SHORT COMMUNICATION

Oryza Vol. 47. No.3, 2010 (257-259)

Characterization of seedling blight on rice variety Sarala

S.R. Dhua, Urmila Dhua* and Apurba Chhotaray

Central Rice Research Institute, Cuttack-753006, India

ABSTRACT

Seedling blight was observed in nursery beds of rice variety Sarala at Central Rice Research Institute, Cuttack during wet season of 2009. The causal organism for this disease was Sclerotium rolfsii. Seedlings were affected in irregular patches. Stem base and roots of affected seedlings turned brown. The growth of infected seedlings was retarded followed by yellowing of leaves and seedling mortality. White mycelial growth and white small round sclerotia were produced on roots as well as on stem bases. The sclerotia later turned brown. Sequence alignment was done for identifying the microbes.

Key words: *rice, Sarala, seedling blight, Sclerotium rolfsi*

Sarala is a long duration rice variety released and notified for commercial cultivation in 2002, for water logged situation of Orissa. Seedling blight of this rice variety was observed in nursery beds of Breeder's seed production plots at Central Rice Research Institute (CRRI), Cuttack, during wet season of 2009. Thompson (1928) found seedling blight in Malysia and since then it was reported from many tropical countries. This pathogen may cause pre-emergence seed rot and post emergence seedling blight. This fungus can penetrate intact/uninjured host tissue. It has many strains (Ou, 1985). Hence, present investigation was carried out to isolate the causal organism and to study its pathogenic capabilities.

Seed bed sowing of the rice variety Sarala was done in raised bed dry seeded condition in May, 2009 at CRRI, Cuttack. Di-ammonium phosphate was applied @ 5kg 100 m^{-2} at the time of bed preparation. Twelve days old seedlings developed irregular patches of yellow plants, which had retarded growth. Symptoms in nursery were recorded until planting. The causal organism was isolated on potato dextrose agar media by "host tissue transplant" method (Booth, 1971). Hot water treated (Burgess *et al,* 1998), surface sterilized seeds of rice variety Sarala were transferred to the petri-plates containing the isolated pathogen. After two days, the germinating seeds were carefully shifted to the petri-plates containing sterilized blotters which were moistened with Hogland and Snyder solution (Yoshida *et al*, 1976). Internal Transcribed Spacer (ITS) region of Ribosomal DNA was amplified and sequenced (White *et al,* 1990) to identify this pathogen. Following ITS primers (Operon), ITS-1 (TCCGTAGGTGAACCTGCGG), ITS-4 (TCCTCCGCTTATTGATATGC) were used for PCR amplification. Sequencing was outsourced to Chromous Biotech. Pvt. Ltd., Kolkata. Sequence alignment was done for identifying the microbes. The sequences were analyzed with the algorithms afforded by blast algorithms for nucleotide or polypeptide homologous sequence analysis in NCBI (Zhang *et al* 2000), BLAST. Phylogenetic analyses were conducted in MEGA4 (Tamura *et al* 2007). The MP tree was obtained using the Close-Neighbor-Interchange algorithm (Nei & Kumar, 2000) with search level 3 in which the initial trees were obtained with the random addition of sequences (10 replicates). All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option).

Seedlings were affected in irregular patches. Stem base and roots of affected seedlings turned brown. The growth of infected seedlings was retarded followed by yellowing of leaves and then seedling

mortality. White mycelia growth and white small round sclerotia were produced on roots as well as on stem bases. The sclerotia later on turned brown. White fungal mycelium and brown sclerotia on the diseased stem base are the diagnostic signs of seedling blight caused by *Sclerotium rolfsii* (Burgess *et al.* 2008). A mature sclerotium of *S. rolfsii* contains several types of differentiated cells (Chet *et al.*, 1969). The cross section of the sclerotia revealed thick-walled rind cells comprising the sclerotial envelope, underlying cortex cells with thinner walls and the inner layer medulla which was composed of cells with extremely thick walls. It

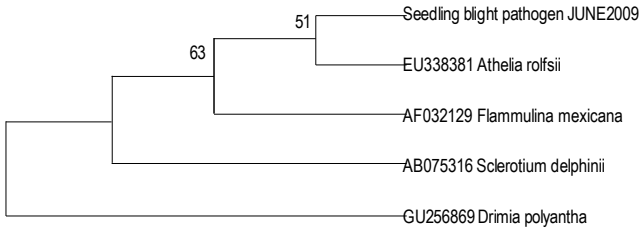

Fig 1. Evolutionary relationships of 5 taxa by Maximum Parsimony method

Table 1. Database sequences searched by Mega BLAST showing pair-wise similarities with fungal isolates causing seedling blight in rice cv Sarala

Database sequences with max. score showing similarity		Maximum Identity	Maximum score	Query coverage	E-value	Reference
Accession	Description					
EU338381	*Athelia rolfsii* (anamorph: *Sclerotium rolfsii*)	88%	769	52%	0	Ling *et.al*., 2007
AB075316	*Sclerotium delphinii*	85%	647	52%	0	Okabe, *et. al.,* 2003
AF032129	*Flammulina mexicana*	92%	56.5	3%	3e-04	Hughes *et. al*., 1997
GU256869	*Drimia polyantha*	81%	462	47%	2e-126	Desai and Kawalkar, 2009

seems that the resistance of sclerotia to biological degradation depends upon the melanin-rich rind as well as the wall structure and organization of cells comprising the inner layers of the sclerotium.

Koch's postulates (1884) were proved. The fungus was found in abundance in all seedlings suffering from the disease, but was not found in healthy seedlings. It was isolated from diseased seedling and grown in pure culture. It caused pre-emergence seed rot when healthy hot water treated, uninjured seeds of rice var. Sarala were exposed to the cultures of *S. rolfsii*. It also caused post emergence seedling blight. This micro-organism was re-isolated from the inoculated, diseased seedlings and identified as being identical to the original specific causative agent. Taxonomy report by Basic Local Alignment Search Tool (BLAST) of NCBI indicated that the pathogen isolated during this investigation belonged to : Fungi; Dikarya; Basidiomycota; Agaricomycotina; Agaricomycetes; Agaricomycetidae; Atheliales; Atheliaceae. It had similarity with *Athelia rolfsii* (anamorph: *Sclerotium rolfsii*), *Sclerotium delphinii Flammulina Mexicana* and *Drimia polyantha* (Table1)

The evolutionary history was inferred using the Maximum Parsimony method (Eck and Dayhoff, 1966). The consistency index was 0.909091, the retention index was 0.800000 and the composite index was 0.765217 (0.727273) for all sites and parsimony-informative sites are given in parentheses. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). There were 10 positions in the final dataset, out of which 5 were parsimony informative. It was clear from this boot strap test (Fig.1) that isolated pathogen had maximum similarity with *Athelia rolfsii* (anamorph: *Sclerotium rolfsii*). This is a virulent strain of *Sclerotium rolfsii*. Though seedling blight of rice is considered as a minor disease, presence of this virulent strain in long duration rice cultivar is a cause of concern.

REFERENCES:

Booth, C. 1971. Introduction to general methods. In: Methods in Microbiology, Volume 4, page 3-5, Edited by C. Booth, Academic Press, London and New York.

Burgess Lester W., Timothy E. Knight, Len Tesoriero, Hien Thuy Phan. 2008. Diagnostic manual for plant diseases in Vietnam. published by ACIAR, page: 112

Chet I., Y. Henis and Namoi Kislev 1969. Ultrastructure of Sclerotia and Hyphae of *Sclerotium rolfsii* Sacc. Journal of General Microbiology 57, 143-147.

Desai, N.S. and Kawalkar, H.M 2009. DNA barcoding studies in Indian Urginea species. Unpublished. Department of Biotechnology and Bioinformatics, Padmashree Dr. D. Y. Patil University, Mumbai, India

Eck RV and Dayhoff MO 1966. *Atlas of Protein Sequence and Structure*. National Biomedical Research Foundation, Silver Springs, Maryland.

Felsenstein J 1985**.** Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

Hughes, K., Methven, A., Petersen, R.H. and McGhee, L. Direct Submission (1997) Botany, University of Tennessee, Knoxville TN 37996-1100, USA

Koch Robert (1884). "2 Die Aetiologie der Tuberkulose". *Mitt Kaiser Gesundh*. pp. 1–88.

Ling, K.S., Kousik, C.S. and Keinath, A.P. 2007. First report of Southern blight on bottle gourd (Lagenaria siceraria) caused by Sclerotium rolfsii in South Carolina. Submitted U.S. Vegetable Laboratory, USDA-ARS, 2700 Savannah Highway, Charleston, SC 29414, USA

Nei M & Kumar S 2000. *Molecular Evolution and Phylogenetics*. pg. 128 Oxford University Press, New York.

Ou S.H. 1985. Rice Diseases. CAB publication, U.K. Page 302-304

Okabe, I. and Matsumoto, N. 2003. Phylogenetic relationship of Sclerotium rolfsii (teleomorph Athelia rolfsii) and S delphinii based on ITS sequences. Journal Mycol. Res. 107 (PT 2), 164-168

Tamura K, Dudley J, Nei M & Kumar S 2007 MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24:1596-1599.

Thompson, A.1928. Notes on Sclerotium rolfsii Sacc.in Malaya. Malaysian Agricultural Journal 16, 45-58.

Winter, W. Baenziger, I., Krebs, H., Rueegger, A., Frei, P., Gindrat, D.1998. Alternatives to the chemical dressing against bunts and Helminthosporium diseases of cereal seeds. *Agrarforschung 5(01), 29-32.*

Yoshida Y., D.A. Forno, J.H. Cock, K.A. Gomez. 1976. Laboratory manual for physiological studies of rice. IRRI, Phillippines, page 62-64