Cellular and molecular pathways involved in the bacterial endophyte-mediated biocontrol of *Sclerotinia sclerotiorum* in *Brassica juncea*

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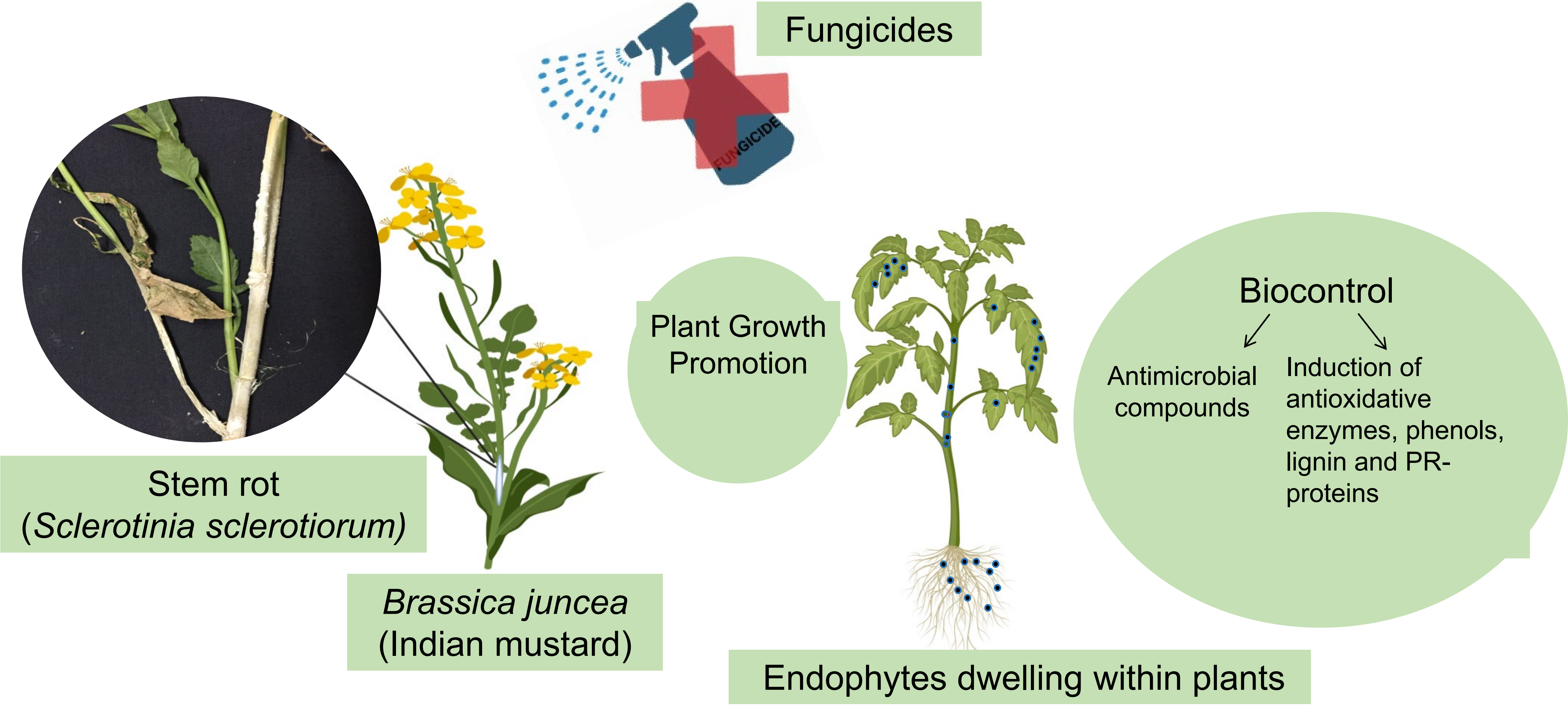
Overview



Fungicides

Stem rot
(*Sclerotinia sclerotiorum*)

Brassica juncea
(Indian mustard)

Plant Growth
Promotion

Biocontrol

Antimicrobial compounds

Induction of antioxidative enzymes, phenols, lignin and PR-proteins

Endophytes dwelling within plants

Biological questions asked

Does the endophytic bacterial strain have the capacity to induce immunity against stem rot in Indian mustard plant?

Is there any relationship between defense metabolites and incidence of stem rot?

Does any correlation exist between lignin accumulation and expression of key genes linked to lignin biosynthesis in stem of Indian mustard?

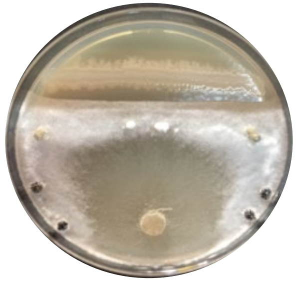
OBJECTIVES OF THE STUDY

- To assess the potential of bacterial endophytes in triggering defense signaling molecules in the management of stem rot in *B. juncea.*

- To examine the mechanisms involved in the bacterial endophyte-mediated biocontrol of *S. sclerotiorum*

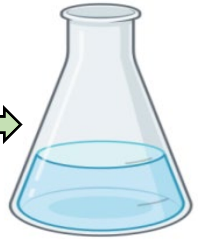
EXPERIMENTAL LAYOUT

Stem rot susceptible mustard variety: PBR 357



Pseudomonas aeruginosa

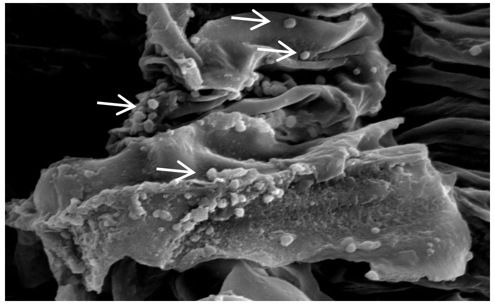
Serratia liquefaciens



Bacterial inoculum

Seed Treatment

Foliar Spray at 50% flowering stage



SEM image showing bacterial colonisation

Stem inoculation with *S. sclerotiorum* at 50% flowering stage

T1	**Control (only fungus inoculation)**
T2	Seed treatment with endophyte consortium + fungus inoculation
T3	Foliar spray with endophyte consortium + fungus inoculation
T4	Seed treatment + Foliar spray with endophyte consortium + fungus inoculation

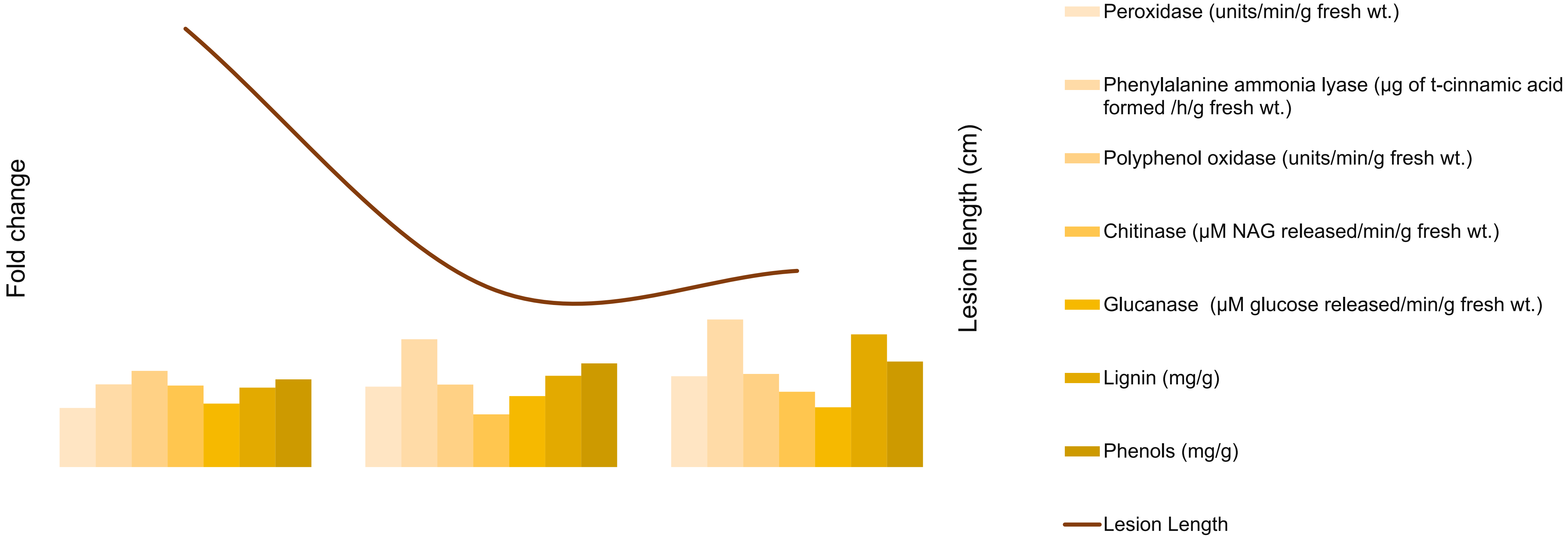
Stem samples harvested at 48,72,120,168 hpi for analysing:
Enzymatic:Peroxidase, Phenylalanine ammonia lyase, Polyphenol oxidase, chitinase, glucanase
Non-enzymatic metabolites:Lignin and phenols
Disease data was recorded after three weeks of fungal inoculation

Selection of effective treatment

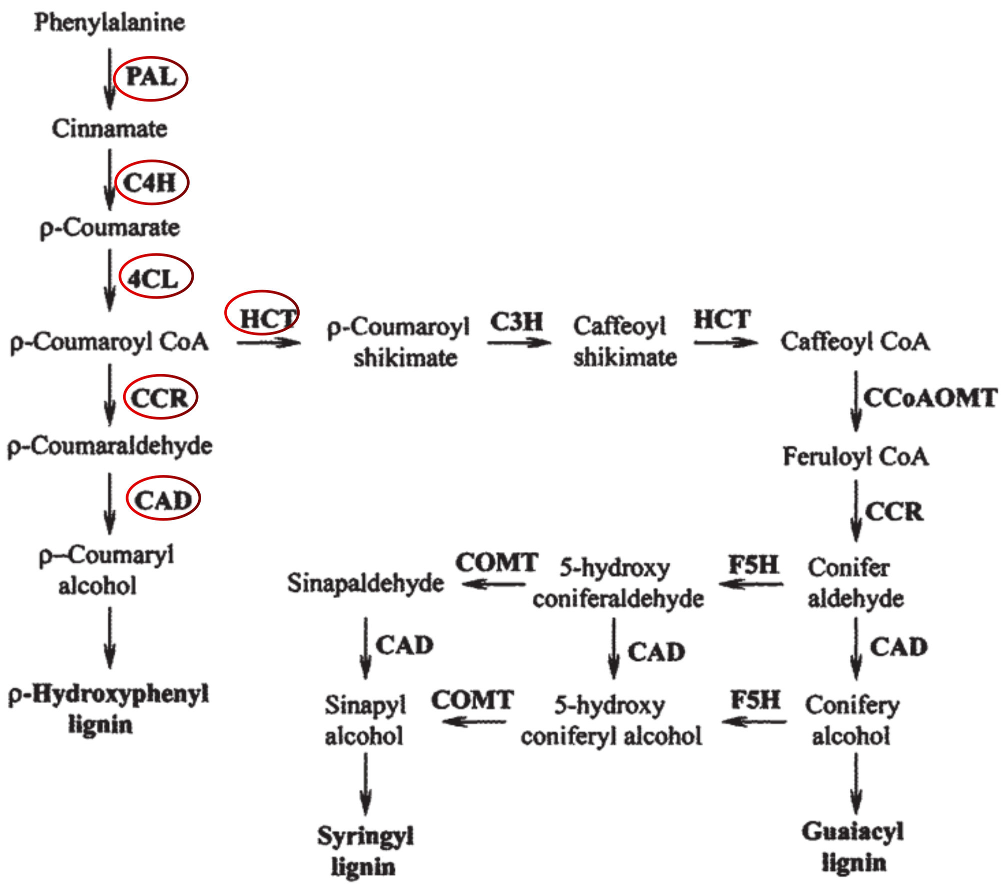
Pot trials

Expression studies of genes involved on lignin biosynthesis

Endophyte-mediated fold change increase in the defense-related metabolites in *B. juncea* (L.) after challenge with *S. sclerotiorum*



Time course analysis of key genes involved in lignin biosynthesis



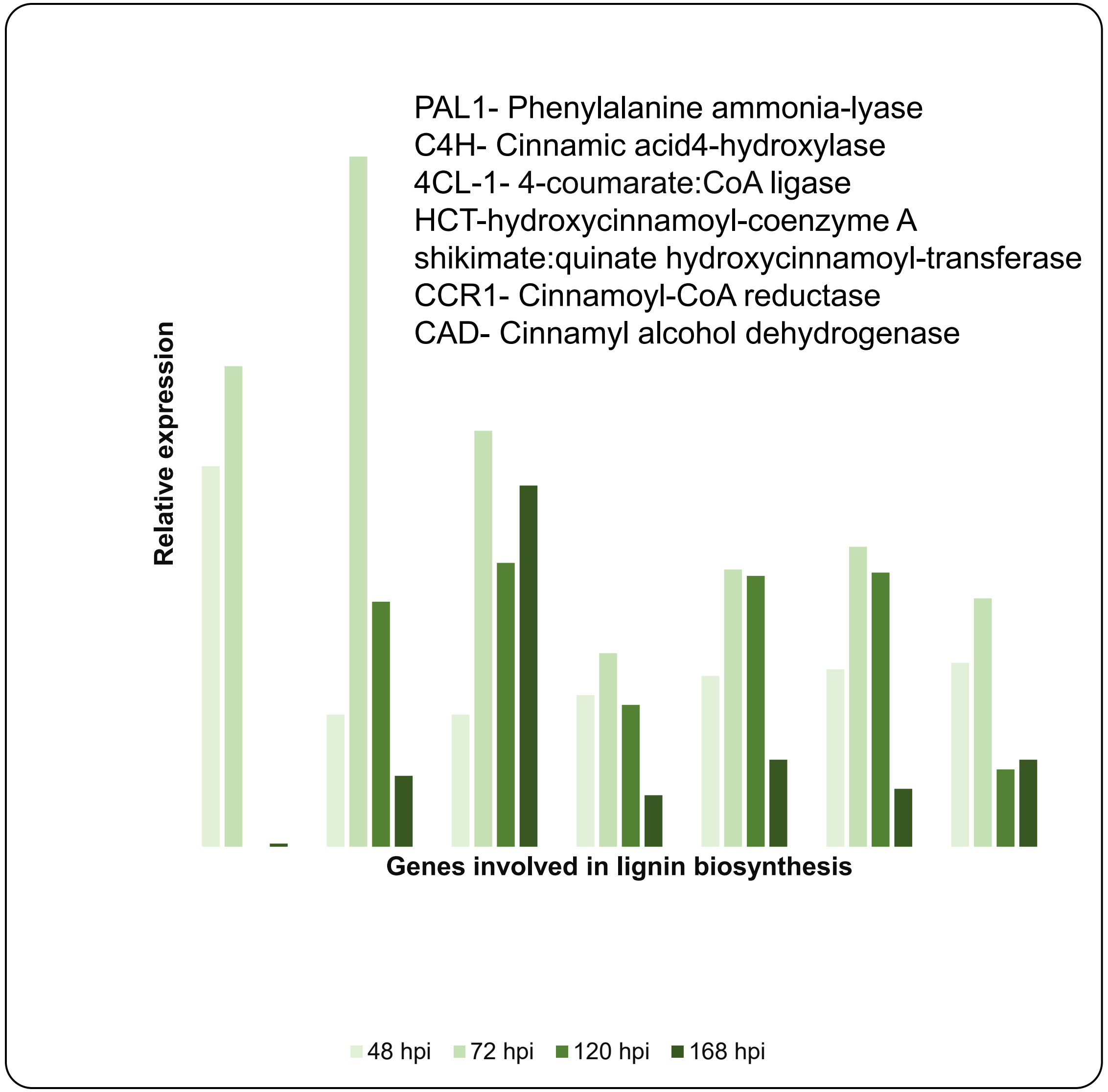
Peng M *et* al (2008) *J Exper Bot* 11:2933-44

Time intervals: 48, 72, 120, 168 hpi

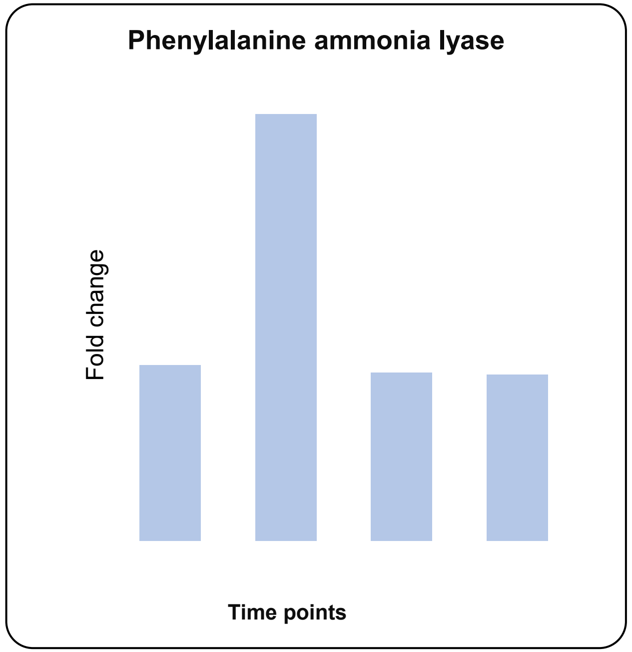
Seed treatment + Foliar spray with endophyte consortium + fungus inoculation

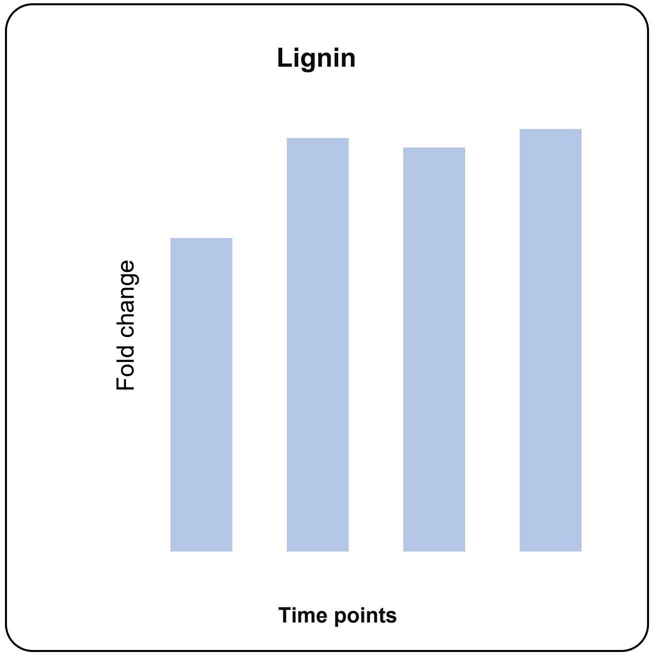
Gene	Primer sequence (5' to 3')	bp
PAL1(F) **PAL1(R)**	TCGTGAGGGAAGAGCTTGGAACA GCTCCGTTCCACTCGTTAAGGCA	23 23
C4H (F) **C4H (R)**	TTACACTGGGAGCCGCTCTTCT AGGCGAGGGTTGTTTCCTTTTC	22 22
4 CL-1 (F) **4 CL-1 (R)**	GGGCTTGTGGTACGGTGGTTAG AGCCAGCCGTCTTTGTCTATGG	22 22
HCT(F) **HCT(R)**	CGACGTGGTATGCTGCTGGACT ACCTGACCCAGCTCGTGATTCC	22 22
CCR1(F) **CCR1(R)**	AAGAACCCTAGAGCCAAGCCGT GGTGGCCCTTCTCTTGCAAGCT	22 22
CAD-C (F) **CAD-C(R)**	CCTTACTTGGCTTGTCTTAAACTCG TCCTCTGTCTCTTTGATGCTCCCA	25 24
CAD-D (F) **CAD-D(R)**	ATTGGGAGCATGAAGGAAACGG CGCAGTGTTCACATAATCCATCTTC	22 25

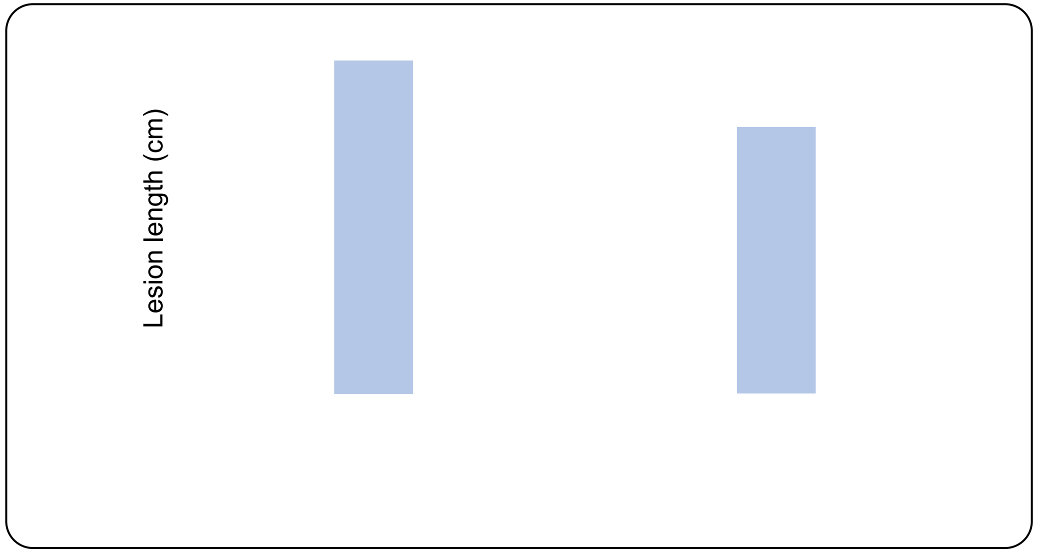
Relative expression of genes involved in lignin biosynthesis confirming defensive response



PAL1- Phenylalanine ammonia-lyase
C4H- Cinnamic acid4-hydroxylase
4CL-1- 4-coumarate:CoA ligase
HCT-hydroxycinnamoyl-coenzyme A shikimate:quinate hydroxycinnamoyl-transferase
CCR1- Cinnamoyl-CoA reductase
CAD- Cinnamyl alcohol dehydrogenase







Outcomes

Increased production of defensive metabolites and shorter lesions in plants treated with selected endophytes demonstrate their ability to induce resistance in the host plant.

Phenylalanine ammonia lyase (PAL) appears to be the key player in the production of phenols and lignin, which are well known to stimulate resistance to biotic stress in host plant.

Up-regulation of key genes *viz., C4H* (Cinnamic acid 4-hydroxylase) and *PAL1* (Phenylalanine ammonia lyase1) in the lignin biosynthetic pathway suggest that lignin deposition results in enhanced cell wall rigidity which may limit the invasion of pathogen.

Future Perspectives

Application of endophytes could assist in the enhanced resistance to sclerotinia stem rot by increased synthesis and deposition of lignin in the plant stem.

Engineering lignin pathway genes in crops could promote disease resistance to enhance crop yield.

Efforts should be made to provide education and training to end users regarding this technology, with the aim of promoting effective and environmentally sustainable plant disease management practices.

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Thank you