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Programmed cell death mediated by non-host resistance in *Arabidopsis* against rice blast

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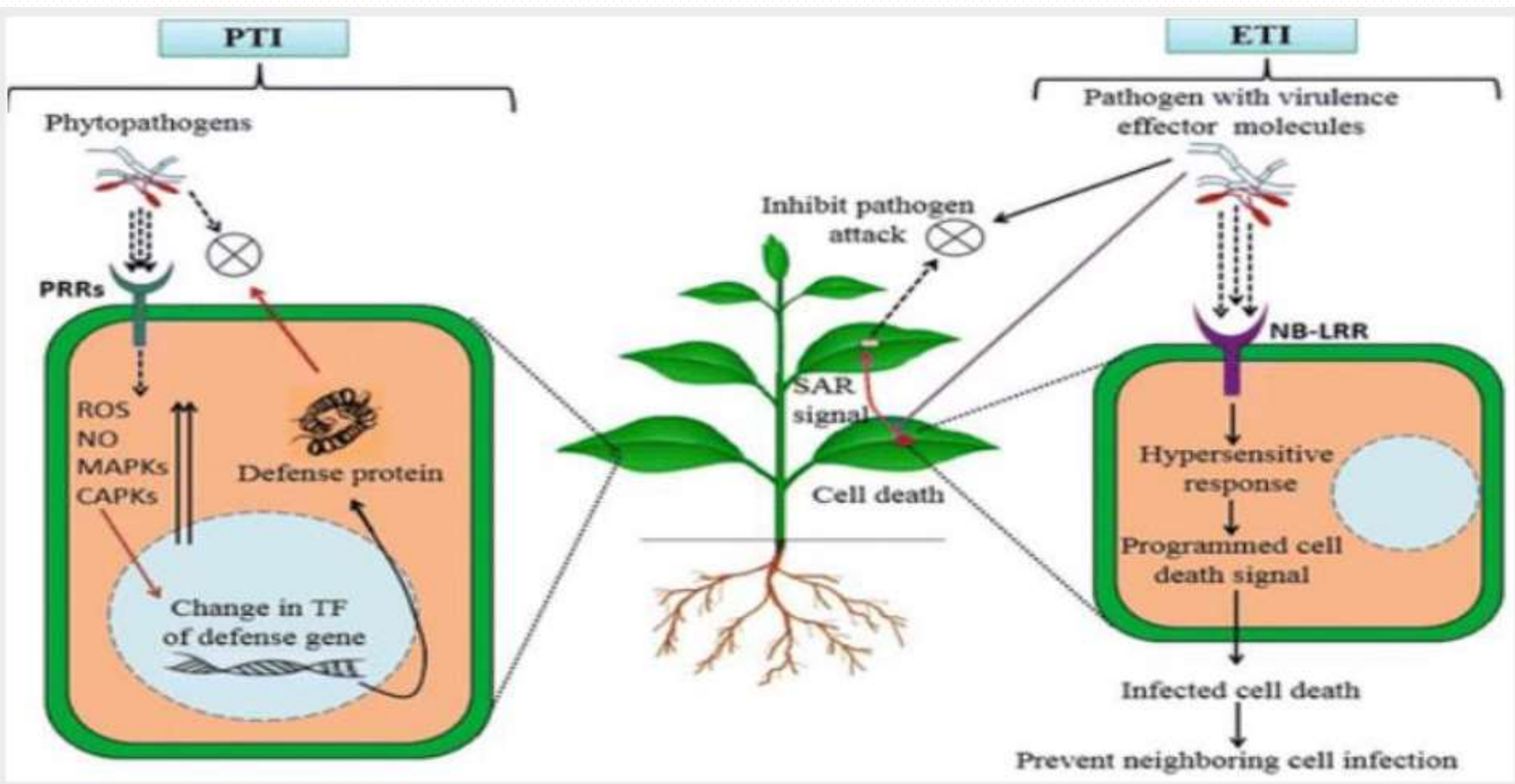
Abstract

Rice blast is one of the major diseases in rice deterring the crop production up to 30% which in turn affects global food security. The devastating disease is caused by filamentous hemibiotrophic ascomycetes, *Magnaporthe oryzae*. Several studies on the identification of the disease resistance genes hitherto resistant cultivar did not solve to culminate the disease from the field. However, there are plants in nature that are not infected by the rice blast pathogen and are known to be non-host. A non-host plant provides broad-spectrum disease resistance against a pathogen race. The infected cells communicate and activate the cell death in an orchestrated fashion. The NHR mechanism results in hypersensitive responses like oxidative burst and programmed cell death (PCD) at the site of infection that restricts further spreading of the disease into adjacent cells. Although disease resistance played by various NHR genes is reported, the underlying molecular mechanism of NHR and PCD remains elusive. Thus, the present study focuses on the comparative oxidative burst, ion leakage, and cell death corresponding to disease resistance in different mutants of *Arabidopsis*. This study can lead to the identification of the active protagonists from the non-host and help in engineering broad spectrum and durable disease resistant rice. Further, the cell death pathway will be studied to delineate its relevance if any in the host.

Background

Fig-1: Depiction of plant defence mechanism through PTI and ETI responses

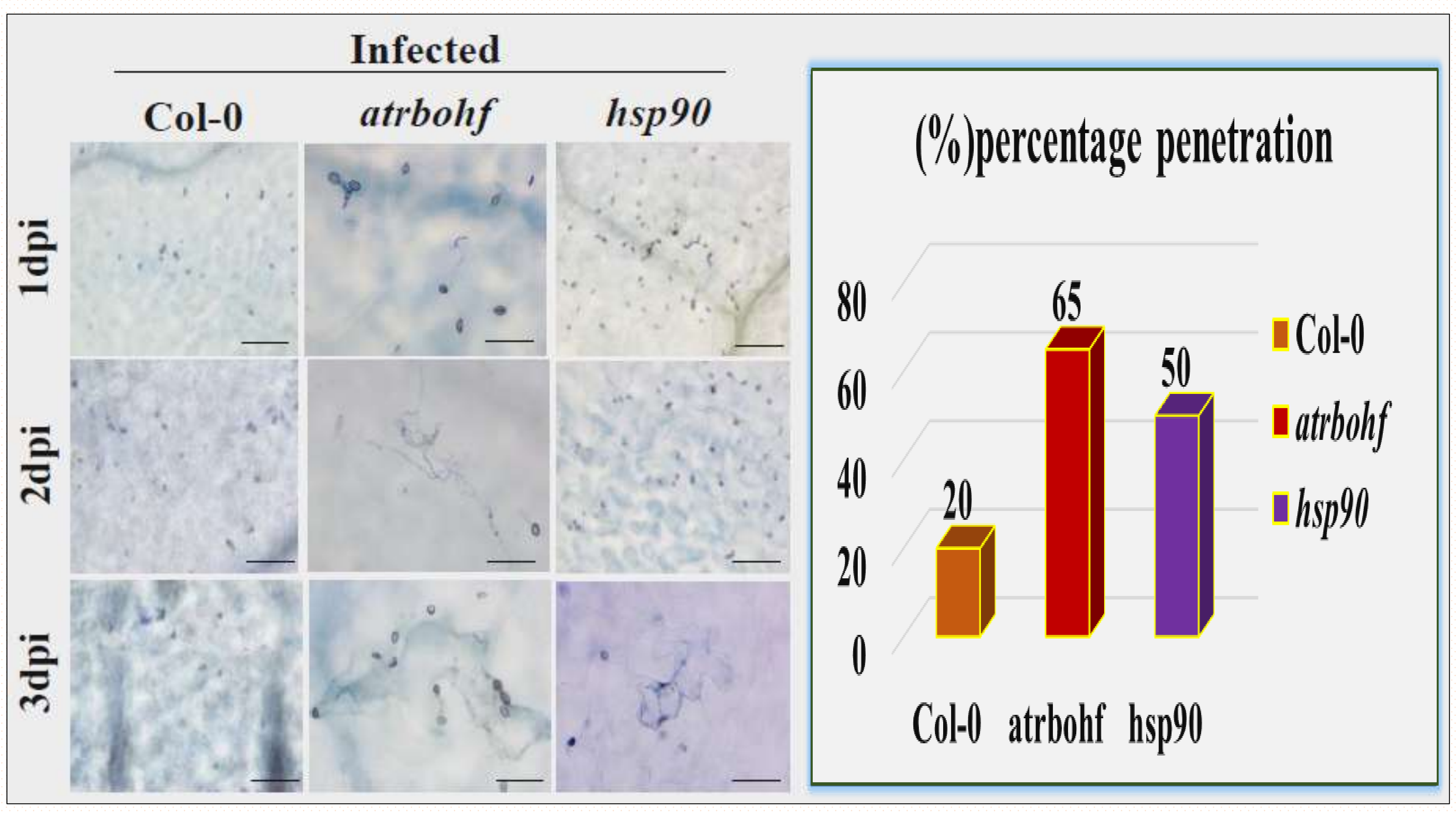


Materials and Methods

1. **<u>Plant and Pathogen growth and Infection</u>:** Arabidopsis wild type & mutants were grown on soil in a plant growth chamber with 14h photoperiod, 22˚C & 60% relative humidity. *M. oryzae* was maintained on oatmeal agar plates and spores were harvested from 10day old culture. 10µl of spore solution of concentration 10^5 spores/ml were inoculated onto the detached Arabidopsis leaves.
2. **<u>Staining assay</u>:** Control and infected leaves were observed under microscope with trypan blue and DAB staining for cell death and ROS production.
3. **<u>Confocal Microscopy</u>:** Leaf disks of each infected ecotype were cut and placed on the slides along with glycerol on it and observed under confocal microscope .

Results

Fig-2: Differential staining pattern of trypan blue in wild type Col-0 and mutants (*atrbohf* & *hsp90*) and its quantitative analysis



Results

Fig-3: Differential staining pattern of DAB in wild type Col-0 and mutants (*atrbohf* & *hsp90*) and its quantitative analysis

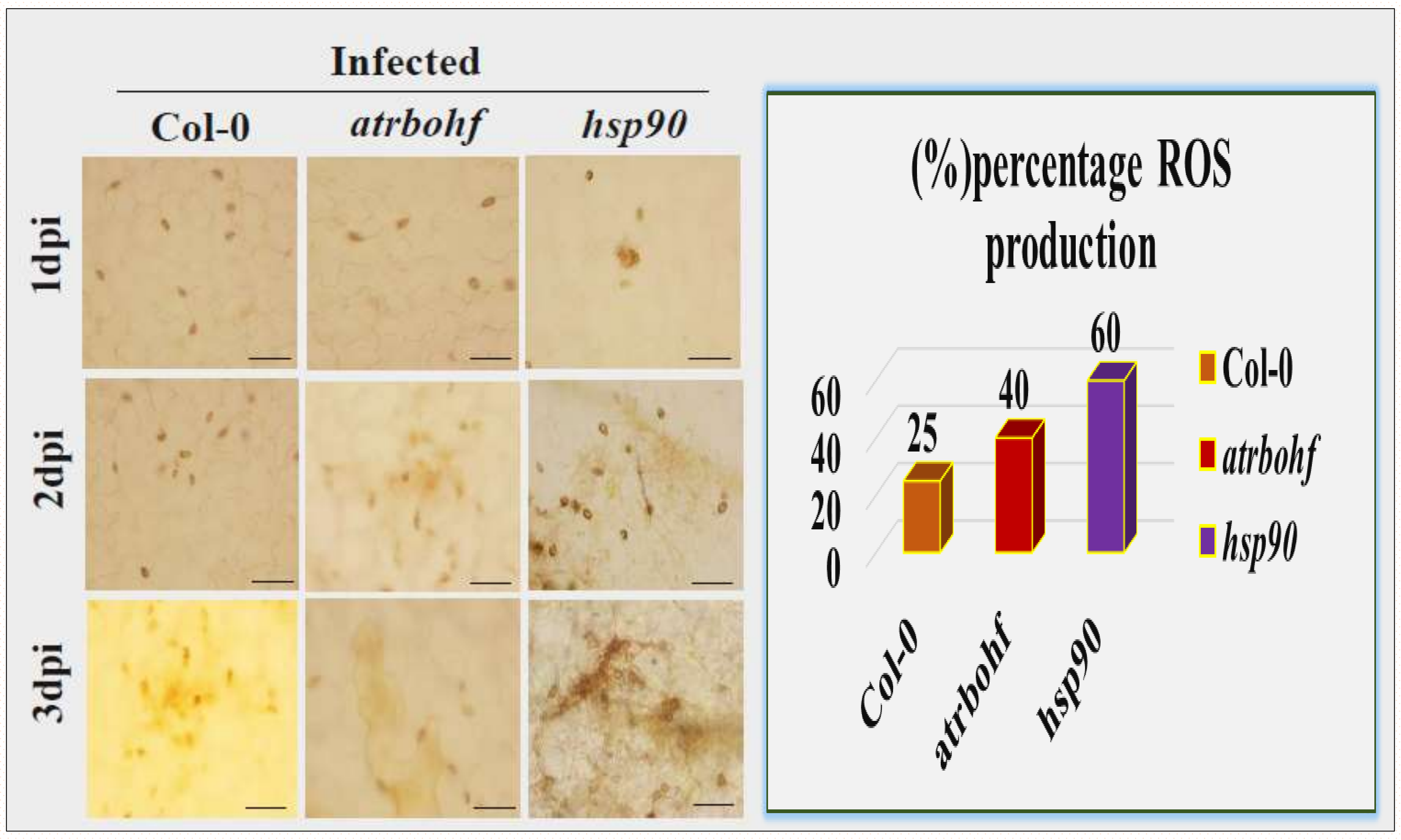
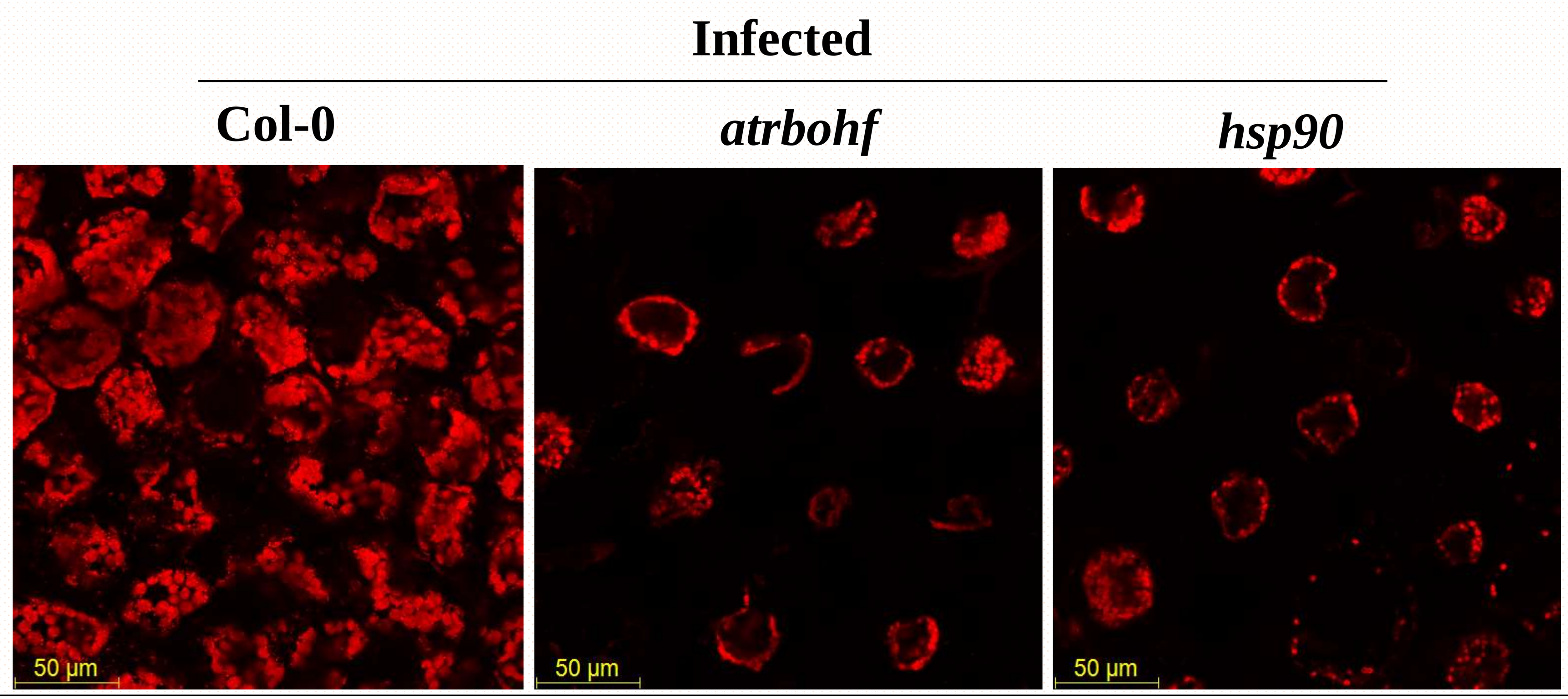


Fig.4: Evaluation of chlorophyll autofluorescence in *M. oryzae* challenged leaves of wild type Col-0 and mutants (*atrbohf* & *hsp90*) . Autofluorescence of chlorophyll was measured using Laser scanning live-cell imaging microscope (Leica, STELLARIS 5) with an excitation of 633nm and emission of 647-721nm. Scale Bar = 50µm.



Conclusions

- Trypan blue staining shows the differential cell death pattern in different *Arabidopsis* mutants.
- DAB staining exhibits the differential ROS production across the mutants.
- Chlorophyll evaluation depicts the degradation of chlorophyll molecules upon pathogen infection indicating higher cell death.

Future Plans

- Biochemical changes during infection progression and cell death.
- Relative expression of defence genes and involved pathways.
- Expression of the NHR gene with relevance to immunity in both host and non-host plant.

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References

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