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Variability and Pathogenicity in Bangladeshi Isolates of *Botrytis cinerea* Causing Botrytis Gray Mold in Chickpea (*Cicer arietinum* L.)

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Abstract: Ten isolates of *Botrytis cinerea* causing botrytis gray mold in chickpea were collected from different agro climatic regions of Bangladesh to study the variability among them. The isolates were characterized in terms of cultural, morphological and pathogenic. The isolates varied significantly in cultural and morphological traits- colony color, shape, margin and texture; production and arrangement of sclerotia on PDA medium. The length of conidia varied from 5.00 to 15.00 μm. Mean length of conidia was found maximum (12.00 μm) in isolate AHI-9 and minimum (7.50 μm) in isolate AHI-1. The breadth of conidia ranged from 5.00 to 10.00 μm. The highest mean breadth (8.25 μm) was observed in isolate AHI-9 and the lowest (6.00 μm) in isolate AHI-4. The isolates exhibited reaction ranged from highly susceptible to resistant to a set of 9 chickpea cultivars and among them AHI-9 and AHI-10 were found the most virulent isolates.

Key words: *Botrytis cinerea*, chickpea, variability, pathogenicity

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important legume crop grown globally in at least 44 countries (Bakr *et al*., 2002; Pande *et al*., 2006). Chickpea is valued for its nutritive seeds with high protein content ranged from 25.3 to 28.9% (Hulse, 1991). Chickpea cultivated on about 11.12 million ha, adding 8.62 million tones of grain to the global food basket (FAO, 2005). Chickpea is suffered from various abiotic and biotic stresses. Among them, Botrytis Gray Mold (BGM) caused by *Botrytis cinerea* Pers. Ex. Fr., is an economically detrimental disease of chickpea (Pande *et al*., 2006). The disease has threatened the chickpea crop so much that the area of the crop has come down to 16,000 ha from more than 1,000,000 ha in Bangladesh within a span of 10 years (BBS, 1999). Cool wet weather favors the disease development (Anonymous, 2006). BGM can devastate chickpea, resulting in complete yield loss in years of extensive winter rain and high humidity (Reddy *et al*., 1988; Pande *et al*., 2002). The disease is of serious concern in Bangladesh, India, Nepal, Pakistan, Australia and Argentina (Bakr *et al*., 1993; Dhar *et al*., 1993; Pande *et al*., 2002; Davidson *et al*., 2004) where 100% yield losses were reported. Although a number of various investigations were conducted on variability of others fungal pathogen but a little work have been done on the variability of *B. cinerea*. Hence, present research work was aimed to carry out detailed investigation on the variability of *B. cinerea* isolates collected from major chickpea growing regions of Bangladesh in respect to cultural, morphological and pathogenic variation and correlate them with the aggressiveness of *B. cinerea*.

MATERIALS AND METHODS

Collection of isolates: The experiment was conducted at Plant Pathology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh during June to December, 2007. *Botrytis cinerea* isolates were obtained from seven different major chickpea growing districts of Bangladesh (Table 1). Purification of *B. cinerea* was done following single spore isolation technique (Mian, 1995). The stock culture of the isolates was maintained on potato dextrose agar in test tubes slant at 4±0.5°C in a refrigerator for further use.

Table 1: List of 10 *B. cinerea* isolates with their locations

	Locations	
Isolates	District	Upazila
AHI-1	Chuadanga	Chuadanga Sadar
AHI-2	Meherpur	Meherpur Sadar
AHI-3	Pabna	Atghoria
AHI-4	Pabna	Ishurdi
AHI-5	Faridpur	Faridpur Sadar
AHI-6	Rajbari	Rajbari Sadar
AHI-7	Kushtia	Bheramara
AHI-8	Kushtia	Bheramara
AHI-9	Pabna	Ishurdi
AHI-10	Rajshahi	Godagari

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Cultural and morphological variations of *B. cinerea* isolates: Cultural characteristics were noted on Potato Dextrose Agar (PDA) medium after three days of incubation at 20°C. Cultural features of *B. cinerea* isolates were observed on the basis of colony color, shape, margin and texture. Morphological variations in terms of sclerotia size, ability to produce sclerotia and their arrangement were also observed on PDA medium. Data on sclerotia production and their arrangement were observed after 18 days of incubation at 20°C without extra light provided. Length and breadth of conidia was measured using a pre-calibrated ocular micrometer. To determine the conidial size each isolates of *B. cinerea* was measured 20 times in length and breadth wise.

Pathogenicity test of *B. cinerea*

Growing condition for raising of chickpea seedlings: Polypropylene (PP) bags (10×14 cm) were used to grow the chickpea plants in the net house. PP bags were filled with sterilized soil aiding well decomposed organic matter. Five seeds of each entry were sown in each bag having 3 replications. The net house temperature was 25±2°C without extra light and temperature controlled. Seedlings were watered whenever necessary. After 15 days of sowing, the chickpea seedlings were shifted in the laboratory for inoculation.

Preparation of inocula: In order to get huge amount of inocula of *B. cinerea*, each isolate was sub-cultured onto marigold petals aided sucrose (3 g L^{-1}) in a conical flask and incubated for at least 10 days in room temperature at 25±2°C. The flasks were flooded with sterile distilled water and were allowed to stand for 5 min. Then the flasks were thoroughly shaken well to dislodge the conidia in a solution. The conidial suspension was filtered through one layer of muslin cloth and the concentration of the conidial suspensions was estimated by a haemacytometer. Conidial suspensions were diluted to get the required concentrations ($6x10^4$ conidia mL^{-1}) by adding sterilized distilled water. From the freshly prepared inoculums, loops full of conidial suspensions were smeared out onto a PDA plate to test the viability of the conidia. After 24 h of incubation at 20°C in an incubator the plates were checked under a microscope to confirm spore germination.

Foliar inoculation of the chickpea plants: Fifteen days old seedlings of chickpea were inoculated with spore suspension ($6x10^4$ conidia mL^{-1}) with a hand sprayer. The inoculated plants were covered with polyethylene bag to maintain high relative humidity and also to prevent natural contamination with other fungal conidia/spores. The plants were kept in a room temperature at 25±2°C and humid condition (>90%) was confirmed by gently spraying sterilized distilled water.

Confirmation of disease: To confirm the symptoms shown on inoculated plants, infected plant parts were collected and causal organisms were re-isolated, following the standard isolation procedure (Nene *et al*., 1981).

Assessment of disease severity: Disease score was recorded after 5 days of inoculation on chickpea cultivars using a 9 point scoring scale as described by Singh (1999), where 1= no infection on any part of the plant; 2 = Minute lesions on lower leaves; 3= Lesions on <5% of the leaves; 4= Lesion and some fungal growth (conidiophores and conidia) can be seen on up to 15 % of the leaves; 5= Lesions and slight fungal growth on up to 25% of the leaves; 6= Lesions and fungal growth on up to 40% of the leaves; 7 = Large lesions and good fungal growth on up to 60% of the leaves; 8 = Large lesions and profuse fungal growth on up to 80% of the leaves and 9 = Large lesions, very profuse fungal growth on up to 100% of the flowers. Based on the score the cultivars was further graded as, 1 = Immune or asymptomatic (I), 2-3 = Highly Resistant (HR), 4-5 = Resistant (R), 6-7 = Susceptible (S) and 8-9 = Highly Susceptible (HS).

RESULTS

Cultural variations: All the isolates exhibited variation in their colony characteristics such as color, shape, margin and texture (Fig. 1).

Cottony white colony color was found in AHI-1, ashy white in AHI-2, AHI-3, AHI-5 and AHI-6; off white in AHI-4 and AHI-10; light ash in AHI-7, AHI-9 and greenish white in AHI-8 (Table 2).

Marked variability was observed in colony shape. Irregular colonies were observed in isolates AHI-1 and AHI-7, whereas regular with sector type of colonies were observed in two isolates AHI-5 and AHI-8. Regular without sector type of colonies were found in isolates AHI-2, AHI-3, AHI-4, AHI-6, AHI-9, and AHI-10. Irregular colony margin was observed in isolates AHI-1, AHI-2, AHI-4 and entire margin in most of the isolates AHI-3, AHI-5, AHI-6, AHI-8, AHI-9, and AHI-10. Only wavy margin was found in isolate AHI-7 (Table 2).

Distinct differences were observed in colony texture. Fluffy texture was found in isolates AHI-1, AHI-2, AHI-3, AHI-8, AHI-10 and velvet texture was found in isolates AHI-4, AHI-5, AHI-6, and AHI-9. Effuse type texture was noted in isolate AHI-7 (Table 2).

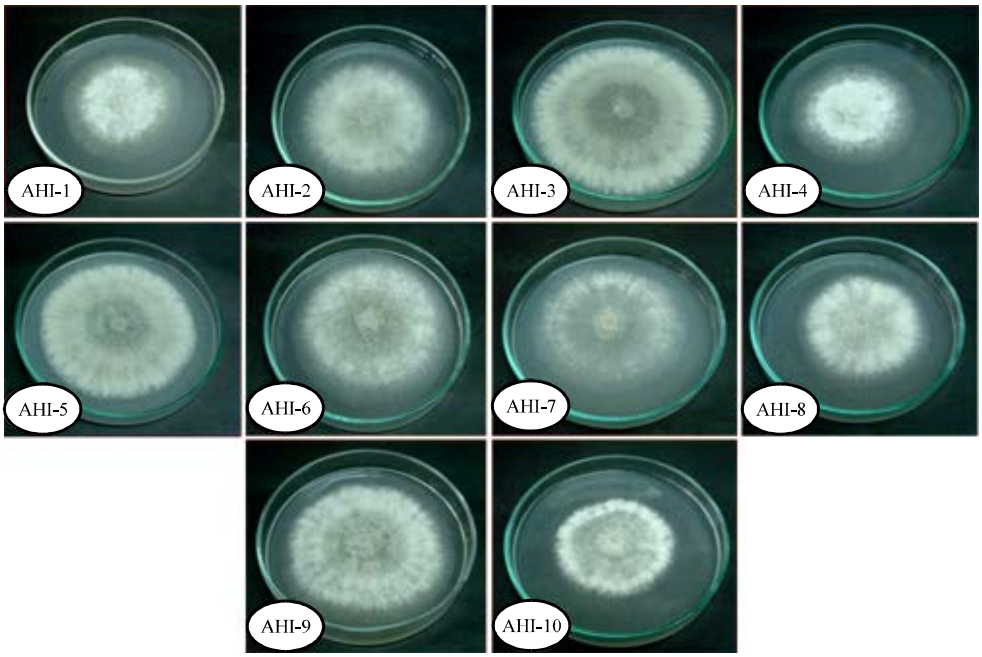

Fig. 1: Variation in cultural characteristics of 10 *B. cinerea* isolates grown on PDA at 20°C after 3 days of incubation

Table 2: Cultural variation of 10 *B. cinerea* isolates after 3 days of incubation on PDA medium

	Cultural characteristics			
Isolate	Colony color	Colony texture	Colony shape	Colony margin
AHI-1	Cottony white	Fluffy	Irregular	Irregular
AHI-2	Ashy white	Fluffy	Regular without sector	Irregular
AHI-3	Ashy white	Fluffy	Regular without sector	Entire
AHI-4	Off white	Velvet	Regular without sector	Irregular
AHI-5	Ashy white	Velvet	Regular with sector	Entire
AHI-6	Ashy white	Velvet	Regular without sector	Entire
AHI-7	Light ash	effuse	Irregular	Wavy
AHI-8	Greenish white	Fluffy	Regular with sector	Entire
AHI-9	Light ash	Velvet	Regular without sector	Entire
AHI-10	Off white	Fluffy	Regular without sector	Entire

Morphological variations: *Botrytis cinerea* isolates were categorized into four groups viz., BC-I, BC-II, BC-III and BC-IV on the basis of their sclerotia production and arrangements on the PDA medium (Fig. 2). Of 10 isolates, two belongs to group BC-I, four to BC-II, one to BC-III and three to BC-IV shown in Table 3. Almost all the sclerotia were blackish in color. The production of sclerotia were very few to high and only group BC-IV produced high amount of sclerotia and spread over the entire plates preferably in peripheral region of petriplates. Incase of group BC-I, very few sclerotia were produced and arranged in the centre of the petriplate and rest of them produced few to medium amount of sclerotia.

Conidial size: The length of conidia varied from 5.00 to 15.00 μm, whereas the highest mean length (12.00 μm) was recorded in isolate AHI-9 and minimum (7.50 μm) in isolate AHI-1. Breadth of conidia ranged from 5.00 to 10.00 μm. The highest mean breadth 8.25 μm was also observed in isolate AHI-9 and the lowest 6.00 μm in isolate AHI-4 (Table 4).

Pathogenic variation of *B. cinerea* isolates: All 10 isolates showed differential reaction on chickpea genotypes. Among the isolates only two (AH-9 and AHI-10) were found to be the most virulent and infected all the tested chickpea genotypes an equal level (Table 5).

Isolate AHI-1 induced susceptible reaction on four varieties (BARI Chola 1, 2 and 4) including a tolerant genotype ICCL-87322 and highly susceptible on 5 varieties (BARI Chola 3, 5, 6, 7 and 8) (Table 5). The lowest disease score (6) was observed in varieties BARI Chola-1, 2, and 4 and the highest disease score (9) was noted in BARI Chola-5, 7 and 8 (Table 6).

Isolate AHI-2 showed susceptible reaction on two varieties (BARI Chola 5 and 6) and highly susceptible on

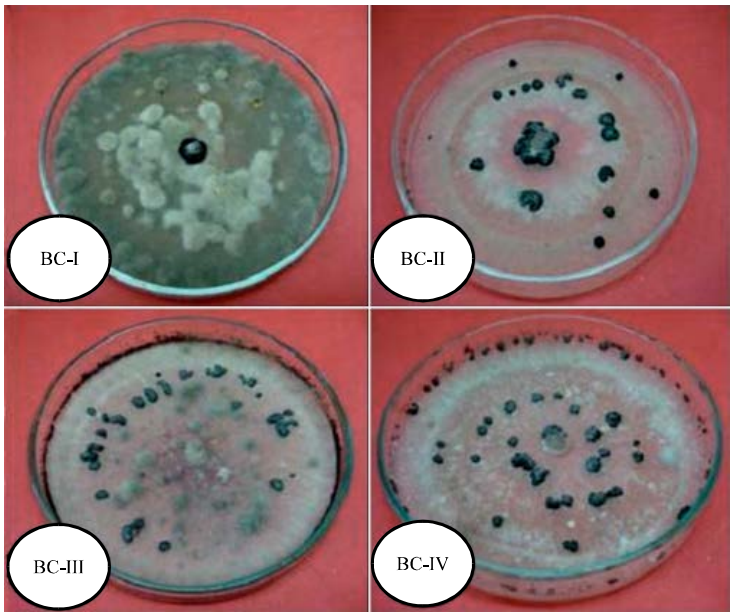

Fig. 2: Grouping of *B. cinerea* isolates on the basis of distribution and pattern of sclerotia formation on PDA medium

Table 3: Grouping of 10 *B. cinerea* isolates based on their ability to produce sclerotia

Isolates	Sclerotial characteristic features	Isolates group
AHI-1 and AHI-4	Very few, moderate size, and grouped in the centre form of clots	BC-I
AHI-2, AHI-5, AHI-7 and AHI-10	Few, moderate to large size, few centre and some scattered in entire plates in the form of clots	BC-II
AHI-6	Medium, large size and spread all over the plates	BC-III
AHI-3, AHI-8 and AHI-9	High, comparatively large size and spread all over the plate preferably peripheral region	BC-IV

Table 4: Size of conidia of 10 *B. cinerea* isolates after 15 days of incubation on PDA at 20°C

	Dimensions (µm) of conidia			
	Conidia length[1] (µm)		Conidia breadth[1] (µm)	
Isolates	Range	Mean	Range	Mean
AHI-1	6.25-10.00	7.50	5.00-7.50	7.50
AHI-2	7.50-11.25	10.00	6.25-7.50	7.25
AHI-3	7.50-12.50	10.00	6.25-7.50	6.75
AHI-4	7.50-11.25	8.00	6.25-7.50	6.00
AHI-5	5.00-13.75	8.25	5.00-7.50	6.25
AHI-6	7.50-12.50	9.50	6.25-10.00	7.75
AHI-7	8.75-11.25	9.75	7.50-10.00	7.50
AHI-8	7.50-12.50	10.50	5.00-7.50	7.00
AHI-9	7.50-15.00	12.00	5.00-10.00	8.25
AHI-10	5.00-10.00	10.25	5.00-10.00	7.50

[1]Means of 20 times observations for each isolate

Table 5: Resistant susceptibility pattern of 10 *B. cinerea* isolates on 9 chickpea cultivars under artificial inoculation condition

	Chickpea genotypes*								
Isolates	1	2	3	4	5	6	7	8	9
AHI-1	S	S	HS	S	HS	HS	HS	HS	S
AHI-2	HS	HS	HS	HS	S	S	HS	HS	HS
AHI-3	HS	HS	R	R	HS	S	S	HS	S
AHI-4	HS	HS	S	S	HS	HS	HS	HS	S
AHI-5	R	HS	R	R	R	HS	HS	HS	R
AHI-6	S	HS	S	S	HS	HS	HS	HS	HS
AHI-7	S	S	R	HS	R	HS	R	S	HS
AHI-8	R	S	R	HS	HS	S	S	S	HS
AHI-9	HS	HS	HS	HS	HS	HS	HS	HS	HS
AHI-10	HS	HS	HS	HS	HS	HS	HS	HS	HS

*1: BARI Chola-1, 2: BARI Chola-2, 3: BARI Chola-3, 4: BARI Chola-4, 5: BARI Chola-5, 6: BARI Chola-6, 7: BARI Chola-7, 8: BARI Chola-8, 9: ICCL-87322

seven varieties (BARI Chola 1, 2, 3, 4, 7, 8, and ICCL-87322) (Table 5). The maximum disease score (9) was noted in varieties BARI Chola-3 and 4 and the lowest disease score (7) was observed in BARI Chola-5 and 6 (Table 6).

Isolate AHI-3 showed susceptible reaction on three varieties (BARI Chola 6, 7 and ICCL-87322), highly susceptible reaction on four varieties (BARI Chola 1, 2, 5 and 8) and resistant reaction on two varieties (BARI Chola 3 and 4) (Table 5). The disease score reached up to the highest level of the scale (9). The highest disease score (9) was observed in BARI Chola 2, 5 and 8 and the lowest disease score (4) was obtained in BARI Chola 4 (Table 6).

Isolate AHI-4 induced susceptible reaction on three chickpea cultivars (BARI Chola 3, 4 and ICCL-87322) and highly susceptible on six cultivars (BARI Chola 1, 2, 5, 6, 7 and 8) (Table 5). The maximum disease score (9) was observed in BARI Chola-1, 2, 6, 8 and ICCL-87322 and the lowest disease score (6) was found in BARI Chola-3 (Table 6).

Table 6: Disease score (1-9 scale) of 9 chickpea genotypes against 10 different isolates of *B. cinerea*

	Disease score on a 1-9 rating scale*								
Isolates	1	2	3	4	5	6	7	8	9
AHI-1	6	6	8	6	9	8	9	9	7
AHI-2	8	8	9	9	7	7	8	8	8
AHI-3	8	9	5	4	9	7	6	9	6
AHI-4	9	9	6	7	8	9	8	9	9
AHI-5	4	9	3	4	5	9	9	9	5
AHI-6	6	9	7	7	8	9	9	9	9
AHI-7	6	7	4	9	5	8	5	6	8
AHI-8	5	6	4	3	8	7	6	9	8
AHI-9	9	9	9	9	9	9	9	9	9
AHI-10	9	9	9	9	9	9	8	9	9

*1: BARI Chola-1, 2: BARI Chola-2, 3: BARI Chola-3, 4: BARI Chola-4, 5: BARI Chola-5, 6: BARI Chola-6, 7: BARI Chola-7, 8: BARI Chola-8, 9: ICCL-87322

Isolate AHI-5 induced highly susceptible reaction on five chickpea cultivars (BARI Chola 2, 6, 7 and 8) and resistant on four cultivars (BARI Chola 3, 4, 5 and ICCL-87322) (Table 5). The maximum disease score (9) was observed in BARI Chola-2, 6, 7, and 8 and the lowest score (3) was noted in BARI Chola-3 (Table 6).

Isolate AHI-6 showed highly susceptible reaction on six chickpea cultivars (BARI Chola 2, 5, 6, 7, 8 and ICCL-87322) and susceptible on three cultivars (BARI Chola 1, 3 and 4) (Table 5). The maximum disease score (9) was observed in BARI Chola-2, 6, 7, 8 and ICCL-87322 and the lowest score (6) were noted in BARI Chola-1 (Table 6).

Isolate AHI-7 induced highly susceptible reaction on three chickpea cultivars (BARI Chola 4, 6 and ICCL-87322), susceptible reaction on three cultivars (BARI Chola 1, 2 and 8) and resistant on three cultivars (BARI Chola 3, 5 and 7) (Table 5). The maximum disease score (9) was observed in BARI Chola-4 followed by BARI Chola 6 and ICCL-87322 (8) and the lowest score (4) were noted in BARI Chola-3 (Table 6).

Isolate AHI-8 should highly susceptible reaction on three chickpea cultivars (BARI Chola 2, 6, 7 and 8), susceptible reaction on four cultivars (BARI Chola 2, 6, 7 and 8) and resistant on two cultivars (BARI Chola 1 and 3) (Table 5). The maximum disease score (9) was observed in BARI Chola 8 followed by BARI Chola 5 and ICCL-87322 (8) and the lowest disease score (3) was noted in BARI Chola 4 (Table 6).

Isolate AHI-9 and 10 both induced highly susceptible reaction on all chickpea cultivars (BARI Chola 1 to 8 and ICCL-87322) (Table 5) and disease score was 9 for all the chickpea cultivars except BARI Chola 7 in isolate AHI-10 where disease score was 7 (Table 6).

DISCUSSION

In the present research, all the ten isolates (AHI-1 to 10) showed variations in respect of their cultural, morphological and pathogenic characteristics. In respect of cultural characteristics, the isolates of *B. cinerea* showed variation in colony color, shape, margin and textures. Remarkable variations found in colony color and shape. Colony color was cottony white and greenish white in AHI-1 and AHI-8, respectively. Others colony colors were ashy white off white and light ash. Colony margin was irregular, wavy and entire. Colony textures were fluffy and velvet in most of the isolates but isolates AHI-8 possessed effuse type of texture. The current findings are well supported by Ahmed *et al.* (2007) who reported grayish and light gray colony color with regular or wavy margin and fluffy, matted or velvet texture of 4 Indian isolates.

Botrytis cinerea isolates also showed variations in terms of sclerotia formation, color and conidia production. The pathogen produced sclerotia on culture media and the sclerotia formation was very much peculiar. On the basis of sclerotia production the isolates of *B. cinerea* are classified into four groups (BC-I to BC-IV). Soheila Mirzaei *et al.* (2009) identified two morphological type of *Botrytis cinerea*, mycelia and sclerotial. Significant variation also observed in conidia size. The length and breadth of conidia ranged from 5.00 to 15.00 μm and 5.00 to 10.00 μm, respectively. Maximum mean length of conidia was observed in isolate AHI-9 (12.00 μm) and minimum in AHI-1 (7.50 μm). The highest mean breadth was noted in isolate AHI-9 (8.25 μm) and the lowest in AHI-4 (6.00 μm). The present findings agreed with the report of Joshi and Singh (1969), who measured the conidia of *B. cinerea* as 4-16×4-10 μm. Bakr *et al.* (2002) also reported that variation exists in morphological and cultural characteristics of different isolates of *B. cinerea*.

Marked variations were observed in all isolates in terms of reactions of *B. cinerea* on different chickpea cultivars. All the isolates were not equally reacted on the chickpea cultivars but the isolates showed reactions in all cultivars. All the variety (BARI-Chola 1 to 8) and a tolerant chickpea genotype (ICCL-87322) showed highly susceptible reaction to isolate AHI-9 and 10. However, isolate AHI-9 and AHI-10 can be selected as different biotypes of *B. cinerea*. The findings agreed with the findings of Rewal and Grewal (1989) and they categorized the *B. cinerea* isolates to five pathotypes on the basis of their reaction on a set of five chickpea differential varieties/lines.

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