Executive Summary of the Final Report

of a Major Research Project supported by

University Grants Commission

New Delhi – 110 002

1. Title of the Project:
   Studies on the morphological and molecular variability of rice blast pathogen in four main rice growing districts of Southern Karnataka

2. Name and address of the Principal Investigator:
   Dr. N.S. Devaki,
   Associate Professor, Dept. of Molecular Biology, Yuvaraja's College (Autonomous), University of Mysore, Mysuru - 570 005, Karnataka

3. Name and address of the institution:
   Dept. of Molecular Biology, Yuvaraja's College (Autonomous), University of Mysore, Mysuru 570 005, KARNATAKA

4. UGC approval letter no. and date: **F. No. 41-408/2012(SR) Dated 16 July 2012**

5. Date of implementation: **06th September 2012**

6. Tenure of the project: **06th Sept 2012 to 31st Dec 2015**

7. Total grant allocated: **Rs. 10,99,600.00**

8. Total grant received: **Rs. 10,90,600.00**

9. Final expenditure: **Rs. 11,18,965.00**

10. Title of the project:
    Studies on the morphological and molecular variability of rice blast pathogen in four main rice growing districts of Southern Karnataka

11. **Objectives of the project:**
    - Collection of disease samples from the field from the four districts of Karnataka
    - Isolation of the pathogen and maintenance of pure culture
    - Study of morphological and biochemical variations
    - Differentiation of the pathogen based on differential varieties. Popular varieties of rice will be screened for resistance for the identified races (isolates collected during the investigation)
    - Molecular characterization of isolates of *Pyricularia grisea* to identify the variations at molecular level.

12. Whether objectives were achieved: **Yes, objectives were achieved**

13. **Achievements from the project:**

- Developed a simple and reliable protocol for isolation and storage of rice blast pathogen *Magnaporthe oryzae*
- Identified 15 new pathogen races based on differential varieties of host
- Showed ITS also can be used to understand the diversity of the pathogen
- Morphological, biochemical and molecular diversity of 72 blast pathogen isolates are elucidated
- Five research papers are published
- One candidate successfully completed his Ph.D. under this project

14. **Summary of the findings:**

According to the latest revision of the UN population prospects, the world population is projected to grow by 34 percent from 6.8 billion today to 9.1 billion in 2050. There is urgent need to increase global production of rice with respect to increasing population with decreasing cultivating land. Rice plant suffers from many diseases caused by different pathogens *viz*., bacteria, fungi, viruses, phytoplasma and nematodes. Among the fungal diseases, the blast is considered as a major threat to rice production because of its worldwide distribution and its destructiveness. Blast disease of rice plant is caused by *Magnaporthe grisea* (anamorph *Pyricularia grisea*) is currently known as *Magnaporthe oryzae.* This disease is recognized to be one of the most serious diseases of rice crop around the world. A total of 171 places were visited and samples were collected from 101 diseased plots from different geographical regions of Karnataka, India during 2012-2014. Disease incidence of 64.44%, 68.75%, 60.71% and 55.55% was recorded from major rice growing districts of south Karnataka *viz*., Chamarajanagar, Kodagu, Mandya and Mysuru districts respectively. Varieties MTU-1001, Jyothi and Sona Masuri remained highly susceptible in these four districts. Newly developed blast resistant varieties namely Rasi, KRH 4, Raksha and Mugad Siri 1253 were also found to be susceptible in some of the regions of Karnataka indicating breakdown of resistance. Thus, the emerging pathogenic

variants among fungal populations trigger serious and incessant threat to the newly released resistant varieties also.

Seventy two monoconidial isolates of *M. oryzae* were successfully established and maintained using a simple, reliable and inexpensive isolation and storage procedure developed by us. The growth pattern and growth rate of all the 72 *M. oryzae* on different media *viz*., oat meal agar and potato dextrose agar was studied to understand the extent of morphological diversity among these isolates. The growth measurement parameter and growth rate of 72 isolates of *M. oryzae* on two individual media and also between two media showed the P-value less than 0.05 ($P \leq 0.05$). This indicated that there is a significant growth difference among isolates grown on individual media as well as between these two growth media.

All the isolates were screened for virulence through pathogenicity test. Twenty-one isolates were found to be highly virulent. Among virulent isolates, 16 were selected for the race (pathotype) identification using eight standard international host differentials. A total of 15 pathotypes were detected among 16 isolates. We have chosen 20 *M. oryzae* isolates which ranked top for their virulence among the 72, for the isozyme analysis. Three isozymes *viz.*, catalase, esterase and protease were screened for their activity and visualized on non-denaturing PAGE through the use of specific stains. The relative mobility (Rm) of each isozyme band on non-denaturing PAGE was calculated. A total of 17 polymorphic bands were observed with an average of 5.66 bands for each marker. In UPGMA cluster analysis, two distinct clusters were formed in which isolates obtained from south districts formed separate cluster and few of the isolates collected from northern region of Karnataka formed separate subgroup in the other cluster.

All the isolates of *M. oryzae* were characterized using ribosomal DNA sequences (ITS). Variability of rDNA sequences was found to be highly polymorphic with 0.068962 nucleotide diversity showing 6 distinct clades. Thirty three haplotype groups were identified with haplotype diversity of 0.8881 and Tajima's neutrality test with a D value of -1.96827 with $P < 0.05$ showing the presence of variations among the sequences of pathogen isolates. This shows the importance of characterizing internal transcribed spacer (ITS) to know pathogen diversity and its fitness. The fertility status of all the 72 isolates was examined using mating type alleles. 44 isolates belonged to MAT1 type (male

fertile) and 28 isolates were of MAT2 (female fertile) and there were no hermaphrodite isolates. In a given geographical location, only one mating type was identified. Results revealed that the isolates obtained from these regions are not sexually fertile showing predominant asexual reproduction.

Pot2 rep-PCR DNA fingerprinting profile showed 27 polymorphic bands with bands ranging in size from 0.65 to 4.0 kb and an average of 10 to 14 bands per isolate. Five distinct clusters were formed. Some of the isolates belonging to clusters 3, 4, and 5 are interlinked as these locations are close to one another sharing common geographical parameters and boundaries.

21 SSR markers are taken to analyse genetic diversity. Significant variation among the isolates of *M. oryzae* was observed with 92 polymorphic bands with an average of 4.38 bands for each marker. Average locus heterozygosity and polymorphic information content (PIC) in a total of 21 SSR markers were 0.73 and 0.60 respectively; Genetic similarity coefficient ranged from 0.20 to 0.63 indicated the existence of high polymorphism among the isolates. Cluster analysis interestingly indicated a correlation of grouping with the geographical boundaries. We have plotted virulence spectrum for each group and found out mixed types of reaction pattern in each group. Hence during breeding program locus heterozygosity, PIC and virulence spectrum have to be considered for obtaining stable resistance cultivars.

When we compiled our morphological, pathogenicity and molecular data altogether it was very clear that there is significant diversity existing among these isolates. However, pooling of the data showed that correlation with geographical boundaries is not significant. Analyses of pathogen diversity using molecular tools are much preferable to understand differences at molecular level as compared to morphological data in a large population study. From this we are concluding that our investigations and output will supplement the data required for accelerating the engineering of blast resistance genes into rice cultivars through molecular breeding.

15. **Contribution to the society:**

Investigation carried out under the current project has generated data on status of the blast pathogen, incidence, fertility status and extent of genetic diversity existing among the isolates collected from southern Karnataka region. This study helped in understanding the variability of the pathogen, which is important for resistant breeding of rice cultivar against the blast pathogen. Thus the output of the present research work will indirectly contribute to the increase in rice productivity of our country.

16. Whether any Ph.D. enrolled/produced out of the project: Yes, as under:
Jagadeesh. D (Submitted Ph.D thesis on December 2018)
Enrollment No. DOR.9.9/Ph.D/JD/435/2012-13 in University of Mysore, Mysuru

17. No. of publications out of the project: **Five**

i. Chandrakanth R, Jagadeesh D, Devaki NS (2014) Black light mediated growth and sporulation of *Magnaporthe oryzae*. **International Journal of Agricultural Science and Research**, 4:25–30

ii. Jagadeesh D, Prasanna Kumar MK, Chandrakanth R, Devaki NS (2018) Molecular diversity of internal transcribed spacer among the monoconidial isolates of *Magnaporthe oryzae* isolated from rice in southern Karnataka, India. **Journal of Genetic Engineering and Biotechnology** 16: 631–638 https://doi.org/10.1016/j.jgeb.2018.05.008

iii. Jagadeesh D, Prasanna Kumar MK, Devaki NS (2018) Status of *Magnaporthe oryzae* infection in different districts of Karnataka, India and establishment of monoconidial cultures for understanding genetic diversity. **International Journal of Agriculture, Environment and Biotechnology** 11:345–355 doi: 10.30954/0974-1712.04.2018.16

iv. Jagadeesh D, Prasanna Kumar MK, Devaki NS (2018) A simple and reliable method for obtaining monoconidial culture and storage of *Magnaporthe oryzae*. **International Journal of Life Sciences** 6:540–543

v. Jagadeesh D, Prasanna Kumar MK, Devaki NS (2018) Population analysis of *Magnaporthe oryzae* by using endogenous repetitive DNA sequences and mating-type alleles in different districts of Karnataka, India. **Journal of Applied Genetics** 59:365–375

18. **Conference attended and paper presented based on this project work**

CONFERENCES/SEMINARS/WORKSHOPS/SYMPOSIA/TRAINING PROGRAMMES ATTENDED		
Year	**Conference/Seminars/Workshop Symposia/Trainings attended**	**Title of paper presented/ Delivered Lecture/ Chaired Sessions**
2018	National conference on "Applications of microbiology in Human welfare" Department of Microbiology, University College of Science, Tumkur University, Tumkur	Presented Oral Presentation on Title " Mating type distribution and genetic diversity of *Magnaporthe oryzae* populations from Karnataka, India"
2015	International Rice Symposium, ICAR-IIRR, Hyderbad	Presented Poster on : Collection and isolation of rice blast fungus *Magnaporthe grisea* for assessment of genetic diversity in four main rice growing districts of Southern Karnataka
2015	National Symposium on" Microbes and Human Welfare" Postgraduate Dept. of Biotechnology in Association with Microbiologist of India (Mysore Chapter) 2015. At JSS College of Arts, Commerce and Science, Ooty Road, Mysore	Presented Oral Presentation on Title "Collection and Isolation of rice Blast fungus *Magnaporthe grisea* in four main rice growing districts of southern Karnataka"
2014	International Conference on Biodiversity, Bioresources and Biotechnology Mysuru	Presented Poster on : A novel method of in vitro sporulation of rice blast fungus *Magnaporthe grisea* * Best Poster awarded
2013	Tools for Genetic Diversity and Mapping Analysis using Molecular Marker Data, at CPCRI, ICAR, Kasaragod, Kerala	Attended Workshop on "Tools for Genetic Diversity and Mapping Analysis using Molecular Marker Data"
2012	CIST Recent Trends in Information Technology and Animation, visual Effects and gaming, University of Mysore, Mysuru	Oral Presentation Title on "Comparision of Sequences of Calmodulin gene responsible for Appressorium formation and Attachment over the host"

5/2/2019

PRINCIPAL INVESTIGATOR

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