**Student “Side Project” Reveals a Strategy for Managing
Alternaria Blight Through Nonhost Resistance**

Urooj Fatima and [Muthappa Senthil-Kumar](skmuthappa%40nipgr.ac.in)

National Institute of Plant Genome Research, New Delhi

<Link to author’s email added above, rather than provide footnote>

<Eliminate this link after confirming link added above works: \* correspondence, Email: skmuthappa@nipgr.ac.in >

Alternaria blight, caused by the necrotrophic fungus *Alternaria brassicae,* is one of the most serious and devastating diseases of oilseed brassicas throughout the world. To date, several disease management strategies—such as applying fungicides in the field and developing disease-resistant varieties by utilizing the host germplasm—have not been effective enough to circumvent this pathogen. This prompted us to identify an alternative source of resistance against this pathogen. In nature, resistance is often wide and disease is the exception. Exploiting sources of resistance outside the host germplasm that are not hosts of the pathogen could be a promising strategy for developing blight-resistant brassica crops.

In the past two decades, the concept of nonhost resistance (NHR) has been widely studied. Researchers have tried to understand the mechanistic basis of NHR in plants against a wide range of pathogens. Our lab has also worked to identify the genes and the mechanisms of NHR in the model system Arabidopsis against pathogens of crop plants. During travel to Rajasthan, Uttar Pradesh, and Assam—important mustard cultivation states in India—we realized that Alternaria blight is a serious problem that causes high yield losses. Considering our background in working with NHR and the fact that many crops co-cultivated with mustard do not have this disease, we put forward the idea of exploiting the NHR mechanisms that could provide durable resistance against *A. brassicae,* and the scientific community much appreciated it.

Later, we casually discussed testing the ideas in our weekly lab meetings. Urooj showed a lot of interest in this project (despite working on a separate topic for her doctoral thesis), and we decided to take it up as a side project. It was a curiosity for us to expand the expertise and knowledge about NHR mechanisms for the fungal pathosystem. We decided to utilize part of the institutional core grant meant for our group to initiate the experiments. Here, we describe the process that went into developing this manuscript for submission to *MPMI.*

**Identifying the Potential Nonhost Plant**

To decipher the mechanisms and the genes involved in NHR against *A. brassicae,* the first and most crucial step was to identify a potential nonhost plant that could reveal stable and exploitable NHR mechanisms for feasible transfer to a host (brassica crop) for imparting resistance. Knowing that disease is the exception, not the norm, we expected a vast source of resistance available in the form of nonhost plants that are not infected by this fungal pathogen. But it was very difficult to identify a nonhost plant based on experimental screening of a large number of plants*.*

So we utilized two approaches for identifying a suitable nonhost plant. Our first approach was based on a literature search about the intercropping systems followed in India, where brassica crops have been cultivated with other plant species for several decades. We ensured through a publicly available database that these plant species are not known to be infected by *A. brassicae*. Through this approach, we found a good number of plant species, belonging to nine different families of monocots and eudicots (including chickpea, lentil, pea, barley, sugarcane, and potato), that were potential nonhosts. We also performed artificial inoculation and confirmed this notion. However, in terms of application, it was not feasible to transfer the resistance genes of these likely nonhost plant species, which are phylogenetically distant from members of the Brassicaceae family, to mustard.

To further streamline the selection process, we did the phylogenetic analysis and compared the evolutionary distances between these plant species. We found that several members of the Fabaceae family, which is closely related to the Brassicaceae, are known to be infected by this pathogen, including guar, lima bean, and pea except for chickpea. Further, our experience and the literature survey clearly suggested that chickpea is often cultivated close to brassica crops in different parts of India. Since both crops have been grown in the same area for many years, the pathogen has had ample opportunities to infect chickpea but failed to do. This further strengthened our selection criteria for identifying chickpea as a potential nonhost plant.

Our search for a nonhost plant made us realize that the molecular biologist cannot work in solitude to address scientific problems related to crops. The interdisciplinary research undertaken by the agronomist and the molecular biologist could provide solutions to the problems posed by the farmers.

**Addressing Curiosities by Using Good Experimental Tools**

To provide answers to scientific questions, it is essential to select the correct experimental tools and techniques. Doing so will not only facilitate the researcher in addressing the problem but will also strengthen his or her manuscript for publishing in a reputable journal.

Our next curiosities were to know how the *Alternaria* pathogen behaves in the nonhost plant and which layer of NHR operates in chickpea against the pathogen. To answer these questions, we chose microscopic imaging techniques. By venturing into the microscopic world, the researcher can gain more confidence in his or her results because “seeing is believing.” We started with fluorescence microscopy of the live cells with the calcofluor that specifically stains the fungal chitin. Although we got images of the fungus growing on the plant, because of background noise, the images did not provide clarity in visualizing the critical fungal structures and the interaction of the fungus with the plant cell.

So we opted for light microscopy of the samples after lactophenol aniline blue staining. We observed the infection process of *A. brassicae* on chickpea and compared it with that of the host plant, brassica. Selecting different points in time allowed us to identify the stages at which the pathogen failed to infect chickpea. The NHR operating in chickpea actively suppressed pathogen development, penetration, and colonization. We found that the preinvasion NHR in chickpea operates mainly via stomatal closure to prevent the entry of pathogens. Around this time, our project and projects of researchers at Assam Agricultural University were externally funded by the Department of Biotechnology, Government of India.

Our next step was to perform the transcriptome analysis through microarray. The microarray chip was designed based on information available from the publicly available Chickpea Transcriptome Database (CTDB), produced by our institute, NIPGR. Further, selecting the time points was an important step for identifying the critical genes involved in a robust NHR response against *A. brassicae* infection. Based on our results from microscopic and morphopathological studies, we decided the time points (24 hpi and 48 hpi). The data analysis was done using the CTDB, which provided us with the most comprehensive information regarding the genes and the pathways involved in NHR against *A. brassicae*. Moreover, the transcriptome analysis usually throws a lot of genes and pathways, and therefore, the experimental validation is always required to gain more confidence in the data. So we validated the function of genes involved in pre- or postinvasive NHR through various experiments.

**Preparing a Manuscript for Submission**

We have a few key points to share with the young researchers that we followed during manuscript preparation for submission to *MPMI.* A researcher must have a thorough understanding of his or her topic to carry out good research. We have extensively studied the literature, including recent and old research papers, review articles, and books related to our topic. Parallel to writing this manuscript, we wrote a review article about the perspective on the host and nonhost resistance mechanism against Alternaria blight. Doing this allowed us to comprehend the topic more completely and to address the scientific problem systematically. Besides, it is important to note that presenting data in a well-organized and comprehensive way is one of the primary factors in getting a manuscript published in a reputable journal. Apart from doing thorough experimentation, we worked critically on writing the manuscript and presenting data in the form of high-quality figures.

Furthermore, data transparency is also important in the research field. To provide more transparency, we submitted the raw data used to create the figures for the manuscript. It is advisable to submit the raw data files and to make them available to the scientific audience, thereby increasing the credibility of your research. By considering the above-mentioned points, we were able to publish our work in a highly reputable journal, *MPMI.*

**Acknowledgment**

We duly acknowledge the funding from the Department of Biotechnology, Ministry of Science and Technology, Government of India and National Institute of Plant Genome Research, New Delhi.