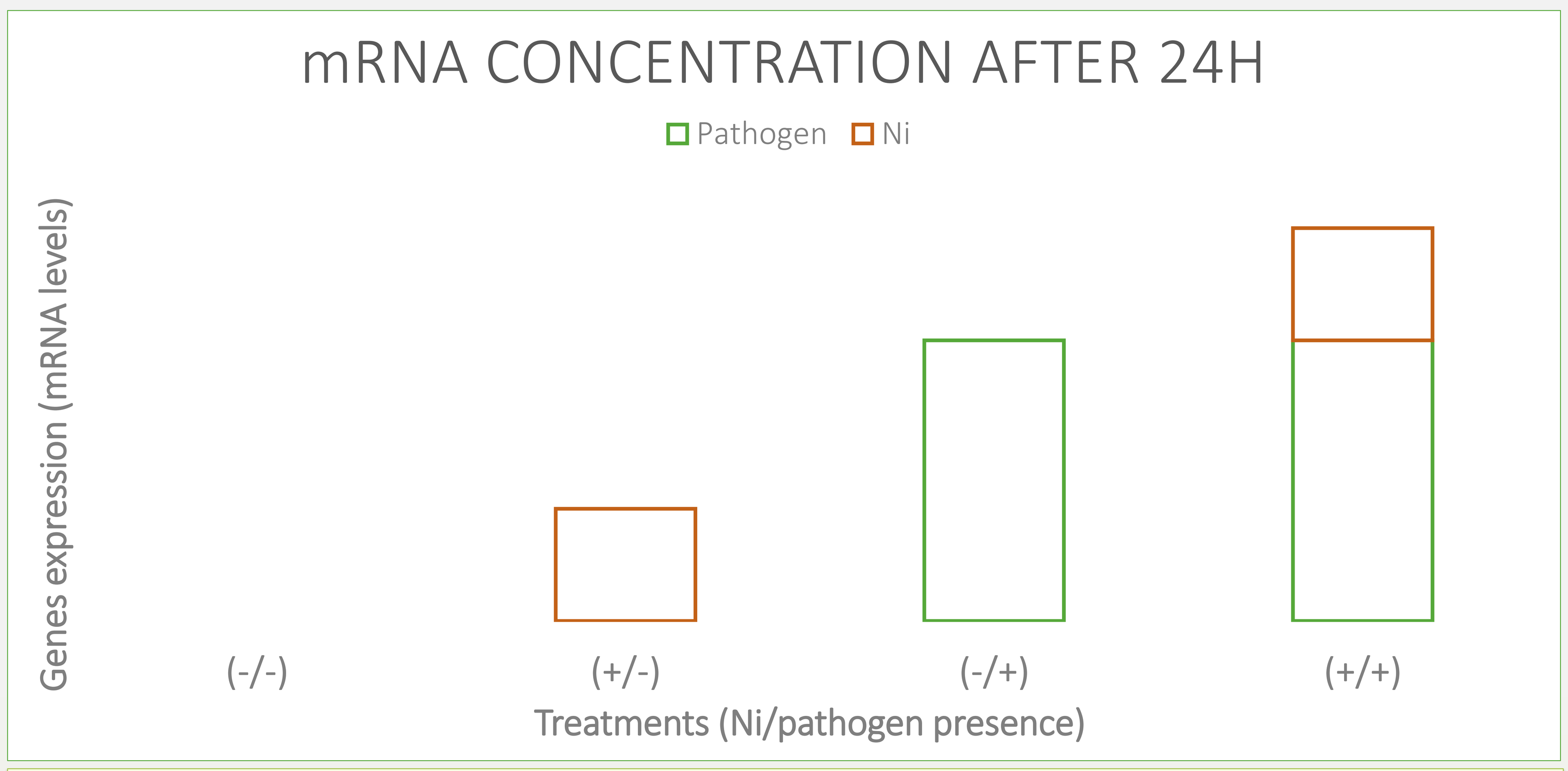
Stress response quantification: qRT-PCR



Expected results



A steeply growth decrease was observed at 3200µg Ni/mL. Pathogens would remain unable to infect *Brassica juncea* hyperaccumulators due to an excessive toxicity produced by high Ni levels.



mRNA concentration was detected in (+/-), (-/+) and (+/+) *B. juncea* plants. Ni is acting as a defence enhancer by increasing SA/JA/ET levels although there was not a pathogen infection = Joint Effect + Elemental defence hypothesis

3. Pathogen specificity and defence

Specific pathogens (*L. maculans, CaMV)* were more resistant to high Ni concentrations and, therefore, inoculation with this pathogens produced higher infection rates despite joint action of SA/JA/ET and Ni toxicity.

Schedule

September	October	November	December	January	February	March	April	May

- *Brassica juncea* collection (September)
- Strains order (October)
- *B. juncea* sown (October)
- *B. juncea* maturation (November – January)
- Pathogen revival (November)
- Elemental Defence test (December)
  - Growth rate + EC_{50}
- Results & Conclusions (January – February)
  - Specific vs. Generalist
- Joint Effect test (February – March)
  - Infecting *B.juncea*
  - Stress response quantification
- Results & conclusions (April – May)
  - Specific vs. Generalist
  - SA/JA/ET levels

Bibliography: Boyd, R.S. (2007). The defense hypothesis of elemental hyperaccumulations: status, challenges and new directions. *Plant Soil*, 293:153-176; De Keyser, E., Desmet, L., Van Bockstaele, E., De Riek, J. (2013). How to perform RT-qPCR accurately in plant species? A case study on flower colour gene expression in an azalea (Rhododendron simsii hybrids) mapping population. *BMC Molecular Biology*, 14(13).; Krämer, U. (2010). Metal hyperaccumulation in Plants. *The Annual Review of Plant Biology*, 61:517-534; Poschenrieder, C., Tolrà, R., Barceló, J. (2006). Can metals defend plants against biotic stress? *TRENDS in Plans Science*, 11(6).