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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

to anastomosis groups by pairing the isolates with 'tester' strains and observing the hyphal fusion. A precondition for the easy and successful observation of fusion between two isolates is a relatively thin agar culture with consequent shallow depth of microscopic field of view. The method described by Banniza and Rutherford (2001) was adopted. Rectangular pieces of tap water agar (TWA) (2 cm × 1 cm) were transferred to a sterile Petri dish and placed at opposite sides. Inside the safety cabinet, microscope slides were dipped into ethanol and placed on-top of the two pieces of agar which thus acted as 'piers'. Two thin plugs of TWA (5-mm dia) were then arranged on the slide at a distance of 2 cm apart. Sclerotia of the tester strain and the isolate to be tested were placed on each of the agar plugs.

The sclerotia were held by the TWA plugs in place and limited amount of nutrients were supplied, which promoted growth. About 5 mL sterilized distilled water was added to each Petri dish which was scaled, and incubated at 25 °C. As soon as hyphae from the tester strain and the unknown made contact, slides were removed from the Petri dish, stained with lacto-phenol cotton blue (0.01%) and examined under the light microscope. Anastomosis was observed at 400× and fusion was confirmed at 1000× magnification. Tester isolates IMI303152 was collected from Plant Pathology Laboratory, Bangladesh Rice Research Institute, Gazipur.

Morphological characterization

Mycelial block of 6 mm diameter from margin of 2-day-old colonies were placed aseptically to potato dextrose agar plates and incubated at 27 °C for 12 d. Nineteen characters described in Table 2 were used for morphological characterization.

DNA extraction for molecular studies

Actively growing mycelial plugs were included into 250 mL conical flask containing 50 mL potato dextrose broth and placed on electric shaker at 120 r/min at 25 °C for 3 d following the procedure of Sambrook and Russel (2001). The squeezed and blotted, flattened mycelium of *R. solani* was folded into an aluminum foil paper and frozen at -20 °C. High quality genomic DNA was extracted from 18 isolates of *R. solani* following the methods of Raeder and Broda (1985). Isolated DNA was quantified on 1% agarose gel. Final DNA concentration for setting up PCR was about 20–30 ng/μL.

Characterization of *R. solani* through VNTR analysis

For standardization of $MgCl_2$ concentration, primer and *Taq* polymerase enzyme, a master-mix of 20 μL reactions was prepared with the PCR components. Two concentrations of each of $MgCl_2$, primer and *Taq* polymerase enzyme were used for a series of experiments and selected concentrations were used for the rest of the study. Three primers MR, RY and GF were selected and used in the VNTR analysis. The details of the primers are listed in Table 3.

The DNA was amplified on the basis of PCR. A master-mix of 20 μL reaction was prepared for PCR. Then 19 μL of this master-mix was aliquot into 0.2 mL PCR tube and 1 μL genomic DNA (5 ng/μL) of selected isolates was added into it. One drop of mineral oil was also added to each PCR tube to prevent evaporation. The PCR machine was then run with 103 °C lid temperature following VNTR-PCR protocol as below: 94 °C for 2 min; then 35 cycles of 94 °C for 20 s, 45 °C for 45 s, 72 °C for 2 min; 72 °C for 5 min and hold at 4 °C. The stained gel was rinsed with water for distaining, illuminated on UV trans-

Table 2. Morphological characteristics description of *R. solani* isolates and their attributes.

Character	Character state
Mycelial color on potato dextrose agar, color of sclerotia	0, hyaline; 1, cream or faint brown; 2, light brown; 3, medium brown; 4, dark brown
Superficial sclerotia (SS) dispersed on whole colony, exudate droplets on sclerotium surface	0, present; 1, absent
Colony reverse: pigment	0, not present; 1, cream or faint brown; 2, light brown
Sclerotia on lid	0, absent; 1, present
Topography of sclerotia	0, immersed; 1, superficial
Shape of sclerotia	0, flattened bottom and round top; 1, superficial; 2, irregularly globose with pitted surface; 3, irregular
Arial mycelial quality	0, absent; 1, all hyphae close to surface of agar; 2, air space in dish half filled; 3, almost all airspace filled
Quantity of sclerotia, pseudo sclerotia, SS discrete, SS aggregated, SS scattered, SS near inoculum, SS near margin, dark brown runner hyphae in aerial mycelium and/or on colony surface, growth on lid	0, absent; 1, few; 2, moderate; 3, abundant

Table 3. List of primers and their sequences.

Primer	Sequence
Variable number of tandem repeat (VNTR)	
MR	GAGGGTGGCGGTTCT
RY	CAGCAGCAGCAGCAG
GF	TCCTCCTCCTCCTCC
Amplified fragment length polymorphisms (AFLP)	
AFLP-C	GACTAGGATACATGCAGGC
AFLP-D	GACTACGTACATGKACKGKAC

illuminator and photographed by gel documentation unit for measuring the bands of amplified DNA fragments.

Characterization of *R. solani* through AFLP analysis

The AFLP method by agarose gel consisted of four stages as follows:

1) Restriction digestion with *Pst* I and ligation to an adapter: A master-mix of 20 μL reaction was prepared. Then 16 μL master-mix for each isolate was transferred into 0.2 mL PCR tube, and 4 μL the target genomic DNA (25 ng/μL, 100 ng) was added into the master-mix separately to make the volume of 20 μL, incubated at 37 °C for digestion and ligation for 6 h.

2) Precipitation of digested and ligated genomic DNA: The digested and ligated genomic DNA was diluted by adding 80 μL sterilized distilled water. Then 250 μL pure ethanol and 125 μL of 3 mol/L sodium acetate were added and centrifuged for precipitation of digested and ligated genomic DNA. The DNA pellet was washed with 200 μL of 70% ethanol, centrifuged and re-suspended in 25 μL of Tris-EDTA.

3) Pre-amplification with adapter A: A master-mix of 20 μL reaction was prepared with the PCR components. The 17.5 μL master-mix for each isolate was transferred to 0.2 mL PCR tube and 25 μL digested and ligated DNA (4 ng/μL) were added into the master-mix. One drop of mineral oil was also added into each PCR tube. The PCR machine was then run with a lid temperature 103 °C under the APLP program as follows: 94 °C for 2.5 min; then 35 cycles of 94 °C for 20 s, 55 °C for 45 s, 72 °C for 2 min; 72 °C for 5 min and hold at 4 °C.

4) Selective amplification: Two primers AFLP-C and AFLP-D were used, and their sequences are listed in Table 3.

Data analysis

Morphological data were recorded according to the character stage of the character and analyzed by Multi Variety Statistical Package 3.1 version using Gower's general similarity. Bands of DNA fingerprints were counted and recorded according to the position of bands for each of the 18 isolates. The banding patterns were scored visually for the presence '1' and absence '0' of band. A similarity matrix based on Gower's similarity coefficient was used and cluster analysis of the matrix was done using un-weighted pair group method with arithmetic mean (UPGMA) by numerical taxonomy and multivariate analysis system version 2.11a (Rohlf, 2000).

RESULTS

Anastomosis reaction

Among the 18 isolates of *R. solani*, anastomosis was occurred between all the isolates and the reference isolate AG1 IA (IMI303152). Again, anastomosis also occurred between all the same isolates. Light microscopic studies of anastomosis reactions revealed that the hyphal tips of the reference and test isolates were attracted (as indicated by the change of growth direction) before contact. After contact, 1–3 cells of the tips of both reference and test isolates were granulated and shrunk, which was considered to be a killing reaction (imperfect fusion). Self-anastomosis was noted abundantly in all isolates. Although hyphal tips came into contact in a few isolates, no sign of cell wall fusion was observed. Based on hyphal fusion, all isolates have been grouped into AG1 and based on their morphological character, and the isolates were again grouped into IA subgroup (Parmeter et al, 1969; Sneh et al, 1991). Artificial inoculation on rice cultivar Purbachi showed that all isolates induced typical symptoms of ellipsoid dark brown spot on rice plants. Compound microscope studies revealed all the 18 isolates having hyphal branching at right angle, constriction at the point of branching of the mycelium and presence of a septum near the branching junction conformed as *R. solani.*

Morphological characterization

After 12 d incubation on potato dextrose agar, isolates of *R. solani* showed great diversity in their growth (Fig. 1). Sclerotia tended to be brown to dark brown in most of the cases. All isolates produced superficial sclerotia, some of which produced dark brown runner hypae, exuded droplet on sclerotial surface. In this study, quality, size, shape and distribution of sclerotia within the colonies showed highly variable. Such as some of those isolates produced sclerotia near the inoculum (GA3), peripheral region (CO2), scattered

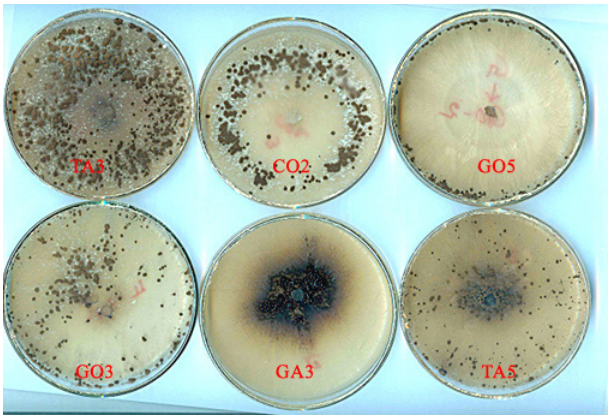

Fig. 1. Morphological variation of *R. solani* showing sclerotial distribution.

isolate (GO3), near margin (GO5), near inoculum scattered (TA5) and also abundant sclerotia (TA3) (Fig. 1). Some isolates produced a moderate quantity of aerial mycelium on the colony surface as well as on the lid and produced at least some sclerotia on the lid. Distribution, size, shape of the sclerotia indicated that variation was present within the isolates. Combined morphological data set of 19 characters with UPGMA resulted four clusters with 0.66 similarity coefficient using the Multi Variety Statistical Package (Fig. 2). Most of the isolates were constellated in cluster I. The isolate GA3 collected from Gazipur was grouped in cluster II, the isolates TA5 and CO2 collected from Tanor and Comilla were in cluster III. The isolates GO1 and GO2 collected from Godagari were in cluster IV. Out of them, the isolates GA1 and GA2 which were collected from Gazipur showed 100% similarities, which indicated that those were the same isolates. Eighteen isolates of *Rhizoctonia solani* showed the variation in their sclerotial characters, such as the pattern of production, size and distribution in the culture. Sclerotial colour was found dark brown in some isolates like GA4, GA5, TA4, TA5 and CO3. All isolates produced superficial sclerotia on potato dextrose agar plates. Flattened bottom and round to oval sclerotia were found in most of the isolates except GA3, GO1, TA1 and TA2. In the present study, the isolates were varied in sclerotial attributes. Mycelium was usually very light brown or whitish, occasionally moderately aerial, white patches scattered over the surface. The number of sclerotia was few to abundant with usually about 1.5 mm diameter on agar surface.

Molecular characterization

Three primers (MR, RY and GF) were used to detect the polymorphism of collected isolates. All of these primers were capable of amplifying multiple polymorphic DNA fragment (Figs. 3–5). The sizes of the amplified DNA products varied from 0.3 to 3.0 kb. The UPGMA dendogram based on the similarity values calculated from the total data set is shown in Fig. 6. All of the isolates gave very similar banding patterns and were clustered at a similarity coefficient above 0.60 except the isolates CO2, TA1 and GO5, which were collected from Comilla, Tanor and Godagari, respectively. Out of those isolates, the isolates GA1 and GA2 collected from Gazipur were identical. At 0.60 similarity coefficient, the isolates were placed under five clusters. Cluster I comprised with 10 isolates, which were the isolates GA1, GA2,

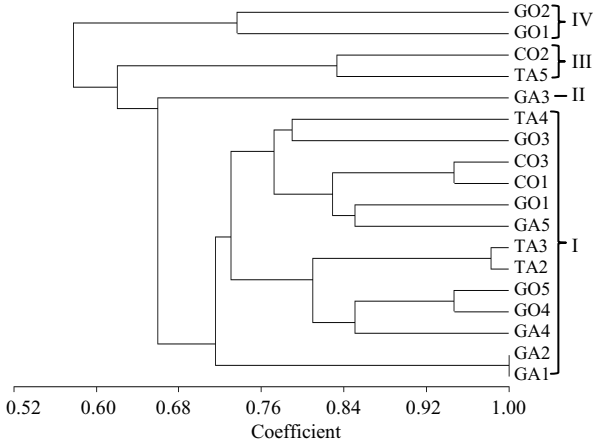

Fig. 2. Un-weighted pair group method with arithmetic mean dendrogram of *R. solani* isolates constructed with Multi Variaty Statistical Package ver. 3.1 using the Gower's general similarity coefficient based on morphological characters.

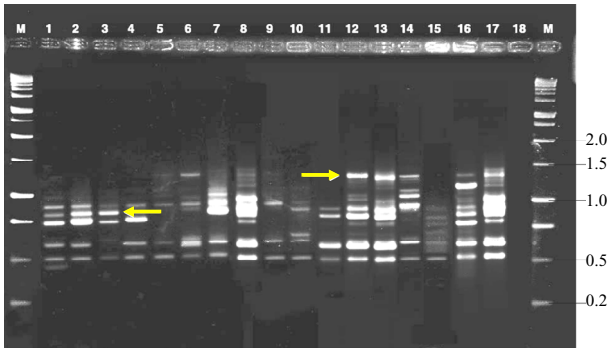

Fig. 3. Variable number of tandem repeat fingerprinting patterns of total genomic DNA of *R. solani* isolates with primer MR.

M, Kilo base (kb) ladder; Lanes 1 to 18, Isolates GA1, GA2, GA3, GA4, GA5, GO1, GO2, GO3, GO4, GO5, TA1, TA2, TA3, TA4, TA5, CO1, CO2 and CO3, respectively.

Arrow indicates polymorphic bands.

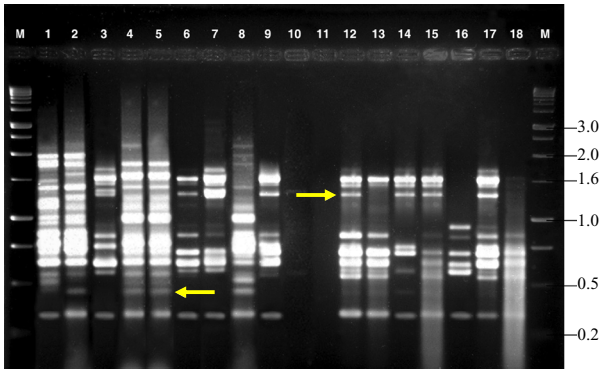

Fig. 4. Variable number of tandem repeat fingerprinting patterns of total genomic DNA of *R. solani* isolates with primer RY.

M, Kilo base (kb) ladder; Lanes 1 to 18, Isolates GA1, GA2, GA3, GA4, GA5, GO1, GO2, GO3, GO4, GO5, TA1, TA2, TA3, TA4, TA5, CO1, CO2 and CO3, respectively.

Arrow indicates polymorphic bands.

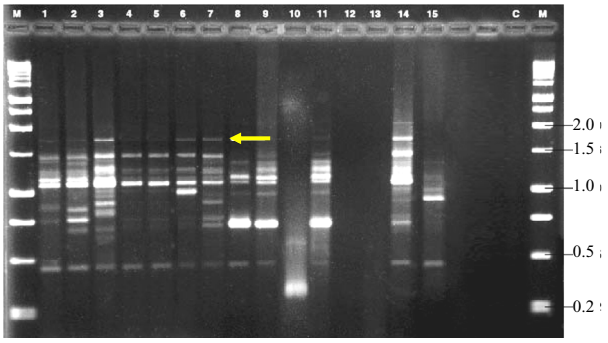

Fig. 5. Variable number of tandem repeat fingerprinting patterns of total genomic DNA of *R. solani* isolates with primer GF.

M, Kilo base (kb) ladder; Lanes 1 to 18, Isolates GA1, GA2, GA3, GA4, GA5, GO1, GO2, GO3, GO4, GO5, TA1, TA2, TA3, TA4, TA5, CO1, CO2 and CO3, respectively.

Arrow indicates polymorphic bands.

GA3, GA4 and GA5 from Gazipur, and the isolates GO1, GO2, GO3 and GO4 from Godagari and TA4 from Tanor of Rajshahi district. Cluster II represented 5 isolates, and TA2, TA3 and TA5 were from Tanor, while CO1 and CO3 from Comilla. But the isolates CO2, TA1 and GO5 collected from Comilla, Tanor and Godagari, respectively, were different from the others and formed individual cluster.

In VNTR-PCR analysis, the primer MR performed better than primers GF and RY. Clear polymorphic difference was observed among the isolates. Isolates showed the morphological variation may be controlled genetically. Five different groups were distinguished among the 18 isolates at 0.60 similarity level in the VNTR analysis. Among the five groups, group I was the largest group which contained the isolates collected from Gazipur and Rajshahi regions, represented 55% of the tested population. Among the

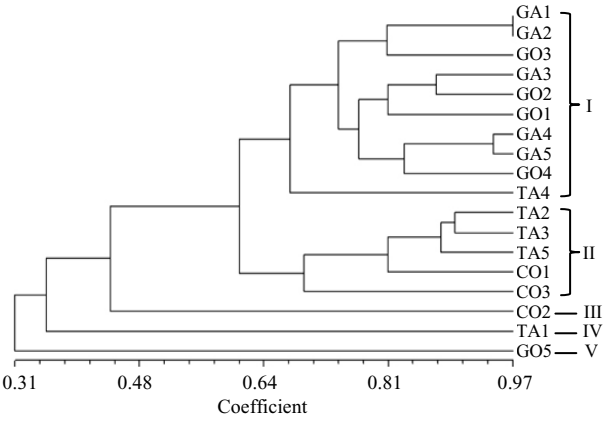

Fig. 6. Un-weighted pair group method with arithmetic mean dendrogram of *R. solani* isolates constructed with Multi Variety Statistical Package ver 3.1 using the Gower's general similarity coefficient based on DNA fingerprinting (variable number of tandem repeat analysis of primers MR, RY, and GF).

isolates, the isolates GA1 and GA2 were identical both morphologically and genetically. The isolates GA4 and GA5 collected from Gazipur were most similar at about 0.94 similarity coefficient. The isolates from Gazipur and Godagari except GO5 were similar above 0.81 similarity coefficient. The isolates GA3, GO2, TA2, TA3 and TA5 were similar at about 0.85–0.90 similarity coefficient.

In AFLP, two markers were used for diversity analysis. The resulting dendogram showed that the isolates were divided into four main clusters at 0.69 similarity coefficient (Figs. 7–9). Cluster I consisted of nine isolates, of which the isolates GA1, GA2, GA3, GA4 and GA5 were collected from Gazipur and the isolate GO1 from Godagari, the isolates CO1, CO2 and CO3 from Comilla. Cluster II comprised with four isolates, the isolates GO2, GO3 and GO4 from Godagari, and TA4 from Tanor. Cluster III obtained four isolates, and the isolate GO5 from Godagari, the isolates TA1, TA2 and TA3 from Tanor. And cluster IV consisted of one isolate, the isolate TA5 alone from Tanor and was distantly related.

DISCUSSION

Variation between isolates from different geographical regions has previously been studied for *R. solani* AG-1 by many researchers (Parmeter et al, 1969; Mekwatanakarn et al, 1999; Sivalingan et al, 2006; Zhou et al, 2007). It is widely recognized that *R. solani* consists of many races, forms or groups of various isolates differing in pathogenicity, morphology in culture and/or physiology (Bonman,

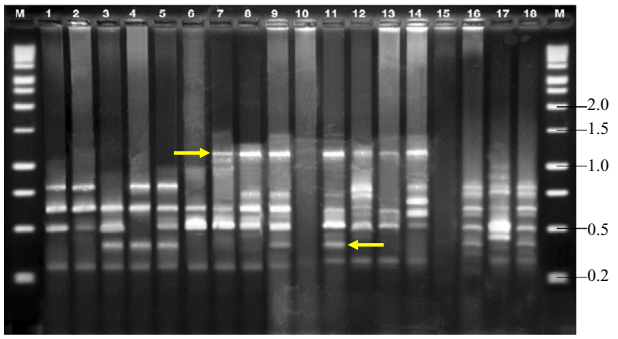

Fig. 7. Amplified fragment length polymorphisms fingerprinting patterns of total genomic DNA of *R. solani* isolates with primer AFLP-C.

M, Kilo base (kb) ladder; Lanes 1 to 18, Isolates GA1, GA2, GA3, GA4, GA5, GO1, GO2, GO3, GO4, GO5, TA1, TA2, TA3, TA4, TA5, CO1, CO2 and CO3, respectively.

Arrow indicates polymorphic bands.

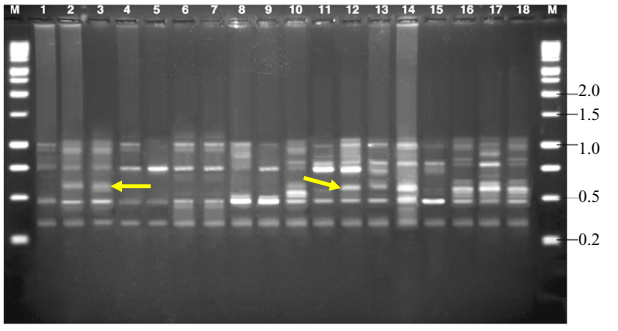

Fig. 8. Amplified fragment length polymorphisms fingerprinting patterns of total genomic DNA of *R. solani* isolates with primer AFLP-D.

M, Kilo base (kb) ladder; Lanes 1 to 18, Isolates GA1, GA2, GA3, GA4, GA5, GO1, GO2, GO3, GO4, GO5, TA1, TA2, TA3, TA4, TA5, CO1, CO2 and CO3, respectively.

Arrow indicates polymorphic bands.

1992). Morphological characters sclerotial distribution and approaches were varied within the isolates has been reported. Similar observation was notated by other investigators (Mekwatanakarn et al, 1999; Pascual et al, 2000; Zhou et al, 2007; Rashad et al, 2012).

The result of the present study reflects that *R. solani* isolates showed morphological variation among the isolates. All the 18 isolates of *R. solani* were genetically differentiated and classified into four main clusters for AFLP and five main clusters for VNTR markers, respectively with few exceptions. The isolates GA1 and GA2 collected from Gazipur were similar in respect to morphological and genetical characteristics and formed the same clusters. The isolates TA2 and TA3 from Tanor and the isolates CO1 and CO3 from Comilla were found identical in the AFLP analysis. The range of different amplification products obtained from the five primers showed variation among different

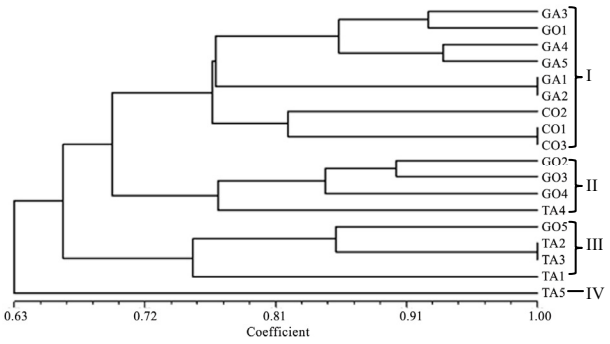

Fig. 9. Un-weighted pair group method with arithmetic mean dendrogram of relatedness of *R. solani* AG1 IA isolates constructed with Multi Variaty Statistical Package ver 3.1 using the Gower's general similarity coefficient based on amplified fragment length polymorphism analysis of primers AFLP-C and AFLP-D.

isolates of *R. solani* collected from different agro-ecological regions and also among the isolates collected from the same agro-ecological region (Sharma et al, 1995). The result of molecular analysis showed that there was no relationship between isolate and geographic location. The isolates collected from a particular location, e.g. Gazipur, were not grouped into the same cluster and the same trend was observed for all other isolates collected from different locations (Singh et al, 1999).

In recent years, the incidence of sheath blight in rice growing regions in Bangladesh has increased through the use of cropping system characterized by high planting density, abundant applications of nitrogenous fertilizers and intensive mono-cropping. Such farming practices, together with pathogen's saprophytic nature and wide host range, and the planting of high yielding but susceptible varieties have led to the dissemination, establishment and persistence of the fungus in all rice producing areas (Singh et al, 2002). An efficient, safe and economic strategy for the farmer is to use resistant or less susceptible varieties. The problem from the view of genetic improvement is that, so far, complete resistance to sheath blight has not yet been detected. Only differences among the cultivars with respect to reaction against the pathogen *R. solani* considered as moderate resistance have been obtained. In the present investigation, both morphological and genetical variations were observed among the isolates collected even from the same field. Therefore, care must be taken in selecting isolates for screening resistant germplasm and effective fungicide.

CONCLUSIONS

The genetic diversity in rice sheath blight fungus *R.*

solani using VNTR and AFLP markers in Bangladesh collected from the same and different agro-ecological regions was studied. Together with morphological characters, these molecular markers revealed diversity not only between isolates from geographically different regions, but also within ones from the same or similar agro-ecological regions. These results suggested the presence of different races within the same geographic regions. Our finding may be helpful for the phylogenetic classification of this complex species and may provide knowledge about the spread of *R. solani* races in Bangladesh. The information may also provide a new insight into the nature of variation in this pathogen. For better understanding of the pathogen population of this important pathogen and the existence of its races, further study is needed with more number of isolates covering all the agro-ecological regions of Bangladesh.

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