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Morphological and Genetical Variability among *Rhizoctonia solani* Isolates Causing Sheath Blight Disease of Rice

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Abstract: Eighteen isolates of *Rhizoctonia solani* collected from infected rice plants in four different locations of Bangladesh were studied by using morphological characters and molecular markers. Anastomosis study with a reference isolate confirmed that all the isolates belonged to *R. solani.* Significant variation was observed in sclerotial size, shape and distribution. Un-weighted pair group method with arithmetic mean dendrogram constructed based on the Gower's general similarity coefficient showed that these isolates were grouped into four clusters at the 0.68 similarity coefficent according to morphological characters. Cluster I was a major cluster consisting of 13 isolates, while clusters II to IV consisted of 1 or 2 isolates. Analyses by variable number of tandem repeat and amplified fragment length polymorphism markers showed that the isolates were grouped into five and three clusters at a similarity coefficient of 0.64 and 0.69, respectively. Although most of the variability was found between isolates from different regions as expected, significant variation was observed within the isolates collected from similar agro-ecological regions. Our results suggest the presence of different races of *R. solani* within the same local geographic regions.

Key words: rice; *Rhizoctonia solani*; fungal variability; molecular marker; morphological character

Sheath blight caused by *Rhizoctonia solani* is one of the most common and destructive diseases of rice in all the rice growing countries, including Bangladesh (Muthumeenakshi and Sreenivasaprasad, 2002). Especially in northern part of Bangladesh, most of the cultivated high yielding rainfed lowland rice varieties are severely affected by this disease. It is a great threat to successful rice cultivation during rainy season in Bangladesh. Outbreak of this disease is a recurrent problem, and it is extremely difficult to control the disease even using costly and environmental hazards synthetic chemicals. In addition, reduction of chemical application is also desire for environmental protection in heavily farmed country such as Bangladesh (Mian et al, 2003). Resistance to sheath blight in rice variety is therefore the most eco-friendly and economic approach for managing it. However, varieties released as resistant have often shown high levels of susceptibility within a few years even shortly after the release, due to the continuous generation of new pathogenic races (Bonman, 1992; Mekwatanakarn et al, 1999; Zhou et al, 2007). The outbreak is explained in the relationships between virulent gene in blast fungus and resistant gene in rice varieties, based on the gene-for-gene theory (Flor, 1971; Silue et al, 1992). To understand the mechanism of outbreak of resistance and built up the durable protection system in rice varieties, knowledge on population structure and diversity of the pathogen

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are important. *R. solani* is a polyphagous fungus that develops different types of symptoms on leaf sheath and also on leaf blade. Its appearance on growing media, virulence in field and physiology are also different within the same population (Muthumeenakshi and Sreenivasaprasad, 2002; Mian et al, 2003). This pathogen overwinters usually as mycelium or sclerotia in soil or in/on plant parts (Sivalingan et al, 2006).

Variability in *R. solani* has also been reported by many investigators, and many attempts have been made to organize its isolate into groups on the basis of morphological, physiological and pathological characteristics (Sherwood, 1969; Parmeter and Whitney, 1970; Ali, 2002; Sharma et al, 2005; Banerjee and Whitney, 2012). Although the classification based on morphological and physiological characters is proved useful and still accepted as a standard, it is laborious and time-consuming (Basu and Gupta, 1992; Khodayari et al, 2009). To understand the genetic relatedness among *R. solani* species, DNA-based analysis has been used in recent year. Molecular techniques, such as restriction fragment length polymorphism, amplified fragment length polymorphism (AFLP) (Toda et al, 1998; Sharma et al, 2005) and also random amplified polymorphic DNA, have been applied extensively for genotype diversity analysis in *R. solani* isolates. Molecular tools are being increasingly used to characterize fungal pathogen, evaluate level of genetic diversity among the isolates and identify particular races of the pathogen. Variable number of tandem repeat (VNTR) includes micro and mini-satellites and hyper variable regions. Micro-satellites are arrays of randomly repeated DNA sequences which are dispersed throughout the genomes (Jeffreys et al, 1985a) and are also referred to as sequence tagged micro-satellite sites. Micro-satellite comprises a class of VNTR loci, in which the repeated sequences are short (< 65 bp) and frequently GC rich (Jeffreys et al, 1985b; Nakamura et al, 1987). Simplified techniques such as VNTR-PCR of the rDNA and internal transcribed spacer region analysis have been utilized for rapid detection of variation in different fungi (Matsumoto et al, 1996). AFLP, a technique for DNA fingerprinting, is used for detection of genetic variation in fungi (Bruns et al, 1991; Majer et al, 1996). To date, AFLP DNA fingerprinting has been applied to identity mating type-correlated molecular markers and the demonstration of heterokaryosis in *R. solani* AG-1(1c) (Julian et al, 1999). Therefore, different levels of genetic diversity of *R. solani* can be best addressed by the use of molecular techniques (Toda et al, 1998). By DNA fingerprinting, it is possible to study pathogen diversity, epidemiology of endemic pathogens and marker-assisted selection in plant breeding. Variability in molecular characters is being utilized for determining resistant cultivar.

Though some studies on morphological and molecular differentiation of *R. solani* have already done in the world, substantial information is not available on morphological characterization along with molecular markers (VNTR and AFLP) of the *R. solani* in Bangladesh. Thus, the present study was undertaken to assess the morphological and molecular variability using molecular markers to distinguish the isolates collected from different agro-ecological regions of Bangladesh.

MATERIALS AND METHODS

Collection and isolation of *R. solani* isolates

Eighteen *R. solani* isolates were collected from heavily infected rice field of Rajshahi (Tonor and Godagari), Gazipur and Comilla districts of Bangladesh during rainy season. Infected leaf sheath or leaf blade or both were collected from rice fields and fresh samples were transferred immediately after collection to the laboratory for isolation. The pathogens were isolated and purified following the standard protocol (hyphal tip culture method) using water agar and potato dextrose agar media (Ali, 2002). The basic information of collected isolates is listed in Table 1.

Pathogenicity test

Pathogenicity test of the pathogen was assessed through artificial inoculation on rice cultivar Purbachi in the fields in T. Aus season (April–August). Artificial inoculation was done at the maximum rice tillering stage using mycelial block of 5-day-old culture. All the collected isolates were confirmed based on the disease symptoms development at 5 d after inoculation.

Anastomosis reaction with reference isolate

Collected 18 isolates of *R. solani* have been assigned

Table 1. List of *R. solani* isolates with geographic locations.

Code	Location	Agro-ecological zone
GA1, GA2, GA3, GA4, GA5	Gazipur	Madhupur tract
GO1, GO2, GO3, GO4, GO5	Godagari	High ganger river flood plain
TA1, TA2, TA3, TA4, TA5	Tanor	High ganger river flood plain
CO1, CO2, CO3	Comilla	Middle Meghna river flood plan