

CROP PROTECTION PROGRAMME

**Validation and promotion of technologies for Rice sheath blight
management**

R 8446 (ZA 0671)

FINAL TECHNICAL REPORT

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Project Leader
Dr. S. Sreenivasaprasad

Warwick HRI, University of Warwick, UK
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Project Collaborators
Dr. M.M. Rahman & Dr. M.A. Taher Mia
Bangladesh Rice Research Institute, Gazipur

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List of Acronyms

AFLP	Amplified Fragment Length Polymorphism
AShS	Aggregate sheath spot
BARD	Bangladesh Academy of Rural Development
BARI	Bangladesh Agricultural Research Institute
BAU	Bangladesh Agricultural University
BINA	Bangladesh Institute of Nuclear Agriculture
BLB	Bacterial leaf blight
BMDC	Bangladesh Management Development Centre
BRRRI	Bangladesh Rice Research Institute
BSMRAU	Bangabandhu Sheikh Mujibur Rahman Agricultural University
CPP	Crop Protection Programme
CRD	Complete randomized block design
DAE	Department of Agricultural Extension
DFID	Department for International Development
FoSHoL	Food security for sustainable household livelihoods
HYVs	High yielding varieties
ICAR	Indian Council of Agricultural Research
IPM	Integrated Pest Management
IRRI-D	International Rice Research Institute, Dhaka
KBA	King's B agar
MVs	Modern varieties
NR Int	Natural Resources International
PCR	Polymerase Chain Reaction
PETARRA	Poverty elimination through rice research assistance
RAPD	Random amplified polymorphic DNA
RDA	Rural Development Academy
RLH	Relative lesion height
RO	<i>Rhizoctonia oryzae</i>
ROS	<i>Rhizoctonia oryzae-sativae</i>
RS	<i>Rhizoctonia solani</i>
SES	Standard Evaluation System
ShB	Sheath blight
ShS	Sheath spot
SI	Severity index
SSR	Simple Sequence Repeat
T.Aman/T.Aus	Transplanted Aman/Aus season
VNTR	Variable number of tandem repeats
Warwick HRI	Department of Warwick HRI, University of Warwick, UK

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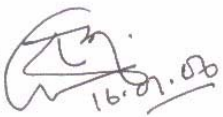
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Our primary recognition is to the farmers who cooperated in the on-farm activities for no direct reward.

Biometricians Signature

I confirm that the biometric issues have been adequately addressed in the Final Technical Report:

Signature:  16.01.06

Name (typed): M. Shahjahan Kabir
Position: Senior Scientific Officer, Biometrics Division, BRRI, Bangladesh
Date: 16.01.2006

Executive Summary

Increasing rice yield per unit area is the number one priority for BRRRI, as part of a recent Bangladesh government initiative to increase food production by 25% within the next 3-5 years. Sheath blight is recognized as a high priority constraint to rice production in Bangladesh. Project R7778 identified and developed outputs that would contribute to the development of technologies for sustainable management of rice sheath blight. The major objective of Project R8446 was validation and promotion of some of these technologies namely the molecular tools for pathogen detection and diagnosis, biological disease control methods and improved rice varieties.

Employing a range of approaches the molecular tools and resources developed for sheath disease pathogen detection and diagnosis have been promoted to the project collaborators at BRRRI, utilizing the molecular laboratory established in a parallel DFID programme. Further training was organized to achieve wider use of the outputs both at BRRRI and other organizations within Bangladesh. Additionally, the knowledge and tools developed on rice blast in West Africa with DFID-CPP funding were also shared with BRRRI pathologists through seminars and training discussions bringing a cross-cutting perspective. This has led to the establishment of knowledge- and skill-pool within BRRRI and other organizations such as BARI and BAU to use the molecular diagnostic technologies in future agricultural R & D programmes. Field work carried out on the application of biological methods showed delayed disease spread and reduction in sheath blight severity at certain location. However, considerable variation was observed in their potential for sheath blight management, suggesting that a number of agro-ecological parameters have a large influence on the use of these methods and additional work is required, potentially through linkages to NARS in India and IRRI, for further development and promotion of these technologies. However, this approach could lead to the beneficial utilisation of waste such as poultry litter, with the development of appropriate preparation and application methodologies. On-farm demonstration work of improved sheath blight tolerant varieties was carried out at locations in Gazipur, Comilla and Rajshahi districts. Farmer field days conducted provided excellent opportunities for close interaction among farmers, DAE staff and BRRRI R & D personnel on the awareness of the disease, management practices and the farmer preferred varieties (e.g. BRRIdhan32 and BRRIdhan34) and their characteristics (e.g. BRRIdhan32, early maturing, disease tolerance, low inputs and good yield; BRRIdhan34, aromatic and disease tolerance). Dissemination materials (leaflet and folder in *Bangla*) on the identification of rice sheath disease complex symptoms and disease management were prepared and distributed to 300 farmers at the trial locations.

Overall, the DFID-CPP funded work on rice sheath blight, in delivering the contracted outputs, also facilitated and created:

- Knowledge- and skill-base at BRRRI on a range of aspects of sheath blight management, and excellent working relationships with and support of senior management at BRRRI and IRRI-D

- Close links with parallel projects and programmes such as weed management and the PETRRA-seed health

- Close links with other R & D and extension organizations such as BARI, BINA, BAU, Univ of Rajshahi, BARD and RDA

- Awareness of recent developments at regional NARS e.g. Universities and Institutes in India and international organizations such as IRRI and Univ of Louisiana in developing biocontrol agents and host resistance.

Along with the outputs of R7778 and R8446, these linkages and the critical mass provide opportunities for developing future R & D in integrated disease management in rice production systems in Bangladesh contributing to increased food production, poverty reduction and the achievement of the Millennium Development Goals.

Background

Sheath blight disease is recognized as a high priority constraint to rice production in Bangladesh. High yielding cultivars such as BR11 and Swarna are widely used and are highly susceptible. *Rhizoctonia solani* (RS) is a versatile soilborne pathogen and is a difficult one to control, particularly in view of the susceptibility of many of the popular cultivars. *R. oryzae* (RO, sheath spot) and *R. oryzae-sativae* (ROS, aggregate sheath spot) have also been recognised as causal agents of rice sheath blight complex in some geographic locations. Knowledge of the epidemiology of the pathogens and also the significance of each of the pathogens are essential to implementing appropriate disease control measures. Accurate diagnosis of these pathogens is also essential to ensure success in developing sheath blight resistant varieties. R7778 addressed these issues.

Sheath blight, locally described as kalopocha/pochon/kholpora is considered as the major rice disease by the farmers in all four districts surveyed (400 farm households). Up to 73 and 11 % farmers during Aman season and up to 60 and 16 % farmers during Boro season reported moderate and high disease, respectively. Losses based on farmers' yield estimates ranged between 30 –32 % in both Aman and Boro in general. Further, above 90 % of the farmers were willing to adopt improved varieties and any new disease management technologies e.g. amendments and/or biocontrol agents.

Molecular diagnostic tools including PCR primers with enhanced specificity to the three *Rhizoctonia* spp., PCR protocols and DNA extraction methodologies for rapid and reliable analysis of fungal and plant specimens and pathogen/disease diagnosis have been designed, tested and developed. Utilisation of these tools in combination with intensive disease surveys confirmed *Rhizoctonia solani* causing sheath blight as the dominant pathogen (more than 80%) and importantly identified wide occurrence of aggregate sheath spot pathogen *Rhizoctonia oryzae-sativae* (48 %) either singly or along with *R. solani* in rice production systems in Bangladesh. A collection of well characterised isolates that form a baseline for long term monitoring and use in resistance screening work was established.

A total of fifteen organic amendments were tested for the control of Sheath blight in T. Aus, T. Aman and Boro during 2002-2003 in Comilla, Gazipur and Rajshahi regions. In Comilla, among the organic amendments tested compost, pulse bran and rice bran showed the better results while urmoi (*Sapium indicum*), bishkatali (*Polygonum hydropiper*) and compost showed good indication for the control of sheath blight and increased yield as well in Gazipur. In Rajshahi, pressmud, sawdust and rice bran reduced disease severity.

Florescent bacteria with high levels of antagonism against ShB pathogen in bioassays and disease control potential in greenhouse experiments were identified. Further, prior inoculation of rice with *R.oryzae* isolates was found to considerably reduce the sheath blight pathogen *R. solani* severity.

A range of varieties such as BR3, BR10, BR22, BR23, BR25, BRR1 dhan29, BRR1 dhan31, BRR1 dhan32, BRR1 dhan34, BRR1 dhan38, BRR1 dhan41, BR6194-27-2-2-1, BR6241-62-2-1, BR6004-75-4-HR1, BINA dhan4, BINA dhan6 showing better resistance to sheath blight compared to commonly grown varieties were identified.

These outputs from R7778 provided a wider framework for improved and sustainable management of rice sheath diseases laying the foundations for further validation and promotion.

Project Purpose

Project R7778 generated a number of outputs including knowledge of the *Rhizoctonia* rice sheath diseases and the pathogen diversity and epidemiology as well as developing disease

management strategies based on host resistance, cultural and biological control. These strategic outputs needed to be further validated and promoted paving way for effective uptake and adoption by BIRRI and national programmes on rice sheath blight management. *Rhizoctonia* spp. diagnostic tool kit promoted to BIRRI and in-country training will also benefit scientists from other institutes who work on *Rhizoctonia* diseases on other crops such as potato, soy bean, wheat and vegetables and have contributed to project activities by providing cultures and participating in stakeholder meetings/workshops.

Development and promotion of improved and sustainable rice sheath blight management package(s) to farmers facilitating improved rice production is a high priority for BIRRI and the outputs generated in the project will feed into BIRRI and associated agencies' R & D programmes on disease management and varietal breeding as well other projects such as IRRI-BIRRI project on System of Rice Intensification. Molecular diagnostic kits promoted and in-country capability developed will be mainly be used at the DFID-PETTRA funded molecular facility. Other organisations such as BSMRAU, BARI and BINA targeted to be involved in the training workshop are also likely to use the technology in their projects and laboratories.

Targeted outputs:

1. Molecular tool kit for *Rhizoctonia* species associated with rice sheath blight promoted to BIRRI and in-country training workshop organised to develop local capacity
2. Efficacy of bacterial and fungal antagonists and organic amendments to reduce sheath blight disease tested and validated
3. Improved and sheath blight tolerant rice varieties promoted.

Research Activities & Outputs

Activities

1. Promotion of molecular tools and resources for detection and diagnosis of *Rhizoctonia* sheath pathogens of rice (additionally knowledge of blast diagnostics from DFID-CPP funded work in West Africa) and training in their application

The activities carried out to achieve this output included 1) shuttle visit to and training at Warwick HRI, 2) promotion of information on *Rhizoctonia* diagnostic primers and protocols for simple and rapid extraction of DNA from fungal cultures and infected tissue and PCR tests to BIRRI, 3) training by project leader Dr. Sreenivasaprasad through seminars, and detailed discussion of the protocols and experimental process with staff in the molecular laboratory in the pathology division, BIRRI and 4) subsequent organisation of a 4 day hands-on training for other scientists.

2. Testing and validation of the efficacy of biological control methods for the management of *Rhizoctonia* sheath pathogens of rice

Bacterial and fungal agents and organic amendments were tested for their efficacy to reduce sheath blight incidence and severity under field conditions at different locations in Comilla, Gazipur and Rajshahi districts during different seasons, following laboratory and greenhouse experiments. For activities 2 and 3, standard statistical design and analysis were followed in consultation with BIRRI biometricians.

3. Promotion of improved and sheath blight tolerant varieties and enhancing farmer awareness of the disease and management

Performance of selected varieties such as BR10, BR23, BIRRIadhan 32, BIRRIadhan 34 and BIRRIadhan 38 along with a widely grown popular variety Swarna was demonstrated in on-farm

trials, through close interaction with farming groups and extension staff and by conducting farmer field days and providing dissemination material on sheath blight symptoms and management.

Outputs & Lessons:

1. A research attachment for BRRRI pathology staff Mohammad Hossain was organized at Warwick HRI during Feb-March 2005, to ensure in-depth training in fungal molecular diagnostics and safe laboratory practices and proper transfer of the technologies. Mr. Hossain was trained in the use of various protocols for diagnosis of *Rhizoctonia* species cultures and direct detection of the pathogen in sheath disease samples. This also led to the establishment of a collection of around 70 *R. oryzae-sativae* (aggregate sheath spot) isolates obtained from rice production systems in Bangladesh. This is first time a large collection of rice aggregate sheath spot pathogen isolates has been characterised internationally, contributing new knowledge on this pathogen which occurs widely (48% of sheath samples) in Bangladesh rice production systems. While at Warwick HRI, Mr. Hossain was also provided an opportunity to work on the molecular diagnostic aspects of the blast pathogen *Magnaporthe grisea* linking into other DFID-CPP funded projects. Following this, list of characterised *Rhizoctonia* spp. Isolates (due to quarantine restrictions characterized cultures cannot be easily sent to BRRRI and in view of this a duplicate set of isolates sent to Warwick HRI were held at BRRRI), PCR primer sequences, protocols for DNA and PCR methods were provided to BRRRI.

During a visit in Dec 2005, by the Project Leader Dr. S. Sreenivasaprasad, a training and discussion session on *Rhizoctonia* diagnostics was organised in the Molecular facility at BRRRI for three BRRRI pathology staff Mr. M. Kamal, Mr. M.A. Latif and Mr.M.S. Mian. This opportunity was utilised to discuss various methodologies developed and used for the characterization of the rice blast pathogen *Magnaporthe grisea* in DFID-CPP funded work in West Africa by Dr. Prasad, enabling a cross-cutting up take of DFID-CPP delivered outputs. BRRRI senior pathologist and seed pathology programme leader Dr. M. A. Taher Mia is keen to build on this successful partnership and Dr. Prasad and Dr. Mia have held discussions on future work on the integrated management of diseases in rice production systems. Subsequently, a 4 day hands-on training including seminars was organised at BRRRI and the trained BRRRI pathologists acted as resource persons. The aspects covered included basic techniques in DNA preparation, gel electrophoresis, PCR based techniques such as VNTR, AFLP, RAPD and restriction digestion in general and for *Rhizoctonia* diagnostics. The trainees included Mohammad Shahjahan Monjil, Asst. Professor, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh; Mohammad Akhlasur Rahman, Scientific Officer, Plant Breeding Division, Bangladesh Rice Research Institute, Gazipur; Md. Shahjahan Kabir, Scientific Officer, Plant Pathology Division, Bangladesh Rice Research Institute, Gazipur; Md. Mynul Islam, Scientific Officer, Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur.

The project not only delivered the promotion of tools and technologies for *Rhizoctonia* diagnostics to BRRRI, but has also contributed to the establishment of a knowledge and skill base at BRRRI for future R & D work in this area as well as to train other scientists. To deliver this, close link was established with the DFID-PETTRA project team at BRRRI and the DFID-funded molecular facility was fully utilised. In addition, both through the stakeholder workshops and the training workshop the molecular technologies have been disseminated to scientists at other divisions such as plant breeding in BRRRI and also other organizations such as BARI, and BAU. Project Leader Dr. Prasad discussed the possibility of using the DFID-funded molecular facility and the staff trained in these projects as a national service and training centre and Dr. M.A. Taher Mia who is responsible for this facility is keen to further develop this idea through future DFID research programmes as well as GoB support.

2. Following revival and re-assessment through *in vitro* antagonism and glasshouse experiments, a bacterial isolate [24/BanShB738(3)] was selected for field based on-farm validation work. Similarly, three *R. oryzae* (RO) isolates that were less virulent on rice varieties and non-pathogenic on 11 vegetables (e.g. eggplant, tomato, potato, cabbage, chickpea and mustard) were initially selected. These were tested by three different application methods (Appendix 12) during on-station experiments at BRRRI, Gazipur for their potential to reduce sheath blight disease. These RO isolates, particularly when applied as broadcast inoculum reduced the ShB incidence

and severity to varying degrees (Appendix 2) and isolate RO545 was used in subsequent on-farm validation experiments. Further, three organic amendments namely, rice bran, saw dust and poultry litter were tested along with two controls [1) fungicide treated and 2) no treatment]. To conduct these experiments farmers' fields were selected at Chandina, Comilla district; Sreepur, Gazipur district and Kaligonj, Tanore and Kakonhat of Rajshahi district based on disease recordings from previous crop (Appendix 12). Application of the fungal and bacterial antagonists as well as organic amendments led to the reduction in ShB disease at some locations both during the T. Aus and T. Aman seasons, particularly in delaying the disease spread and reducing the severity. However, at other locations, the same biocontrol agents were not effective in reducing ShB severity suggesting the influence of various agro-ecological parameters (Appendices 3-6). This shows that either bacterial antagonists with a broader spectrum of efficacy or a mixture of these antagonists is needed to provide effective protection against ShB in different locations across Bangladesh. Similarly, with organic amendments, at the stakeholder workshop colleagues from BARI involved in developing biocontrol agents raised treatment prior to applications as an approach to improve the uniformity. For example, an initial decomposition over a 15-30 day period was suggested for poultry litter. Further, BARI have a collection of more than 500 isolates of Trichoderma and Bacillus and are interested in utilising agro-industrial wastes such as tea waste, wheat bran for multiplying these biocontrol agents using low cost technologies. Similar approaches have been successfully developed in the region for ShB control. For example, scientists at TNAU and the University of Madras, Tamil Nadu, India have developed talc-based formulations of bacterial antagonists for ShB control. SMEs have been actively involved in farmer uptake of these types of biocontrol agents for rice ShB control in Tamil Nadu. Similarly, ICAR institutes have developed bacterial and fungal biocontrol agents for ShB in other parts of India. These experiences point the way forward and that closer linkages with regional NARS in India as well as IRRI, and local SMEs would be beneficial to further develop the biocontrol agents in Bangladesh. GoB has identified SMEs as a priority sector and as the driving force for industrialisation and a national taskforce and well as a special cell have been set up with strong support at the highest policy and political levels. Further, Dr. Hamid Miah, IRRI Liaison Scientist suggested that future efforts could focus around development of technology visibility and impact at particular sites and that funds linked to IRRI programmes might be available for this work. Closer linkages with BARI and other BARC-Institutes would also help in developing disease control packages targeting rice production systems rather than a commodity and disease-specific approach.

3. Demonstrations trials for the promotion of improved and ShB tolerant varieties were conducted in all three districts. Varieties BR10, BR23, BRRIdhan32, BRRIdhan34 and BRRIdhan38 were grown as per farmers' practices during T. Aman season, along with their own varieties (e.g. Swarna) in close interaction with the farmers (Appendix 7). At crop maturity, field days were organised to enable farmers and their community to view the comparative performance of the varieties and differences in ShB severity along with popular varieties and also to obtain the farmer preferences and views. Farmer led varietal preference exercise identified BRRIdhan32 as the most preferred followed by BRRIdhan38 and BRRIdhan34. BRRIdhan32 was preferred mainly due to early maturity, tolerance to diseases particularly ShB, low fertilizer requirement and good yield. BRRIdhan34 was preferred due to the aroma and fine grain in addition to other attributes. Farmers are keen to grow the aromatic and fine grain varieties as they can get support for production and procurement from BMDC, in view of the export potential of these rice varieties. During the final stakeholder workshop held at BARRI in Dec, Mr. A.K.M. Enamul Haque, Director, DAE, Dr. Baqui, BARRI-DR and Dr. Mustafi, Head, BARRI-Agricultural Economics Division were supportive of the idea of the project team to develop additional linkages and closer ties with NGOs and DAE to establish community based seed multiplication and distribution systems. Dr. Noel Magor, Head of IRRI-Dhaka office, who was out of station, later confirmed through e. mail communications with the Project Leader Dr. Prasad that "seed is a major issue in farmers demand and in the new FoSHoL project farmers have good seed is important".

The varietal promotion trials and field days also provided an opportunity for close interaction among farming community, extension staff and R & D scientists and to increase farmers' awareness of the ShB disease and its management (Appendices 8 and 9). Men and women farmers, local government representatives, *Upazilla* Agricultural Officers, DAE staff including senior members (e.g. Mr. M.A. Sattar Mollah, Deputy Director, DAE, Gazipur and BRRRI scientists including senior staff (e.g. Dr. M.A. Baqui, Director-Research and Dr. M.A. Nahar, Chief Scientific officer, Pathology) participated in the farmers' field days emphasizing the importance given to the interaction with and promotion of technologies to farmers. The resource personnel including BRRRI and DAE staff explained to the farmers ShB symptoms, conditions and factors (e.g. close planting, susceptible varieties and inappropriate use of fertilizers such as top dressing with urea) that favour the disease and management practices. Application of split-K, particularly in intensive rice production systems leads to improved crop management and contributes to ShB management. New BRRRI varieties BR44 and BR45 are tolerant ShB and other diseases such as BLB. Farmers were also given the opportunity to examine the crop and identify the disease (which they describe as *kholpocha*). DAE staff emphasised to the farmers the importance of planting improved varieties in the place of *Swarna* and BR11, as these have become susceptible to various diseases including ShB. Farmers were also particularly requested to disseminate the information in their community with each farmer aiming to spread the knowledge among ten neighbours. A leaflet to help with the identification of rice sheath blight symptoms and a folder explaining sheath blight management were prepared in local language *Bangla* (Appendices 10 and 11) and disseminated to 300 farmers at three locations to facilitate further promotion of the knowledge and technologies.

Contribution of Outputs to Developmental Impact

In Asia, population growth rate is estimated to be between 1.3 – 2.4% through 1990-2030 and an estimated 800 million to one billion rough rice will be needed annually by 2030. ShB is recognized as a high priority constraint to rice production, particularly in Bangladesh. The project, which was initiated as a direct collaboration with BRRRI on ShB, has led to the establishment of close links with various R & D organisations and extension agencies such as BARI, BINA, BSMRAU, BAU, Univ. of Rajshahi, DAE, BARD and RDA through on-ground activities as well as involvement in stakeholder and training workshops as well as demonstration trials and farmer field days. Direct interaction with farmers and farming community was developed in Comilla, Rajshahi, Bogra and Gazipur districts with the knowledge dissemination activities involving more than 300 farmers. At BRRRI, knowledge- and skill-base in ShB management was established including the promotion and use of molecular technologies for the pathogen diagnosis and disease detection. These molecular technologies were also promoted to scientists from other R & D organisations such as BARI, BINA, BSMRAU, BAU and other divisions within BRRRI through the involvement in stakeholder and training workshops. Linkages with parallel DFID-CPP funded projects and programmes were established and the molecular facility established at BRRRI through the DFID-PETTRA programme was utilized for the promotion of and training in molecular technologies for ShB identification. In addition, knowledge and tools developed in the DFID-CPP funded blast work in West Africa were also promoted to BRRRI scientists providing a cross-cutting perspective to programme outputs. Performance of improved and ShB tolerant varieties and ShB management practices were demonstrated and promoted to farmers and farming communities in Comilla, Rajshahi and Gazipur districts. Considerable influence of agro-ecological parameters on biological disease control technologies developed has been observed and the way forward in further improving and up-scaling of these technologies has been identified including closer interaction with in-country R & D agencies such as BARI and closer interaction with NARS in India and IRRI as well as local SMEs. Availability of seed of the farmer preferred varieties including those with export and income generation potential is a major issue and closer interaction with extension and enterprise organizations such as DAE, BMDC and NGOs is essential to develop a community based seed multiplication and distribution systems. These outputs, linkages and the critical mass provide opportunities for developing future R & D in integrated disease management in rice production systems in Bangladesh contributing to

increased food production, poverty reduction and the achievement of the Millennium Development Goals. Three areas mentioned below would provide excellent opportunities for further development of the outputs and to achieve wider impact: community based seed multiplication and distribution systems for promoting farmer and market preferred varieties through additional linkages and closer ties with NGOs and DAE, DFID-funded molecular facility, molecular tools established and the staff trained in these projects to be used as a national service and training centre and further develop the biocontrol agents in Bangladesh through closer linkages with regional NARS in India as well as IRRI, and local SMEs.

Publications/Dissemination list

MUTHMEENAKSHI, S., SREENIVASAPRASAD, S., SHARMA, N.R., ALI, A. AND AKTER, S. 2004. *Rhizoctonia* species populations associated with rice sheath diseases. BMS meeting, fungi in the Environment, Nottingham, UK, 13-14 Sep 2004.

SREENIVASAPRASAD, S. 2004. Fungal molecular diversity, development and interactions. Invited lecture, School of Biotechnology, 9th August 2004, Madurai Kamaraj University, India.

Workshop on validation and promotion of rice sheath blight management. 10 presentations on outputs by the project staff and a presentation by the project leader on 'Recent trends in the management of sheath blight and other fungal diseases of rice', BRRI, 11 Dec 2005

Molecular training workshop on rice sheath blight disease detection and pathogen diagnosis. 26-29 Dec 2005.

Rice Sheath blight complex (*Rhizoctonia* spp.): pathogen epidemiology and management. Article in Perspectives on Pests II, pp. 159-161, DFID-CPP, 2006 (in press).

Appendices