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VARIATION IN CULTURAL CHARACTERISTICS, MYCELIAL COMPATIBILITY AND PATHOGENIC POTENTIALITY AMONG THE ISOLATES OF *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY THE CAUSAL AGENTS OF STEM ROT OF MUSTARD (*BRASSICA* SPP.)

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Abstract

Variability among 15 isolates of *Sclerotinia sclerotiorum* (Lib.) de Bary isolated from stem rot of mustard (*Brassica* spp.) collected from five mustard growing areas of Bangladesh was studied. The isolates varied in colony morphology, mycelial growth rate and number of sclerotia formation. Variation of mycelial compatibility among the isolates was also observed and recorded. Different isolates of same district showed compatible reactions and the sclerotia produced in a regular circle of culture plate. But different isolates of different districts showed incompatible reaction line, even sclerotia formation and showed difference in zone circle. The pathogenic aggressiveness of different isolates were also studied and found isolates of Joypurhat (Joy1, Joy2 and Joy3) were virulent, produced symptom (27.69 - 29.69 mm) in extent on leaves of a susceptible variety *Brassica rapa* (BARI shorisha-14) within 72 hrs of inoculation. The isolates obtained from different districts showed variability in their pathogenic potentiality and produced symptom within 3 - 4 days of inoculation. Isolates of *S. sclerotiorum* obtained from samples of Habiganj district (H1, H2 and H3) were less pathogenic compared to other isolates of the fungus and produced symptom after 7 days of inoculation which was very small (10.67 - 12.33 mm).

Introduction

Sclerotinia stem rot is one of the major diseases of mustard. *Sclerotinia sclerotiorum* (Lib.) de Bary is the causal agent of the disease. The fungus has a wide host range and worldwide distribution on numerous crops (Purdy 1979, Boland and Hall 1987). The initial infection occurs in the late winter and the fungal mycelia grow within and between plants. The fungus produces many black fleshy sub-circulars to elongated vegetative structure called sclerotia, which survive from one cropping season to the next (Adam and Ayers 1979). Mycelial compatibility is the ability of two strains of filamentous fungi to anastomose colony and from one continuous colony, is synonymous with vegetative compatibility. A sharp distinction must be maintained between two isolates and heterokaryon compatibility unless it is known that two strains not only anastomose but also from a stable heterokaryon. As an easy test for self-recognition, vegetative compatibility has been extremely useful in intraspecific strain comparisons (Saupe 2000). Compatible pairing formed one confluent colony. Incompatible pairing produced a visible reaction in the interaction zone, such as a red, green or black line visible on the colony reverse, or a line of fluffy, aerial mycelium or thin mycelium on the colony surface (Glass *et al*. 2000). Pathogenic diversity and variation in isolate aggressiveness may influence the success of screening hosts for resistance to *S. sclerotiorum* in controlled environments, and for long-term disease management. Therefore, it

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is important to understand the diversity of this pathogen when developing screening strategies, mainly when results from researchers are shared between widely diverse geographic regions. The objectives of this research were to identify mycelia compatibility, evaluate variability in *S. sclerotiorum* populations from different locations to determine virulence of isolates associated with mustard plant.

Materials and Methods

A total of 15 *Sclerotinia sclerotiorum* isolates were obtained from the infected mustard samples collected from five different mustard growing districts of Bangladesh during the growing season of 2014 - 2015. The districts were Joypurhat, Kustia, Bogra, Jamalpur and Habiganj. Plant samples were collected from four corners and centre of each plot. Selected samples were separately placed in polyethylene bag and properly labeled and brought to the laboratory.

Single sclerotia of the fungus from infected host tissue was picked up with sterilized scalpel and dipped in 10% chlorox for 3 - 5 minutes and then rinsed three times with sterilized distilled water, using dry out blotter for 3 minutes, and inoculated on PDA medium. The plates were incubated at 25^{o}C in darkness for 3 days. A 5 mm mycelial disc was taken from the edge of the actively growing colonies transferred to the Petri plates containing PDA to obtain pure culture of the pathogen. Each isolate was purified by transferring onto fresh medium, and generated sclerotia were stored at –20^{o}C until used (Atallah *et al*. 2004). Colony diameter was measured after 2 days of inoculation, number of sclerotia and size of sclerotia of each isolates were recorded on 10 days of inoculation.

Mycelium compatibility grouping was performed as described by Schafer and Kohn (2006) and Minghe *et al*. (2010). Isolates were paired on modified potato dextrose agar (PDA) amended with 175 µl per litre of red food coloring and 50 mg/l of bromophenol blue. A 5 mm diameter mycelial block of 3 days old PDA culture from each of three isolates from same district and two isolates of different districts was placed on the same Petri dish and incubate at 25^{o}C for 7 days. After incubation the reaction between each isolate pair was evaluated. If two isolates could grow together without any obvious line between them, they were considered compatible with each other. Otherwise, if a reaction line of either hyphal tufts or red or green barrage zone of sparse growth was observed between paired isolates, they were considered incompatible (Kohn *et al*. 1995).

For pathogenicity test seeds of *Brassica napus* cv. BARI sarisa-14 were sown in 12 cm diameter pots and placed in greenhouse maintaining 20 ± 2^{o}C temperature and 16 hrs day length. The same size true and second leaves were used to assess pathogenicity of the isolates. In a 9 cm Petri dish, leaves were put on three layers of moisten filter paper. Three days old of 5 mm fungal PDA disc was inoculated on the center of leaf and 3 replications were made for each isolate. The diameter of necrotic lesions was measured at 48 hrs after inoculation.

Results and Discussion

Differences in all the morphological characters of *Sclerotinia sclerotiorum* were observed (Table 1). Based on radial growth, the isolates were classified into three groups; very fast growing, intermediate and slow growing. Data were recorded on 3 days of incubation revealed that the isolates Joy1, Joy2, Joy3, K1, K2, K3, H1, H2, H3 represented significantly fast growing, isolates B1, B2, B3 were intermediate, and J1, J2, J3showed slow radial colony growth. After 5 days of incubation all isolates were fully covered the Petri plate. Variability among the isolates of *S. sclerotiorum* has already been reported by Carpenter *et al*. (1999). On the basis of number of sclerotia produced by the isolates of *S. sclerotorum* K1, K2 and K3 ranked as highest producer of

sclerotia, Joy1, Joy2, Joy3, B1, B2, B3,H1, H2, H3 were intermediate and J1, J2, J3 showed least number of sclerotial formation after 10 days of inoculation.

Table 1. Variations in colony morphology of different isolates of *Sclerotinia sclerotiorum* on PDA medium after 2 days of inoculation.

Source of isolates	Isolate name	Colony color	Colony diameter (mm) on 48 hrs of inoculation	Growth rate	No. of sclerotia	Sclerotia size (mm)
Bogra	B1	White	52	Intermediate	24	2 - 10
"	B2	"	50	"	25	2 - 13
"	B3	"	56	"	27	2 - 10
Hobiganj	H1	"	60	Fast growing	20	2 - 4
"	H2	"	62	"	25	2 - 4
"	H3	"	59	"	20	2 - 4
Jamalpur	J1	"	30	Slow growing	10	2 - 8
"	J2	"	35	"	12	2 - 8
"	J3	"	30	"	13	2 - 7
Kustia	K1	Bright white	65	Fast growing	34	2 - 7
"	K2	"	62	"	38	2 - 9
"	K3	"	61	"	37	2 - 7
Joypurhat	Joy1	Dark white	66	"	25	2 - 8
"	Joy2	"	64	"	28	2 - 8
"	Joy3	"	64	"	32	2 - 9

As for size of sclerotia is concerned, isolates B1, B2, B3 produced large size sclerotia with length ranged from 2 - 13 mm. Isolates Joy1, Joy2, Joy3, K1, K2, K3 and J1, J2, J3 were intermediate while H1, H2, H3 showed least size of sclerotia (2 - 4 mm). On the basis of cultural and morphological characteristics, 15 isolates were grouped into five clusters. Each clusters consisted of three isolates and observed that the members of same cluster were compatible in most of the cases, whereas it was not true for the isolates with different background on the basis of cultural and morphological characteristics (Table 2). Punja and Damiani (1996), Zarani and Christensin (1997) reported differences in growth rates among different isolates obtained from various host species. Based on sclerotial diameter several workers recorded variation in size of sclerotia among different isolates of the fungus (Dhingra and Sinclair 1973, Mirza *et al*. 1985).

The combinations with antagonistic reactions with each other formed a thin band of living mycelia (Figs 1 and 2). Different isolates of same district showed compatible reactions and the sclerotia produce in a regular circle of culture plate. But different isolates of different district showed incompatible reaction line, even sclerotium formation and showed difference in zone (Table 2 and Fig. 2). Barari *et al*. (2012) identified three levels of incompatibility were distinguished. Level 1 incompatibility (not completely compatible): when a sharp distinct thin band of mycelia was observed in the interaction zone, level 2 incompatibility (not completely compatible) reaction line was visible as abundant tufts, white patches of aerial mycelia in the

reaction zone on the colony surface and level 3 incompatibility (100% incompatible): when a red reaction line observed between the interfering paired isolates.

Table 2. Mycelial compatibility of 15 isolates of *Sclerotinia sclerotiorum.*

Isolates No.	Compatible/non compatible
B1×B2×B3	Compatible
H1×H2×H3	"
J1×J2×J3	"
K1× K2×K3	"
Joy1×Joy2×Joy3	"
B1, B2, B3×H1, H2, H3	Non compatible
B1, B2, B3×J1, J2, J3	"
B1, B2, B3×K1, K2, K3	"
B1, B2, B3× Joy1, Joy2, Joy3	"
H1, H2, H3×B1, B2, B3	"
H1, H2, H3×J1, J2, J3	"
H1, H2, H3×K1, K2, K3	"
H1, H2, H3×Joy1, Joy2, Joy3	"
J1, J2, J3×B1, B2, B3	"
J1, J2, J3×H1, H2, H3	"
J1, J2, J3×K1, K2, K3	"
J1, J2, J3×Joy1, Joy2, Joy3	"
K1, K2, K3×B1, B2, B3	"
K1, K2, K3×H1, H2, H3	"
K1, K2, K3×J1, J2, J3	"
K1, K2, K3×Joy1, Joy2, Joy3	"
Joy1, Joy2, Joy3×B1, B2, B3	"
Joy1, Joy2, Joy3×H1, H2, H3	"
Joy1, Joy2, Joy3×J1, J2, J3	"
Joy1, Joy2, Joy3×K1, K2, K3	"

The pathogenic variability of the isolates of *S. sclerotiorum* was estimated using detached leaf assay produced symptom on leaf at 24 hrs of inoculation. Isolate virulence within the five locations showed that there were differences among virulence of isolates (Table 3 and Fig. 3). Isolate virulence within the five locations showed that there were differences among virulence of isolates. The isolates obtained from different district showed variability in their pathogenic potentiality and produced symptom within 3 days of inoculation. The pathogenic aggressiveness of different isolates also studied and found that isolates of Joypurhat (Joy1, Joy2 and Joy3) were extremely high virulent and produced symptom 29.67, 27.67 and 29.33 mm in extent on leaves of

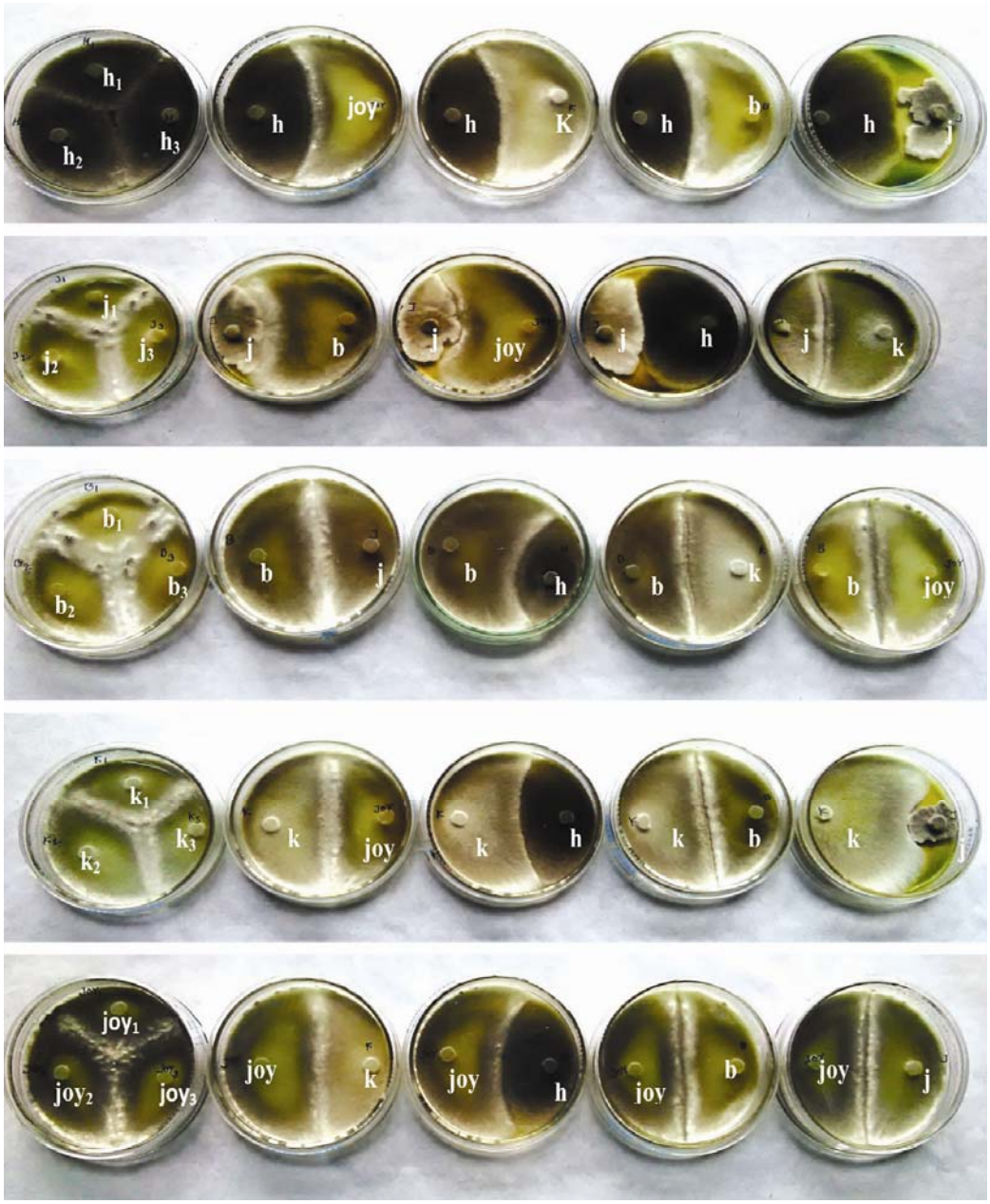

Fig. 1. Interaction between different isolates of *S. sclerotiorum* collected from different districts on PDA medium amended with bromophenol blue. (Name of isolate: b = Bogra, j = Jamalpur, h = Hobiganj, k = Kustia and joy = Joupurhat).

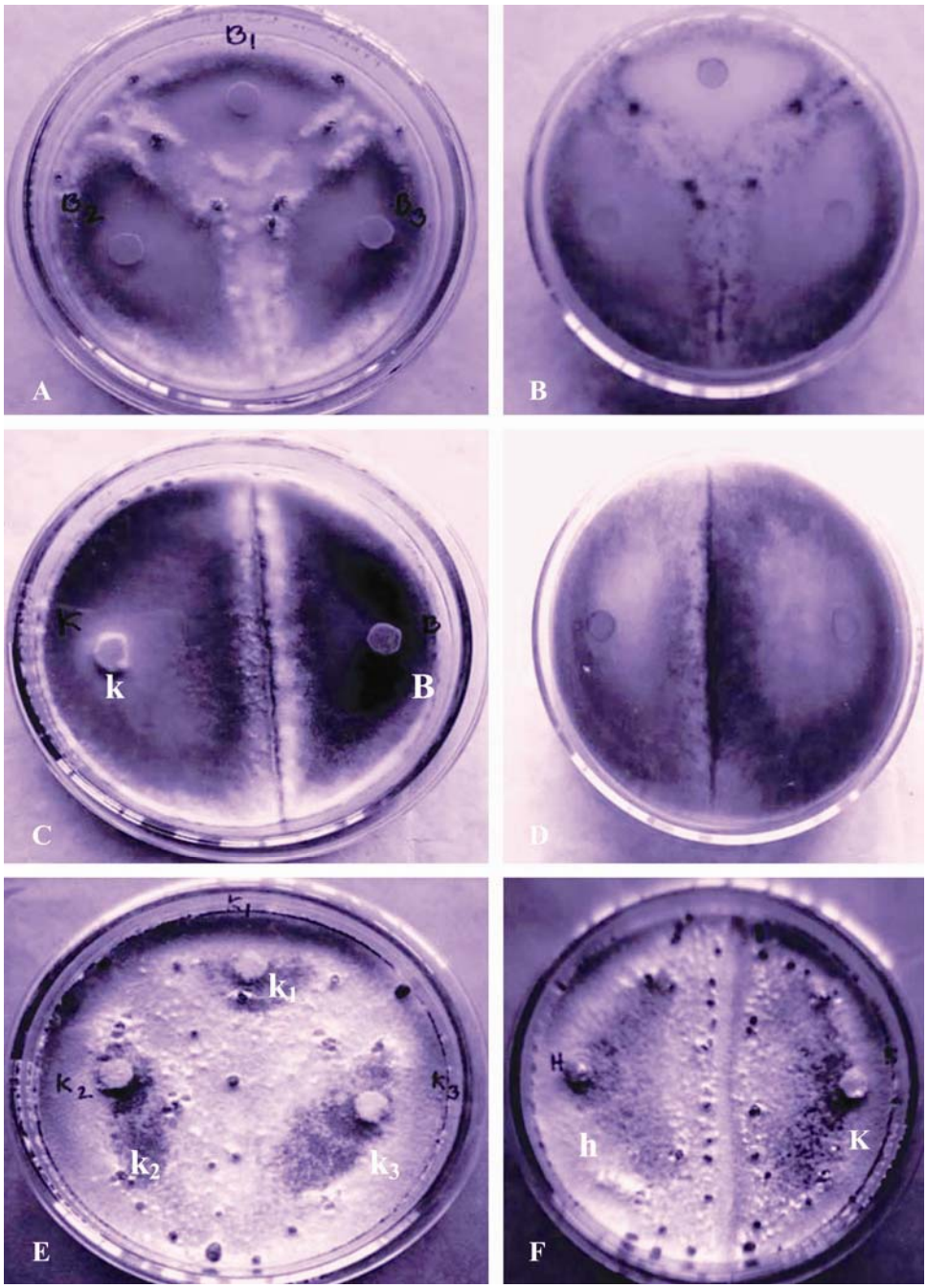

Fig. 2. (A - B). Three isolates of *S. sclerotiorum* of same district did not produce reaction zone; (C - D). Two isolates of different district produce reaction zone; E. Three isolates of *S. sclerotiorum* of same district produce sclerotia in scattered and F. Two isolate of *S. sclerotiorum* in different districts produce sclerotiaon different zone.

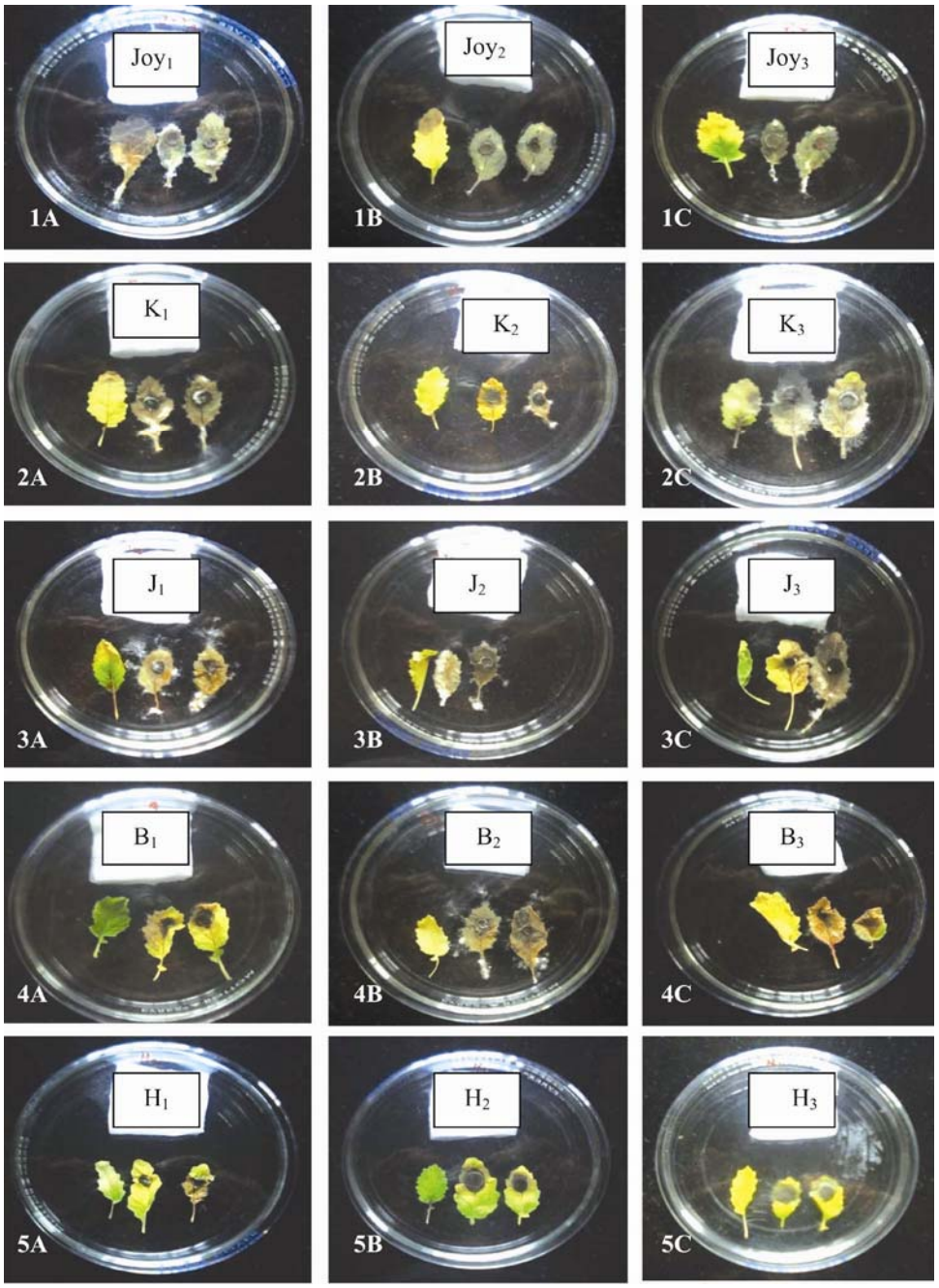

Fig. 3. Photographs showing necrotic lesion 3 days of inoculation; 1(A-C): Three isolates from Joypurhat; 2(A-C): Three isolates from Kustia; 3(A-C): Three isolates from Jamalpur; 4(A-C): Three isolates from Bogra and 5(A-C): Three isolates from Hobiganj.

a susceptible variety *Brassica rapa* (Bari shorisha-14) after three days of inoculation. Isolates of *S. sclerotiorum* obtained from samples of Hobiganj (H1, H2 and H3) was less pathogenic compared to other isolates of the fungus and produced very small size lesion on leaves were 7.33, 8.67 and 9.00 mm, respectively. The isolates of Jamalpur and Kustia (J1, J2, J3 and K1, K2, K3) also showed highly virulent and produced lesion 14.00, 17.33, 16.33 and 10.67, 11.33, 11.00 mm in size after 3 days of inoculation, respectively. Intermediate aggressiveness showed the isolates (B1, B2, B3) of Bogra district 9.00, 10.33 and 9.67 mm lesion size produced three days of inoculation. After seven days of inoculation isolates of Joypurhat (Joy1, Joy2 and Joy3) produced large size lesion (40.33, 36.67 and 36.67 mm) on leaves and covered with white cottony mycelium which followed by isolates of Kustia. Yusuf and Abdurrahman (2011) reported the pathogenic difference of isolates and mycelial compatibility were found related with their different locations and in all localities, statistically significant. The least aggressive isolates (D 27 and D 36) were obtained from cucumber plants in Demre and the most aggressive isolate (K6) were from cucumber plant in Kumluca.

Table 3. Lesion size and relative aggressiveness of 15 isolates of *Sclerotinia sclerotiorum* isoletes on mustard.

Isolates	District	Host	Lesion length (3 dpi)	Lesion length (7 dpi)	Aggressiveness
B1	Bogra	Bari sarisa - 14	9.00ef	29.00g	Intermediate
B2	"	"	10.33ef	30.67fg	"
B3	"	"	9.67ef	30.00fg	"
H1	Hobigonj	"	7.33f	10.67 h	Low
H2	"	"	8.67ef	12.67h	"
H3	"	"	9.00ef	12.33h	"
J1	Jamalpur	"	14.00cd	31.33fg	High
J2	"	"	17.33b	34.67cd	"
J3	"	"	16.33bc	31.67ef	"
Joy1	Joypurhat	"	29.67a	40.33a	Extremely high
Joy2	"	"	27.67a	36.67bc	"
Joy3	"	"	29.33a	36.67bc	"
K1	Kustia	"	10.67e	31.33fg	High
K2	"	"	11.33de	34.00de	"
K3	"	"	11.00de	37.33b	"
CV (%)			7.35	5.29	
LSD (0.05)			1.809	2.583	
Level of sig.			*	*	

Means with different laters (a - h) are significantly different at $p \leq 0.05$.

In brief there were high morphological diversity on *S. sclerotiorum* populations and high variations on pathogenecity of isolates and mycelial compatibility group. So characterizing of *S. sclerotiorum* population and variability in isolates aggressiveness can guide development of management strategies, reducing the loss in yield and quality of crops caused by the pathogen. A

distribution of mycelial compatibility grouping in *S. sclerotiorum* isolates from Bangladesh was for the first time reported in here.

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