Exploring non-host resistance against rice blast via programmed cell death in *Arabidopsis*

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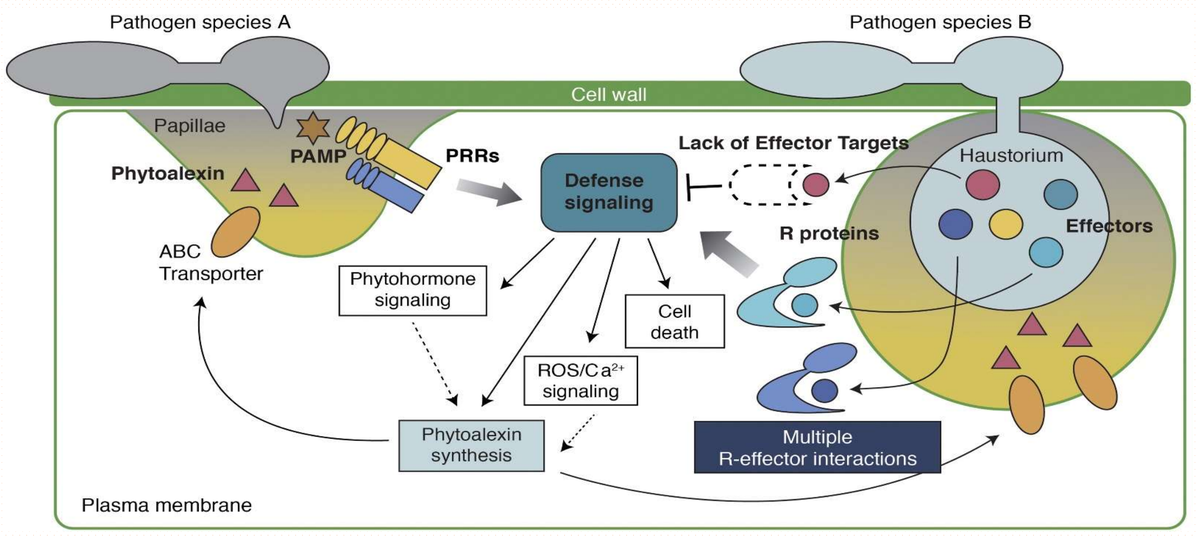
Abstract

Rice blast is a devastating disease caused by a filamentous fungal pathogen, *Magnaporthe oryzae*. It belongs to ascomycetes and is hemi-biotrophic in nature. The global food security is immensely affected by the deterioration of 30% of the crop production due to this disease. Although there are numerous previous studies on the disease resistance genes, but these could not solve to eliminate the disease from the field. Nevertheless, in nature there are plants which are not infected by rice blast and considered as non-host plants. Non-host plants have broad spectrum disease resistance against a pathogen race. Therefore, the NHR activates several defence strategies through cell communication resulting in hypersensitive responses, such as oxidative burst and programmed cell death (PCD) in an orchestrated manner at the infection site. These defence responses do not allow further spreading of the disease into the neighbouring cells. However, many NHR genes are reported earlier, still the exact mechanism of NHR and PCD in plants are not well understood. Thus, the present work focuses on the relative oxidative burst, ion leakage, and cell death corresponding to disease resistance across 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 (Lee et al., 2017)



Materials and Methods

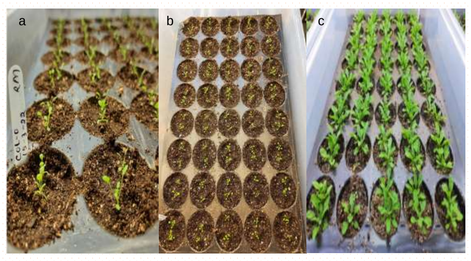


Fig-2: Growth and maintenance of *Arabidopsis*. (a)*Arabidopsis* seedlings at 10th day, (b)Transplantation of each seedlings into individual cups on tray, (c)Well grown *Arabidopsis* plants

Fig-3: Culture of *M. oryzae* in Oatmeal agar medium. Culture conditions maintained in dark, at 27°C.

Fig-4: Infection assay in detached *Arabidopsis* leaves using *M. oryzae*

Fig-6: Microscopic observations of infected leaves using staining methods

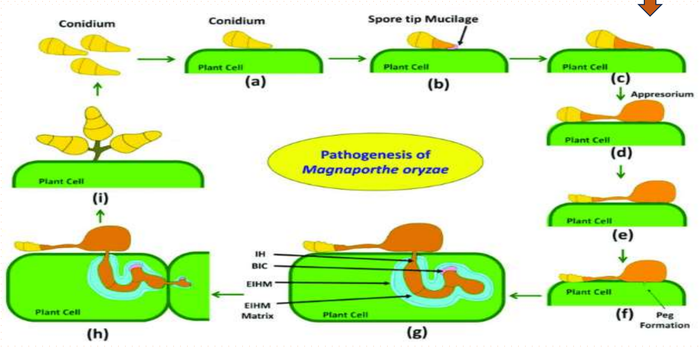
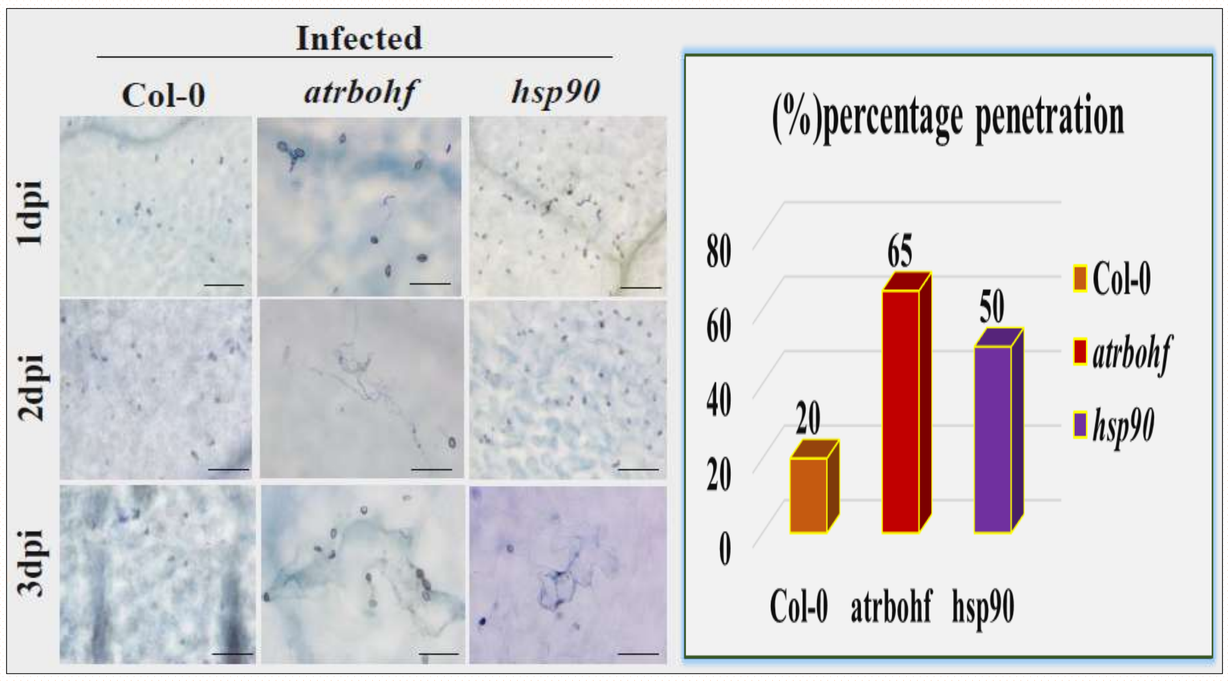


Fig-5: Infection cycle of *M. oryzae* (Eseola et al., 2021)

Results

Fig-7: Differential staining pattern of trypan blue in wild type Col-0 and mutants (*atrbohf* & *hsp90*) and its quantitative analysis



Results

Fig-8: Differential staining pattern of DAB in wild type Col-0 and mutants (*atrbohf* & *hsp90*) and its quantitative analysis

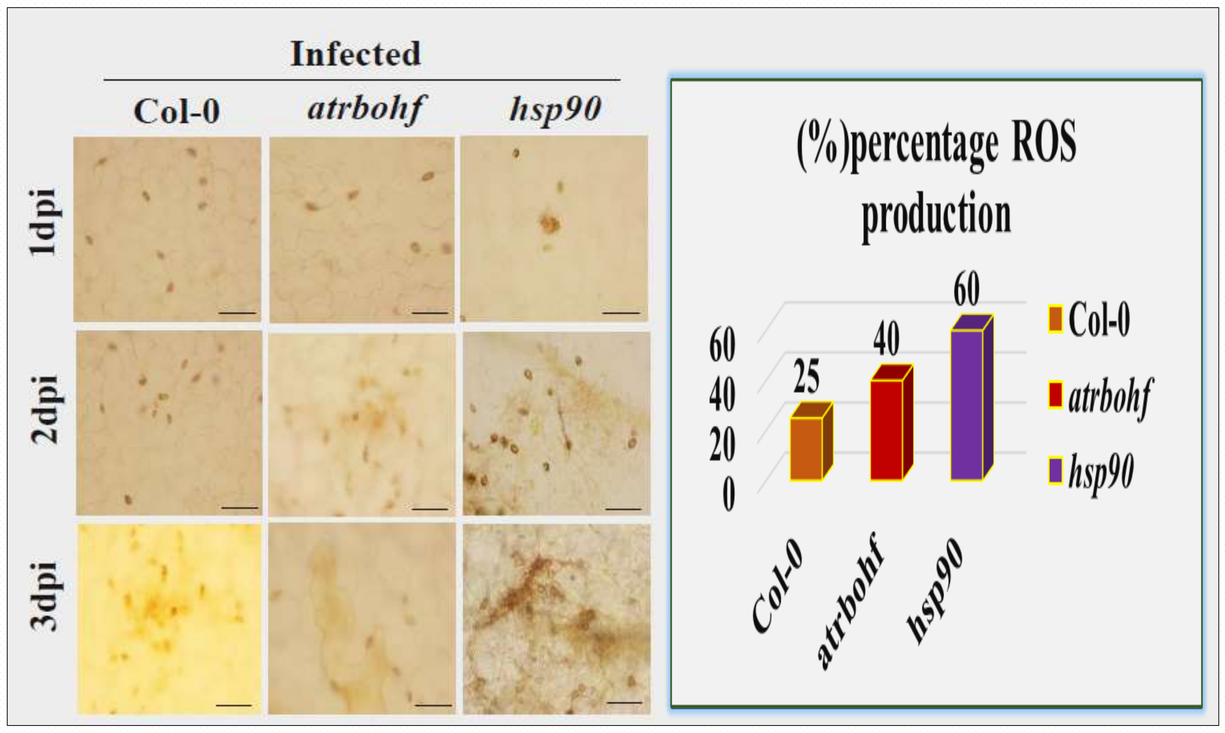


Fig.9: Evaluation of chlorophyll autofluorescence in *M. oryzae* challenged leaves of wild type Col-0 and mutants (*atrbohf & hsp90)* . Autofluorescence of chlorophyll was measured using Laser scanning live-cell imaging microscope (Leica, STELLARIS 5) with an excitation of 633nm and emission of 647-721nm. Scale Bar = 50µm.

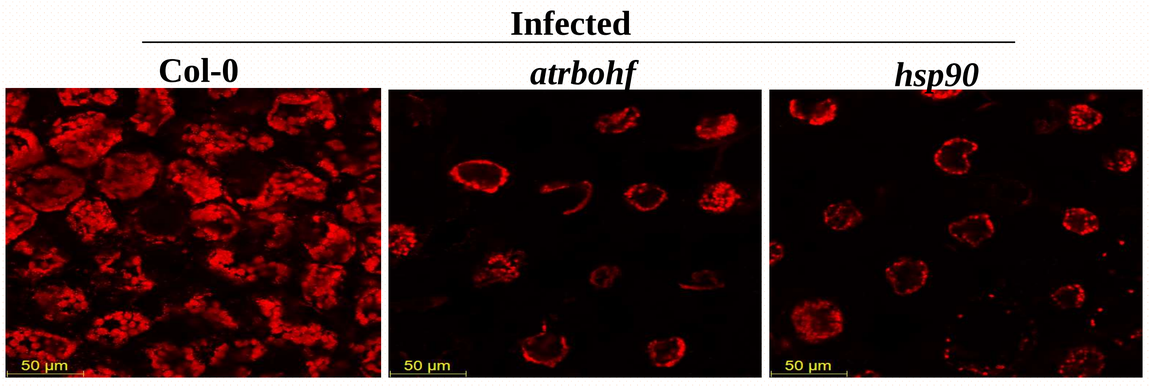
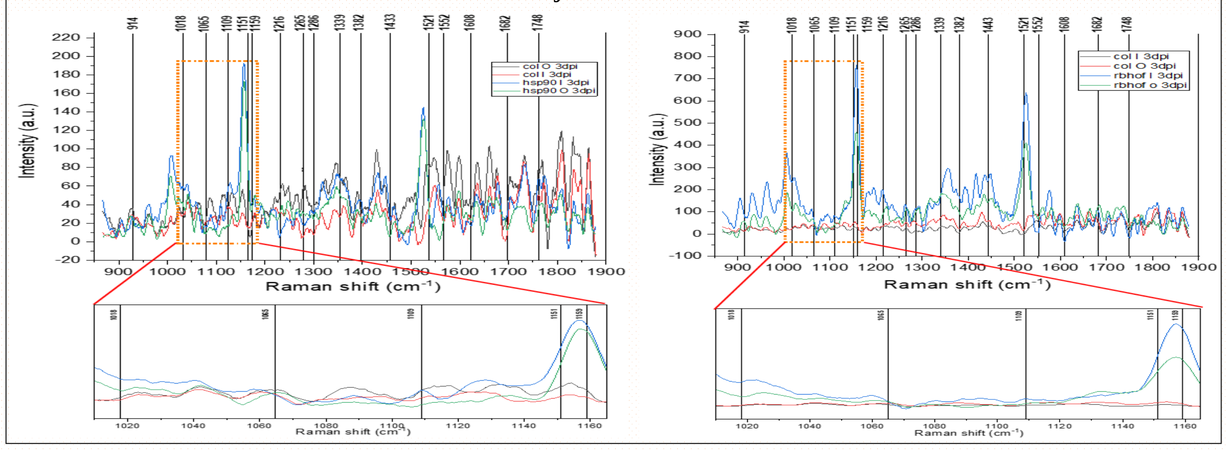


Fig.10: Raman spectra analysis in control & *M. oryzae* challenged leaves of wild type Col-0 and mutants (*atrbohf & hsp90).* The parameters of Raman spectra are as follows: Spectral range:800-1800 cm^{-1} , Acquisition time: 10s, Laser power:20 mW, Magnification objective: 20X



Conclusions

- Trypan blue & DAB staining exhibit the differential cell death pattern and ROS production across different *Arabidopsis* mutants.
- Chlorophyll evaluation depicts the degradation of chlorophyll molecules upon pathogen infection indicating higher cell death.
- Raman spectra analysis further proves the chlorophyll and carotenoid degradation during pathogenesis at the infection site

Future Work

- 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.

Acknowledgement

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References

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