White mold on pea caused by *Sclerotinia sclerotiorum*: a new threat for pea cultivation in Bangladesh

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Abstract:

A new disease causing the tan to light brown blighted stems and pods has occurred in 2.6% pea (*Pisum sativum* L.) plants with an average disease severity rating of 3.7 in Chapainawabganj district, Bangladesh. A fungus with white appressed mycelia and large sclerotia was consistently isolated from symptomatic tissues. The fungus formed funnel-shaped apothecia with sac-like ascus and endogenously formed ascospores. Healthy pea plants inoculated with the fungus produced typical white mold symptoms. The internal transcribed spacer sequences of the fungus were 100% similar to that recovered from an epitype of *Sclerotinia sclerotiorum*, considering the fungus to be the causative agent of white mold. Mycelial growth and sclerotial development of *S. sclerotiorum* were favored at 20°C and pH 5.0. Glucose was the best carbon sources to support hyphal growth and sclerotia formation. Bavistin and Amistar Top inhibited the radial growth of the fungus completely at the lowest concentration. In planta, foliar application of Amistar Top showed the considerable potential to control the disease at 1.0% concentration until 7 days after spraying, while Bavistin prevented infection significantly until 15 days after spraying. A large majority (70.93%) of genotypes including tested released pea cultivars were susceptible, while six genotypes (6.98%) appeared resistant to the disease. These results could be important for management strategies aiming to control the incidence of *S. Sclerotinia* and eliminate yield loss in pea.

**Keywords:** Apothecium, ascospores, sclerotium formation, carbon source, fungicides, resistance source

Introduction

Pea (*Pisum sativum* L.) is an herbaceous annual plant in the family Fabaceae or Leguminosae. It is an important and popular food crop grown in many countries all over the world, especially for its edible seeds. Globally pea is the fourth leading legume in terms of consumption with a total production of 10.2 million tons [1]. Pea is appreciated primarily for its high nutritional value with 23 to 25% protein [2].
It is rich in lysine and other vital amino acids [3]. Moreover, some important minerals such as calcium, phosphorus, and iron are present in plentiful quantities in pea, which are usually, absent in cereals [4]. Pea seeds also are the source of 4-10% sugars, 20-25% starch, 0.6-1.5% fat, and 2-4% minerals [5]. Green peas straw is used as nutritional fodder for livestock. In Bangladesh, the pea is produced on 18000 acres of land with an annual production of 7191 MT [6]. Pea cultivation continues to rise in the country and it ranks sixth in terms of area and production among the pulses. Pea is grown after the harvest of monsoon-rice in the winter season (October-March).

The establishment of healthy plants is the basis for a good yield in pea crop. Pea is a short-term, early-season crop and often susceptible to diseases. A number of bacterial, fungal, viral, and nematode diseases infect pea and reduces its health. Important pathogens infecting pea include *Rhizoctonia solani* Kühn, *Fusarium oxysporum* f. sp. *pisi*, *Meloidogyne* spp., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Erysiphe pisi*, *Botrytis cinerea* Pers.:Fr., *Uromyces fabae*, *Ascochyta pisi* Lib., *Pseudomonas syringae* pv. *pisi*, *Alfalfa mosaic virus*, *Bean leaf roll virus*, etc [7]. These diseases, under the right conditions, significantly decrease both yield and quality of pea [8]. In January 2018, a comprehensive survey for pea diseases was conducted in farmers’ fields in Chapainawabganj district of Bangladesh. A new disease was detected in some of the fields. A preliminary investigation identified the disease as white mold caused by *Sclerotinia sclerotiorum*. This was the first evidence of white mold infection in pea in the field [9].

White mold caused by *S. sclerotiorum* is an important disease with an accelerating incidence on many cultivated crops [10]. The disease has a worldwide occurrence and causes significant yield loss. In the USA, soybean yield losses from 1996 to 2009 by *S. sclerotiorum* equaled up to 10 million bushels (270 million kg) [11]. In India, *S. sclerotiorum* has resulted in a yield reduction of up to 40% in some areas [12]. In peanut, yield loss from *S. sclerotiorum* may exceed 50% [13]. In canola, yield is reported to be reduced by 0.5% (equivalent to 12.75 kg/ha) for every 1% increment in Sclerotina stem rot disease [14]. *S. sclerotiorum* has a large dispersal in many countries, infecting more than 400 plants such as beans, okra, potato, sunflower, soybean, marigold, pea, jackfruit, etc. [15-17,10]. The pathogen infects all aboveground plant parts such as stems, leaves, flowers, and pods. It produces prominent black
sclerotia within mycelium and in cavities of infected plant parts. Sclerotia is a vital part of the disease cycles. Sclerotia can germinate vegetatively and produce mycelium for infection, or they most often carpogenically form apothecia, a sexual fruiting body produced after a period of dormancy. Within the apothecia, the tubular asci develop which finally discharge ascospores into the air. The airborne ascospores initiate infection on host foliage. Temperature and pH value of substrates have a strong influence on mycelial growth, cellular morphology, metabolite biosynthesis, and sclerotial formation of the fungus [18]. Equally, nitrogen and carbon resonate mycelial growth and sclerotial formation of the fungus and are considered as initial nutrient sources for infection, [19,10]. Knowledge of the environmental conditions conducive to S. sclerotiorum inoculum formation is essential for the successful management of white mold disease.

The disease needs to be satisfactorily controlled to reduce yield losses. Attaining good control of white mold is a challenging task in all crops. Disease management requires the application of a wide range of strategies. In practice, fungicide application is the core strategy to protect crop plants from white mold [20]. Similarly, host resistance is considered the safest and most economical way of controlling the disease. Since the white mold of pea is new in Bangladesh, information about pathogen characteristics, effective fungicides, and resistance sources need to be clarified for successful management of the disease. The present study was designed to isolate and identify S. sclerotiorum causing white mold in pea, determine conditions conducive to optimum growth and sclerotia formation, decide effective fungicides for controlling the pathogen, and explore effective sources of resistance.

Materials and Methods

Disease survey in the field and sample collection

A survey was conducted for pea diseases in farmers’ fields in Chapainawabganj district, Bangladesh from 21 to 23 January 2018. A total of 15 farmers’ fields were randomly selected. Pea fields were at
the late growth stage when plants were bearing pods but had not senesced. In each field, five 1.5 m × 1.5 m quadrats located at each of four corners and the center were used for visual examination of disease. Disease incidence and severity were recorded simultaneously in each of the fields. Disease incidence was assessed as the percentage of symptomatic plants compared to total plants. Severity was calculated in the field by adopting a scale of 0–5, where 0 = no symptoms, 1 = up to 10%, 2 = 11–35%, 3 = 36–65%, 4 = 66–90%, and 5 = 91-100% of the foliage affected by the pathogen [21]. Diseased stems and pods were collected from each of the infected fields and returned to the laboratory for examination. Sclerotia were separated from infected plant parts and assessed for their morphological characteristics.

**Fungal isolation and preservation**

Three infected plants with blighted stems and pods with sclerotia and visible mycelium were arbitrarily taken for fungal isolation. Two infected plant parts (one stem section and one pod) and four sclerotia were sampled from each of the three plants. Infected plant parts were cut into 1 cm pieces, while sclerotia were divided in half. The cut pieces were sterilized in 75% ethanol for 1 min, washed three times in sterile distilled water, and dried between two layers of blotter paper. Cut off sclerotia and infected plant pieces were aseptically plated onto acidified potato dextrose agar (PDA) medium amended with 1 ml of 85% lactic acid in 9-cm-diameter Petri plates. Similarly, two fragments of mycelium were removed from the surface of infected stems and pods of each of the three plants with forceps and placed directly on PDA. All plates with infected plant parts, sclerotia, and mycelium fragments were incubated at 25±1°C in the dark for 5 days. Emerging mycelial colonies were transferred to new PDA plates and pure isolates were obtained by hyphal tip isolation. Pure isolates were cultured on PDA slants and preserved at 4°C.

**Morphological characterization of the isolate**
Each of the isolates was transferred on PDA in 9 cm diameter Petri dishes and cultured at 25°C in the dark for a week. Colony characteristics of the fungus were studied with the naked eye. Permanent slides were prepared from the colony and hyphal characteristics were examined under a light microscope at different magnification. Sclerotia produced on the medium were examined for morphological features.

Production of apothecia from sclerotia

Sclerotia were collected from each culture plate and after surface sterilization, 50 sclerotia were placed in five 9 cm-Petri dishes filled with wet sterile sand and incubated at 4°C for 6 weeks. The Petri dishes having germinating sclerotia were transferred to another incubator at 20°C under scattered fluorescent irradiation (260 μmol/m²/s) until apothecial discs were developed [22]. Fifteen apothecia and the ascospores produced from sclerotia were examined under the microscope (40× and 100×) for morphological features.

Pathogenicity test of the isolates

A pathogenicity test was conducted to satisfy Koch’s postulates, and it was repeated twice. The main cultivation varieties of BARI Motorshuti 3 were used for testing. Three pure isolates, one each obtained from infected tissues, sclerotia, and mycelial fragment, were selected for the pathogenicity test. Seeds of pea variety ‘BARI Motorshuti 3’ were surface sterilized and sown in earthen pots filled with sterile soil in the net house. In each of two trials, five plants were inoculated with each isolate and the same numbers were included as a control. When the seedlings were four-week-old (five to seven fully
expanded leaves), 5 mm-diameter plugs from actively growing margins of fungal colonies were placed on superficially wounded stem approximately 10 cm above the soil line. For five control plants, only sterile PDA plugs were used. All plugs were sealed with moistened cheesecloth. The inoculated plants were placed in a dark humid chamber for 48 h at 22±1°C. Then the inoculated seedlings were shifted to a net house and grown under observation for 20 days. Pathogenicity of the isolates was also tested on pea pods and leaves by placing a mycelial plug of the fungi onto an incised pod and leaf surface in a similar manner. The suspected pathogen was re-isolated from inoculated tissues that exhibited symptoms of white mold. It was determined whether the original and the re-isolated strains were identical.

*Molecular identification of S. sclerotiorum isolate PSP-1*

Total genomic DNA was extracted from the mycelia of *S. sclerotiorum* isolate PSP-1 as described by Toda et al. [23]. Polymerase chain reaction (PCR) was conducted with forward primer ITS-1 (5′-TCC GTA GGT GAACCT GCG G-3′) and reverse primer ITS-4 (5′-TCC TCC GCT TAT TGATAT GC-3′) [24] to amplify rDNA-ITS regions of the fungal isolates. PCR was done following the method described by Hayakawa et al. [25]. Two complete sequences were obtained for each ITS region and the BLAST search program was used to search for nucleotide sequence homology in GenBank. Highly homologous sequences were aligned using Clustal-X version 2.0.11 and manually adjusted as required. Neighbor-joining trees were generated using MEGA X version. Bootstrap replication (1000 replications) was used as statistical support for the nodes in the phylogenetic trees. Based on maximum sequence homology percentage, query coverage, and the lowest E value, ITS sequence data of four isolates of *S. sclerotiorum* were selected for phylogenetic analysis. Additionally, three isolates of each of *S. minor* and *S. trifoliorum* were included in this analysis, while *Hypocrea lixii* was used as an outgroup taxon (GenBank accession no. FJ 861393.1).
Effect of temperature on mycelial growth and sclerotia formation of S. sclerotiorum isolate PSP-1

The effect of temperature on mycelial growth of S. sclerotiorum was determined by growing the fungus on PDA at different temperature regimes 5, 10, 15, 20, 25, and 30°C in two independent trials. In each trial, PDA plates (9-cm-diameter) were prepared and inoculated at the center with a 5 mm-diameter mycelium plug excised from a 6-day old S. sclerotiorum culture. There were three replicates for each temperature regime and each replicate consisted of three Petri plates. Inoculated plates were incubated at each of 5, 10, 15, 20, 25, and 30°C in the dark. The radius of colonies was measured at 24, 48, and 72 h after incubation. Cultures were continued for 15 days for maximum sclerotial yield. The number, diameter, and weight of sclerotia were taken.

Effect of pH on mycelial growth and sclerotia formation of S. sclerotiorum isolate PSP-1

The growth and sclerotia formation of S. sclerotiorum was examined at a diverse pH range. PDA media were prepared in 9-cm-diameter PDA plates and adjusted their pH to 3.0, 5.0, 7.0, and 9.0 using 0.1 N HCl and NaOH. There were three replicates for each pH range and each replicate consisted of three Petri plates. Petri plates were inoculated at the center with a 5-mm-diameter mycelium plug excised from a 6-day old S. sclerotiorum culture. The fungal cultures were incubated at 20°C in dark. The radius of colonies was measured at 24, 48, and 72 h after incubation. Cultures were monitored for sclerotial development and then the number, diameter and weight of sclerotia were taken after 15 days. The experiment was repeated twice.
Effect of carbon sources and pH on mycelial growth and sclerotial formation of *S. sclerotiorum* isolate PSP-1

A minimal medium composed of peptone at 4.0 g/ l, carbon sources (sucrose, mannitol, glucose, fructose or soluble starch) at 20 g/l, MgSO₄ at 0.5 g/l, KH₂SO₄ at 1.0 g/l, and agar at 20 g/l was used. The pH of the medium was adjusted to 4.0, 5.0, 7.0, and 9.0 using 0.1 N HCl or NaOH before autoclaving. There were three replicates for each pH and three 9-cm-diameter Petri plates were prepared for each replicates Mycelium plugs of 5-mm-diameter were cut from the growing edge of a 6-day *S. sclerotiorum* culture and placed at the center of each plate. The cultures were then incubated at 20°C in dark. The mycelial growth of the fungus was determined by measuring colony diameters at 72 h after incubation. The same cultures were continued for 15 days to induce maximum sclerotial formation. Sclerotial number and weight were taken. The experiment was repeated twice.

*In vitro bioassay of fungicides for fungitoxicity to *S. sclerotiorum* isolate PSP-1*

Six most widely used protectant fungicides, Amistar Top (Azoxystrobin + Difenoconazole), (Syngenta Bangladesh Ltd., Dhaka, Bangladesh), Bavistin (Carbendazim 50% WP) (BASF Bangladesh Ltd., Dhaka, Bangladesh), Dithane M-45 (Mancozeb) (Bayer CropScience Ltd., Dhaka, Bangladesh), Ridomil Gold MZ (Mefenoxam + Mancozeb), (Syngenta Bangladesh Ltd., Dhaka, Bangladesh), Kumulus DF (Sulphur) (BASF Bangladesh Ltd., Dhaka, Bangladesh) and Rovral (Iprodione) (Bayer CropScience Ltd., Dhaka, Bangladesh) were tested against colony growth of *S. sclerotiorum*. Fungicides were added at a concentration of 10, 50, and 100 ppm in an autoclaved PDA medium following poisoned-food techniques [26]. The pH was adjusted to 5.0 using 0.1 N HCl. Agar disk of 5 mm diameter of fungal culture was excised from a 3-day-old culture and placed in the center of Petri plates having different fungicidal concentrations. There were three replicates of each treatment and each
replicate consisted of three plates. The plates without fungicides worked as control. The inoculated plates were incubated at 20°C. After 7 days of incubation when the fungus in control plates covered the whole plate, the diameter of the radial growth was recorded. The percent inhibition (PI) of the fungus over the control was calculated using the formula of Sundar et al. [27].

\[
\% \text{ Inhibition of growth} = \frac{X - Y}{X} \times 100
\]

Where,

\(X\) = Mycelial growth of the pathogen in absence of fungicides

\(Y\) = Mycelial growth of pathogen in presence of fungicides

In planta efficacy of fungicides against *S. sclerotiorum* isolate PSP-1

Amistar Top and Bavistin were found to be the most effective fungicides in inhibiting mycelial growth of *S. sclerotiorum* in dual culture assay. *In vivo* evaluation of their efficacy against the white mold disease was done on pea plants cv. BARI Motorshuti 3. Pea plants were raised in 9-cm earthen pots and at 28 days of age, the two fungicides were applied as a single foliar spray on three separate sets of 9 plants at a concentration of 0.1%. Plants sprayed with water only were included as controls. Three fully expanded leaves were detached from each plant in each set at 1, 7, and 15 days after spray and placed on two-layered wet paper towels in Petri Dishes. The mycelial plug of 3 mm diameter excised from the growing edge of the fresh culture (6-day old) of *S. sclerotiorum* was placed centered with fungus-side down on one side of the main vein of each leaf and slowly pressed to ascertain good contact with the leaf surface. The inoculated leaves were kept in dark at 20°C for 48 h. Inoculated leaves were transferred to a growth chamber with a light cycle of 16 h light/8 h dark at 20-22°C. Five days after pathogen challenge, disease severity was estimated by measuring the percent area diseased on the leaves [28]. Based on disease severity, the level of protection attained by each fungicide was calculated as follows:
Protection (%) = \[\frac{(A-B)}{A} \times 100\]

Where,

A = disease severity in untreated control plants

B = disease severity in treated plants

Screening of pea genotypes against *S. sclerotiorum* isolate PSP-1

Eighty-six pea genotypes were screened against the isolated fungus using a detached leaf assay as described by Prova et al. [28]. The genotypes included both pea lines and a released cultivar. IPSA Motorshuti 1, and BARI Motorshuti 1, 2, and 3 were released varieties from Bangabandhu Sheikh Mujibur Rahman Agricultural University and Bangladesh Agricultural Research Institute, respectively. Others were experimental lines collected from the Plant Genetic Resources Centre (PGRC) of Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. Surface sterilized seeds were sown in earthen pots filled with sterile soil and grown for 4 weeks in the net house. In each genotype, four fully expanded leaflets were cut at the stem from each of four plants and placed on water-soaked two-layered blotter papers in 9-cm-diameter Petri Dishes. A mycelial plug of 3 mm diameter excised from the growing edge of the fresh culture of *S. sclerotiorum* was placed centered with fungus-side down on one side of the main vein of each leaf and slowly pressed to ascertain good contact with the leaf surface. The inoculated leaves were kept in dark at 20°C for 48 h. Additional leaves were mock inoculated with autoclaved PDA plugs as controls. Inoculated leaves were transferred to a growth chamber with a light cycle of 16 h light/8 h dark at 20-22°C. Five days after pathogen challenge, disease severity was estimated by measuring the percent area diseased on the leaves [28]. Based on disease severity, each genotype was further categorized as Immune (I), resistant (R), moderately resistant (MR), moderately susceptible (MS), or susceptible (S) as shown in Table 1.
Design of Experiments and Analysis of Data

Statistix 10 (Analytical Software, FL, USA) was used for statistical analysis. The experimental design was completely randomized, consisting of at least 3 replications for each treatment. The experiment was repeated at least twice and treatments were compared via ANOVA using the least significant difference test (LSD) at 5% ($P \leq 0.05$) probability level. Data were transformed as and when necessary using the arcsine transformation method.

Results

Detection of the disease in the field

In our survey, pea plants with tan to light brown blighted stems and pods were visually detected in all the surveyed fields (100%) in Chapainawabganj district, Bangladesh. The disease incidence accounted for 2.6% infected plants with an average disease severity rating of 3.70. Infected plants were at the late growth stage, but had not senesced. Some of the infected plants died prematurely and showed a typical wilting symptom. The most conspicuous symptom was the occurrence of the necrotic pod surface covered by a thick white fuzzy mycelial growth with large black sclerotia (Fig. 1A). When splitting the infected stem longitudinally, white mycelial growth with sclerotia was also detected along the stem pith. The sclerotia were round to irregular in shape and measured 3.5-14.8 × 1.9-5.3 mm in size.

Fungal isolation and characterization of the pathogen
All mycelial colonies from sclerotia, mycelium fragments, and symptomatic tissues yielded identical cultures on PDA. A white closely appressed and thin mycelial growth radiated over the PDA plate, but it was relatively thick at the colony margin (Fig. 1B). The reverse colony showed a salmon buff color. The hyphae were aseptate, hyaline, branched and multinucleate (Fig. 1C). No conidium or conidiophore was produced. However, sclerotia were produced in all cultures after 10 days. Sclerotia were commonly formed in-ring and usually close to the periphery of the colony. Three distinct phases of sclerotial development were observed. Whitish aggregates of mycelia appeared (initiation stage) after 5 days, turned beige-colored after 7 days (development), and developed into the dark (maturation) after 10 days of growing (Fig. 1D-F). The number and weight of sclerotia produced per PDA plate ranged from 18 to 31, and 7.38 to 15.81 g, respectively. Sclerotia were of different shapes and sizes with a black outer rind and a white inner cortex. Individual sclerotium measured up to 7 mm long and 4 mm wide.

*Production of apothecia, asci, and ascospores*

Apothecia were produced in 5 to 6 weeks of incubation. One to four apothecia arose from a single sclerotium. Apothecia were tan to beige. Receptacles were flared, somewhat concave when immature, but funnel-shaped at full maturity. Young apothecia were 1 to 2 mm in diameter, while mature apothecia were 4 to 7 mm in diameter with a stipe length of 7 to 15 mm. Asci from apothecia were cylindrical with a tapered base. Each ascus had eight ascospores (Fig. 1G). Ascospores were uniseriate, hyaline, one-celled, smooth, ellipsoid, and uniform in size (Fig. 1H-I). Paraphyses were abundant in the hymenium.

*Pathogenicity tests*
In both pathogenicity assays, all the isolates of suspected causative agent caused an infection on inoculated plants. The inoculated plants started showing water-soaked and light tan lesions around the point of inoculation by 72 h post-inoculation. Between 5 and 8 days after inoculation, the lesions progressed upward and downward in the stem and created a distinct necrotic lesion. Eleven days after inoculation, all inoculated plants wilted and died. Whitish mycelium and prominent sclerotia were visible inside of dead stems. Inoculated pea pods also produced typical white mold symptoms identical to field symptoms within 2 weeks. Whitish mycelia and sclerotia typical of *S. sclerotiorum* emerged from collapsed tissues kept in moist chambers in the laboratory. When emerging colonies were transferred to PDA, they proved to be identical to those recovered from field-infected plants. No disease symptoms were seen in non-inoculated control plants or pods.

*Molecular identification of the pathogen of *S. sclerotiorum* isolate PSP-1*

While the length of ITS sequences obtained in this study were 572 bp, the homology search through GenBank DNA database revealed a 100% sequence identity with ITS rDNA sequences of several *S. sclerotiorum* isolates (accession nos. MG249967.1, DQ329537.1, KY073614.1, MH457168.1). This confirms that the isolated fungus is a member of this group. The nucleotide sequence was deposited to GenBank under accession No. MN216247. In the phylogenetic tree constructed with isolates of *S. sclerotiorum*, *S. minor*, *S. trifoliorum*, and *Hypocrea lixii*, the fungus isolated from pea plants grouped with strains of *S. sclerotiorum* with strong bootstrap support (Fig. 2). This result identified the candidate fungus as a strain of *S. sclerotiorum*.

*Effect of temperature on mycelial growth and sclerotial formation of *S. sclerotiorum* isolate PSP-1*
The mycelial growth of *S. sclerotiorum* was affected by the variation of temperature ranging from 5°C to 30°C. At 5°C and 10°C, the growth of the isolate was very sparse as it was visible 72 h after incubation. When the temperature increased to 15°C, the growth occurred faster (Fig. 3A) and increasing temperature further to 20°C resulted in maximum mycelial growth of the fungus. As the temperature increased to 25°C, the growth declined more than three folds 72 ha after incubation. Further increasing temperature to 30°C arrested mycelial growth completely and no redial growth was observed 72 ha after incubation (Fig. 3A). There was also a significant effect of temperature on sclerotia formation. No sclerotium was observed in plates incubated at 5, 10 and 30°C, while the maximum number of sclerotium (32/plate) was formed in the culture at 20°C followed by 15°C (20/plate) and 25°C (18/plate). Similarly, individual and total sclerotial weight was maximum at 20°C (6.20 mg and 51.36 cg) compared to 15°C (4.75 mg and 32.42 cg) and 25°C (2.60 mg and 22.33 cg) (Fig. 3C).

**Effect pH on mycelial growth and sclerotium formation of *S. sclerotiorum* isolate PSP-1**

The pH of the culture media had a significant effect on mycelial growth of *S. sclerotiorum*. Generally, the fungus was able to grow over a wide pH range, with the highest growth observed at pH 5.0 (Fig. 3B). When the pH was lesser or greater than 5.0, the mycelial growth of the pathogen decreased. Accordingly, the lowest mycelial growth of the pathogen was observed at pH 3.0 followed by pH 9.0 and 7.0. Sclerotia formation has also been shown to be affected by pH conditions. Although sclerotia were formed at diverse pH ranges between 3.0 and 9.0, the highest sclerotial yield resulting from the higher sclerotial number and greater individual sclerotial weight was recorded at pH 5.0 followed by pH 3.0. Oppositely, the lowest sclerotial yield resulted from the fewer sclerotial numbers and lower individual sclerotial weight was observed at pH 9.0 followed by pH 7.0 (Fig. 3D). This suggests that
the sclerotium formation of *S. sclerotiorum* is favored under acidic than under neutral and alkaline conditions.

**Effect of carbon sources and pH on mycelial growth and sclerotium formation of *S. sclerotiorum* isolate PSP-1**

In all carbon source media, the fungus grew and produced sclerotia under acidic, neutral, and alkaline conditions. However, good growth and sclerotial yield were achieved under acidic than neutral or alkaline conditions. Precisely, the highest mycelial growth rate and sclerotial yield were attained at pH 5.0 regardless of which carbon source was used. Decreasing pH to 4.0 or increasing pH to 7.0 or 9.0 resulted in progressively slowed mycelial growth and lower sclerotial yield. Accordingly, the lowest mycelial growth and sclerotial yield were observed at pH 9.0. Among the carbon source media, glucose and mannitol favored the maximum mycelial growth, while starch supported the least. Similarly, the highest sclerotial yield in terms of the number and weight of sclerotia was observed in glucose followed sucrose, whereas the lowest was recorded in starch (Table 2).

**In vitro efficacy of fungicides on the growth of *S. sclerotiorum* isolate PSP-1**

Six fungicides were tested at three concentrations (100, 50, and 10 ppm) for their ability to inhibit mycelial growth of *S. sclerotiorum*. Among the tested fungicides, Bavistin and Amistar Top were the most effective in inhibiting the radial growth. Mycelial growth was completely inhibited by them at all three concentrations (Table 3). Dithane M-45 inhibited 88.33% and 48.22% of mycelial growth at 100 and 10-ppm concentration, respectively. The percent inhibition of mycelial growth by Rovral and Kumulas ranged between 89.85 to 48.73 and 72.59 to 27.41, respectively, where the highest inhibition
was achieved at 100 ppm and the lowest was at 10-ppm concentration. Agar amended with Ridomil showed no inhibition and thereby, had no effect on the mycelial growth *S. sclerotiorum*.

*In planta efficacy of fungicides against S. sclerotiorum isolate PSP-1*

Experiments on detached pea leaves indicated that Amistar Top and Bavistin provided excellent preventive control efficacy against *S. sclerotiorum* (Table 4). Application of Amistar Top and Bavistin one day before pathogen inoculation provided 100% control efficacy against *S. sclerotiorum*. When pathogen inoculation was done 7 days after fungicide application, the control efficacy of Amistar Top remained unaffected. Bavistin was also observed to be significantly effective against the pathogen and the control efficacy was more than 95%. When the interval between spraying and inoculation increased to 15 days, the disease severity on fungicide treated leaves became greater. Leaves of Amistar Top-treated plants showed disease severity levels that were not statistically different from the control plants. However, Bavistin was able to restrict the disease significantly and the control efficacy was 80%.

*Screening of pea genotypes against S. sclerotiorum isolate PSP-1*

Visible water soaking and leaf necrosis symptoms appeared under the plug after 48 h. By 96 h, the water soaking and necrotic regions reached the leaf margin in some leaves with green leaves becoming necrotic and discoloring to a brown. No such symptoms were observed in the non-inoculated control leaves. A significant difference in disease response was observed across tested cultivars (Fig. 4). Among 83 genotypes tested, 29 genotypes namely BD-4152, BD-4170, BD-4184, BD-4194, BD-4192, BD-7211, BD-4188, BD-4135, BD-4167, BD-4195, BD-11209, BD-11204, BD-4137, BD-4168, BD-4141, BD-4149, BD-11205, BD-4162, BARI Motorshuti 1, BD-4165, BD-4163, BARI Motorshuti 2, BD-
4153, BARI Motorshuti 3, BD-4175, BD-4158, BD-4160, BD-4161 and IPSA Motorshuti 1 showed disease severity between 50 to 100% and considered susceptible (S) to *S. sclerotiorum*. Thirty two genotypes namely BD-4230, BD-4186, BD-4220, BD-7216, BD-4179, BD-4143, BD-4222, BD-7213, BD-4173, BD-11208, BD-4196, BD-4180, BD-4492, BD-4171, BD-4219, BD-4177, BD-4191, BD-4182, BD-4187, BD-4154, BD-4494, BD-4169, BD-4174, BD-4189, BD-4176, BD-11202, BD-7210, BD-4184, BD-11206, BD-4136, BD-4159 and BD-7217 were found to have disease severity between 20.0-49.99% and grouped as moderately susceptible (MS). A total of nineteen genotypes (22.62%), BD-4193, BD-4190, BD-4144, BD-4496, BD-4181, BD-4150, BD-4227, BD-4146, BD-4232, BD-4183, BD-6944, BD-4166, BD-4145, BD-4142, BD-6945, BD-7218, BD-7208, BD-7214 and BD-7215 showed a disease severity level of 5.0-19.99% and considered moderately resistance (MR). Relatively low disease severity (1 to 4.99%) was observed in six genotypes such as BD-4157, BD-4151, BD-4231, BD-4225, BD-7778, and BD-4147 (Fig. 4) and were considered resistant (R). The lowest disease severity was found in genotype BD4157 (2.50%) followed by BD4151 (2.75%), BD4231 (3.75%), BD4225 (4.50%), BD7778 (4.50%) and BD4147 (4.75). No genotype was found as immune (I) to *S. sclerotiorum* (Table 5).

**Discussion**

Pea is an important crop and widely used for feed and food. A new disease with a significant yield loss was detected in the pea field in Chapainawabganj district, Bangladesh since 2018. The suspected disease showed a range of characteristic symptoms that are distinctive from other pea diseases such as downy mildew or Ascochyta blight [29]. Proper characterization of the causal pathogen and subsequent identification of effective fungicides and resistant sources are important to gain a clearer understanding of the disease and to minimize future losses. This study represents the first attempt to characterize a new disease of pea according to their morphological traits, pathogenicity tests, and phylogenetic analyses of ITS regions.
Based upon the observed field symptoms of the disease, it was presumed that the new disease would be white mold caused by *S. sclerotiorum* [30]. The finding that the isolated fungus has cultural and morphological characteristics similar to those of *S. sclerotiorum* e.g., a white appressed mycelial growth, the formation of a ring of sclerotia on the plate, lack of conidia and conidiophore, existence of multinucleated hyphae, and production of apothecia, asci, and ascospores provided further evidence for white mold disease [31,32]. Moreover, the induction of white mold symptoms in pea, indistinguishable from those observed in the field, by the isolated fungus fulfilled Koch's postulates and confirmed the pathogenicity establishment of *S. sclerotiorum*. Finally, sequence comparisons and phylogenetic analyses showed that the fungus was most closely related to *S. sclerotiorum*. Thus, these results established that the disease under inspection on pea is white mold caused by a fungus *S. sclerotiorum*. Although *S. sclerotiorum* has been reported to be the pathogen of the white mold of pea in other countries [33,34], this is the first documented occurrence to our knowledge of a strain infecting pea in Bangladesh [9]. In Bangladesh, *S. sclerotiorum* was previously reported on hyacinth bean, jackfruit, and okra [10,17,28,34], implying that the pathogen has the potential to expand to other host species. The wide host range of the pathogen poses an epidemiological threat and makes the management of the disease very challenging.

The white mold pathogen *S. sclerotiorum* is sensitive to temperature. Temperature influences the growth and development of the fungus because of its effect on the enzymes involved. Although *S. sclerotinia* can grow under a wide range of temperatures, the optimal temperature for mycelial growth is 20°C. The radial growth rate of the mycelium decreased at temperatures below 10°C and above 25°C. Similarly, the formation of sclerotia was at a temperature between 15 to 25°C, with an obvious maximum at 20°C. These findings were in agreement that *S. sclerotiorum* has a preference for growth in subtropical climates [28,32]. The 20°C optimum reported for growth coincides with the temperatures reported as favoring infection and pathogenesis by *S. sclerotiorum* [36]. Knowledge about environmental factors affecting the pathogen could contribute to the development of new methods of managing the disease. Therefore, early sowing might be effective at controlling white mold in pea.
The external pH puts a significant influence on fungal morphogenesis through a highly complex mechanism [37]. Moreover, pH has been involved as a major regulatory factor for procedures linked to development, pathogenicity, and virulence of S. sclerotiorum [18]. It is known that pH may act in several different ways, such as influencing enzyme action, altering metal solubility, modifying surface reactions, and preventing or facilitating the entry of vitamins, organic acids, and minerals into the hypha [38]. In general, fungi tolerate relatively narrow ranges of pH. However, mycelial growth and sclerotium formation of the S. Sclerotiorum isolate in this study were found to occur over a broad pH range, from pH 3 to 9, suggesting that S. Sclerotiorum isolate pathogenic to pea is likely to proliferate in most agricultural soils in which peas are grown. Yet, the optimal growth and sclerotium formation of the fungus was observed at a slightly acidic pH value of 5.0, indicating that S. sclerotiorum isolate exhibited the best growth characteristic of a fungus specific for acidic habitats. Since pH is not a unitary actor, the reasons for the preference for slightly acidic conditions for growth and sclerotial development of the fungus are unclear and warrants further investigation. Several hypotheses have been postulated concerning the effect of pH on mycelial growth and sclerotia production. Membrane permeability is affected by pH, therefore the ability of the fungus to take up nutrients required for mycelial growth and sclerotial production may have been affected at different media pH [39]. Oxalic acid production by S. sclerotiorum is also governed by pH [40]. The fungus may produce substantially greater amounts of oxalic acid and support mycelial growth and sclerotial formation as long as the pH of the culture is 5.0 [28].

Carbon assimilation is necessary for pathogenic fungi to survive, grow, and persist within a host. The carbon metabolism of S. sclerotiorum is strongly influenced by the nature of the carbon compound. Carbon compounds perform two important functions in the metabolism of fungi. They supply the carbon needed for the synthesis of cell components and form the primary source of energy through their oxidation [41]. There are no available accounts of the carbon requirements of S. sclerotiorum isolate infecting pea. In this study, S. sclerotiorum isolate metabolized a broad range of sugars. This indicates the efficient assimilation of different carbon sources by the fungus, hence enhancing the fitness of this pathogen in the host. Of the six-carbon sugars, glucose appears to be the
best carbon source that supported the highest mycelia growth and sclerotium formation at pH 5.0. Glucose is a biologically most important carbon compound that is utilized for growth and energy by virtually all cultivated fungi [42]. Good growth and sclerotium formation were also observed with mannitol and sucrose. Simple carbohydrates (glucose, mannitol, and sucrose) are utilized by S. sclerotiorum and converted to oxalate [43,44,18]. Oxalate-deficient mutants grow 19% to 28% slower than wild type S. sclerotiorum and do not produce sclerotia [42], indicating that oxalate production is necessary for faster growth and sclerotium production of S. sclerotiorum.

Fungicides are especially valuable as white mold disease in most crops is principally controlled by the application of fungicides. Effective fungicidal control of the disease requires precise information on the sensitivity of the pathogen to the available fungicides. Since white mold on pea is a newly reported disease, the response of the pathogen to fungicides is still missing. Evaluating the effect of fungicides on the growth of the fungus in vitro is the first step for acquiring such information. Six fungicides were screened to evaluate their ability to inhibit the growth of S. sclerotiorum mycelia in vitro. The strongest inhibition of mycelial growth was observed in cells treated with Amistar Top and Bavistin. Mycelial growth of S. sclerotiorum was inhibited by low concentrations (10 ppm) of Amistar Top and Bavistin, and hence, they are potentially useful fungicides against the disease. The enhanced fungitoxicity of Amistar Top is largely due to the additive activity of two different modes of action fungicides, Azoxystrobin and Difenoconazole. Azoxystrobin affects alternative oxidase (aox) gene expression and the alternative respiration pathway capacity development in mycelia of S. sclerotiorum [45]. Difenoconazole acts against the fungus by inhibition of demethylation during ergosterol synthesis [46]. The fungicidal potentials of Carbendazim and Azoxystrobin were reported against S. sclerotiorum in previous studies [47-49].

Although many fungicides show in vitro fungitoxic activity, a few of them prove effective against pathogens in plants [50]. The efficacy of Amistar Top and Bavistin WP showing the best performance in vitro needs to be tested in planta assay. Both fungicides controlled the disease development with great efficacy in planta screening, although a certain variability in their lengths of activity was observed. In particular, Amistar Top proved to be the most active molecule against the
white mold until 7 days after application, while the fungitoxic effect was lost in 15 days post-treatment, possibly due to early degradation of fungitoxic molecules. Bavistin, on the other hand, was relatively less efficient than Amistar Top particularly in 7 days post-treatment, but the fungitoxicity remained significantly active even up to 15 days. This indicates that spray of Bavistin at a 15-day interval is sufficient for successful control of the disease. However, frequent applications of Amistar Top such as spray at 7-day intervals might be beneficial for its control efficacy.

Information on responses of pea genotypes to infection by *S. sclerotiorum* is currently absent. The pea accessions screened showed varied responses to *S. sclerotiorum* infection. None of the 83 pea genotypes screened were found immune (I) or highly resistant to *S. sclerotiorum*. The majority of the genotypes (61 genotypes or 70.93% genotypes) were moderately susceptible (MS) to susceptible (S). Among the remaining genotypes, 122.01% fell in moderately resistant (MR) category, while only 6.98% genotypes proved resistant. These results indicate that resistance or tolerance to *S. sclerotiorum* exists among the tested pea genotypes, although the frequency of occurrence is less. Resistance to *S. sclerotiorum* has been reported only in a few pea accessions in India [51]. The popular pea cultivars in Bangladesh appeared highly susceptible. Hence, it is desirable to include newly identified sources of resistance in the national breeding program to improve the existing pea germplasm.

The data acquired in the present study resolved the new disease from the pea field as white mold caused by *S. sclerotiorum*. The potential of *S. sclerotiorum* to become a pathogen of a pea in the field is demonstrated. Hence, it may be postulated that the ability of *S. sclerotiorum* to become a devastating pathogen of pea is high as environmental conditions are conducive to disease development. Adequate characterization of the pathogen, its biology, fungicidal control, and host resistance may contribute to important information about the epidemiology of the disease and can be useful in implementing effective management strategies for the disease.

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Figure legends

Fig. 1. Field infected pea and the isolated fungus. (A) Infected pea pod showing white mycelium growth and sclerotial development (shown within circles). (B) Pure culture of the infected fungus showing white fluffy mycelium and sclerotial ring. (C) Micrograph of the mycelium of *S. sclerotiorum* strain PSP-1. (D) Initiation stage of sclerotium (5 days after incubation). (E) The development stage of sclerotium (7 days after incubation). (F) Maturation stage of sclerotium (10 days after incubation). (G) Induced formation of mature apothecia. (H) Rows of asci containing ascospores. (I) Ascus with eight ascospores.
Fig. 2. Phylogenetic construction of *Sclerotinia sclerotiorum* isolate PSP-1 and related species based on ITS sequences. Sequence recovered from the causative agent is in bold.
Fig. 3. Radial growth and sclerotium formation of *S. sclerotiorum* isolate PSP-1 on PDA at different temperatures and pH. (A&C) Radial growth (A) and sclerotium formation (C) of *S. sclerotiorum* of at 5°C, 10°C, 15°C, 20°C, 25°C, and 30°C temperature. (B&D) Radial growth (B) and sclerotium formation (D) of *S. sclerotiorum* of at pH 3, 5, 7, and 9. In each of two trails, five 9 cm-diameter PDA plates were prepared for every single temperature and pH regime and inoculated with *S. sclerotiorum* culture. The radius of colonies was measured at 24, 48, and 72 h after incubation. The number, diameter, and weight of sclerotia were taken 15 days after incubation.
Fig. 4. Disease severity of white mold caused by *S. sclerotiorum* isolate PSP-1 in pea genotypes. In each genotype, four fully expanded leaflets were cut from each of four plants and inoculated with the mycelial plug of *S. sclerotiorum*. Five days after pathogen challenge, disease severity was estimated by measuring the percent area diseased on the leaves. The data presented are from representative experiments that were repeated at least twice with similar results.
Table 1 Classification of disease reaction based on percent disease severity

| Disease severity range (%) | Type of disease reaction       |
|----------------------------|--------------------------------|
| 0.0% (No lesion)           | Immune (I)                     |
| ≤4.99                      | Resistant (R)                  |
| 5.0-19.99                  | Moderately Resistant (MR)      |
| 20.0-49.99                 | Moderately Susceptible (MS)    |
| ≥50.0                      | Highly Susceptible (HS)        |
Table 2 Effect of different carbon sources on radial growth and sclerotial developments of *S. sclerotiorum* isolate PSP-1 at different pH

| Carbon source | pH | Mycelial growth (mm) | No. of sclerotia | Sclerotial weight (g) |
|---------------|----|----------------------|-----------------|----------------------|
| Glucose       | 4  | 31.25 ± 0.39 d       | 24.00 ± 0.29 ab | 0.48 ± 0.012 a       |
|               | 5  | 39.25 ± 0.48 a       | 29.00 ± 0.51 a  | 0.51 ± 0.014 a       |
|               | 7  | 32.50 ± 0.23 cd      | 20.21 ± 0.50 b  | 0.43 ± 0.07 ab       |
|               | 9  | 30.00 ± 0.02 d       | 16.00 ± 0.26 cdefg | 0.38 ± 0.02bc       |
| Sucrose       | 4  | 36.00 ± 0.58 abc     | 18.50 ± 0.40 bc | 0.46 ± 0.09 a        |
|               | 5  | 37.50 ± 0.04 ab      | 27.50 ± 0.69a   | 0.49 ± 0.04 a        |
|               | 7  | 30.20 ± 0.01 d       | 18.25 ± 0.39 bcd| 0.41 ± 0.08 abc      |
|               | 9  | 21.50 ± 0.22 c       | 17.00 ± 0.48 bcd| 0.30 ± 0.02 bc       |
| Mannitol      | 4  | 34.00 ± 0.71bcd      | 11.00 ± 0.29 defgh | 0.20 ± 0.01 cde     |
|               | 5  | 39.21 ± 0.43 a       | 17.25 ± 0.48 bcdef | 0.24 ± 0.02 bcd   |
|               | 7  | 30.20 ± 0.01 d       | 10.00 ± 0.08 fgh | 0.16 ± 0.01 cd      |
|               | 9  | 23.25 ± 0.15 e       | 8.00 ± 1.08 h   | 0.10 ± 0.01 e        |
| Starch        | 4  | 30.00 ± 0.52 d       | 8.25 ± 0.20 h   | 0.21 ± 0.08 cd       |
|               | 5  | 30.50 ± 0.55 d       | 10.25 ± 0.85 fgh | 0.23 ± 0.02 cd      |
|               | 7  | 12.50 ± 0.47 f       | 8.50 ± 0.84 h   | 0.24 ± 0.03 bc       |
|               | 9  | 10.20 ± 0.837 g      | 8.40 ± 0.84 h   | 0.09 ± 0.01 e        |

*Values are the Mean ± SE. Mean values in each column with the same letter(s) do not differ significantly by LSD (P ≤ 0).*
Table 3 *In vitro* performance of different fungicides in mycelial growth inhibition of *S. sclerotiorum* isolate PSP-1

| Fungicide   | Concentrations (ppm) | % Inhibition of mycelial growth of *S. sclerotiorum* a |
|-------------|----------------------|------------------------------------------------------|
| Bavistin    | 100                  | 100.00±0.00a                                         |
|             | 50                   | 100.00±0.00a                                         |
|             | 10                   | 100.00±0.00a                                         |
| Dithane M-45| 100                  | 88.33±4.29b                                          |
|             | 50                   | 56.85±5.50d                                          |
|             | 10                   | 48.22±4.14f                                          |
| Rovral      | 100                  | 89.85±0.80b                                          |
|             | 50                   | 75.13±2.03c                                          |
|             | 10                   | 48.73±1.68f                                          |
| Amistar Top | 100                  | 100.00±0.00a                                         |
|             | 50                   | 100.00±0.00a                                         |
|             | 10                   | 100.00±0.00a                                         |
| Kumulas     | 100                  | 72.59±0.00C                                          |
|             | 50                   | 54.82±1.51de                                         |
|             | 10                   | 27.41±0.78g                                          |
| Ridomil     | 100                  | 0.00±0.00h                                           |
|             | 50                   | 0.00±0.00h                                           |
|             | 10                   | 0.00±0.00h                                           |
| Control     | 0.00                 | 0.00±0.00h                                           |

a Values represent mean ± SE (*n* = 3); one replication consists of three plates. Within each column, different letters indicate a statistically significant difference between treatments (Fisher’s LSD, *P* ≤ 0.05). The experiment was conducted twice. Significant treatment effects were identical in the two trials. Data shown are from one trial.
Table 4 Disease severity of white mold in pea leaves caused by *S. sclerotiorum* isolate PSP-1 and the corresponding protection afforded by two fungicides

| Treatment | 1 day after spray | 7 days after spray | 15 days after spray |
|-----------|-------------------|--------------------|--------------------|
|           | Disease severity  | % Protectionb      | Disease severity   | % Protection      | Disease severity | % Protection |
| Control   | 88.33±4.41a       | -                  | 85.23±4.21a        | -                 | 89.33±4.51a      | -            |
| Amistar   | 0.00±0.00b        | 100.00±0.00        | 0.00±0.00b         | 100.00±0.00       | 84.33±2.33a      | 4.53±2.64    |
| Bavistin  | 0.00±0.00b        | 100.00±0.00        | 4.33±0.33b         | 95.09±0.38        | 17.67±1.45b      | 80.00±1.64   |

\[\text{Protection} (\%) = \left[\frac{(A-B)}{A}\right] \times 100 \] in which A = disease severity in untreated control plants and B = disease severity in treated plants.

aValues are mean±SE (n = 9). Data within the same column followed by different letters are significantly different (LSD, p ≤ 0.05).

bProtection (%) = [(A-B)/A] × 100 in which A = disease severity in untreated control plants and B = disease severity in treated plants.
| Genotype | Source | Flower color | Plant size | Maturity (days) | Reaction | Genotype | Source | Flower color | Plant size | Maturity (days) | Reaction |
|----------|--------|--------------|------------|----------------|----------|----------|--------|--------------|------------|----------------|----------|
| BD4157   | PGRC, BARI | Pink         | Medium     | 81             | R        | BD4187   | PGRC, BARI | Pink         | Medium     | 78             | MS       |
| BD4151   | PGRC, BARI | Pink         | Short      | 78             | R        | BD4154   | PGRC, BARI | Pink         | Medium     | 74             | MS       |
| BD4231   | PGRC, BARI | Pink         | Medium     | 81             | R        | BD4494   | PGRC, BARI | Pink         | Short      | 79             | MS       |
| BD4225   | PGRC, BARI | Pink         | Large      | 74             | R        | BD4169   | PGRC, BARI | Pink         | Medium     | 77             | MS       |
| BD7778   | PGRC, BARI | White        | Large      | 75             | R        | BD4174   | PGRC, BARI | Pink         | Short      | 77             | MS       |
| BD4147   | PGRC, BARI | Pink         | Medium     | 78             | R        | BD4189   | PGRC, BARI | Pink         | Short      | 79             | MS       |
| BD4193   | PGRC, BARI | Pink         | Medium     | 77             | MR       | BD4176   | PGRC, BARI | Pink         | Medium     | 81             | MS       |
| BD4190   | PGRC, BARI | Pink         | Short      | 77             | MR       | BD11202  | PGRC, BARI | Pink         | Medium     | 81             | MS       |
| BD4144   | PGRC, BARI | Pink         | Medium     | 80             | MR       | BD7210   | PGRC, BARI | Pink         | Short      | 79             | MS       |
| BD4496   | PGRC, BARI | White        | Large      | 77             | MR       | BD4184   | PGRC, BARI | Pink         | Medium     | 77             | MS       |
| BD4181   | PGRC, BARI | Pink         | Medium     | 77             | MR       | BD11206  | PGRC, BARI | White        | Large      | 78             | MS       |
| BD4150   | PGRC, BARI | Pink         | Medium     | 75             | MR       | BD4136   | PGRC, BARI | Pink         | Medium     | 74             | MS       |
| BD4227   | PGRC, BARI | Pink         | Medium     | 80             | MR       | BD4159   | PGRC, BARI | Pink         | Medium     | 77             | MS       |
| BD4146   | PGRC, BARI | Pink         | Medium     | 78             | MR       | BD7217   | PGRC, BARI | Pink         | Medium     | 78             | MS       |
| BD4232   | PGRC, BARI | White        | Large      | 78             | MR       | BD4152   | PGRC, BARI | Pink         | Short      | 78             | S        |
| BD4183   | PGRC, BARI | Pink         | Medium     | 77             | MR       | BD4170   | PGRC, BARI | Pink         | Medium     | 78             | S        |
| BD6944   | PGRC, BARI | Pink         | Short      | 78             | MR       | BD4185   | PGRC, BARI | Pink         | Short      | 75             | S        |
| BD4166   | PGRC, BARI | Pink         | Medium     | 80             | MR       | BD4194   | PGRC, BARI | Pink         | Short      | 75             | S        |
| BD4145   | PGRC, BARI | Pink         | Medium     | 77             | MR       | BD4192   | PGRC, BARI | Pink         | Medium     | 76             | S        |
| BD4142   | PGRC, BARI | Pink         | Medium     | 75             | MR       | BD7211   | PGRC, BARI | Pink         | Medium     | 78             | S        |
| BD6945   | PGRC, BARI | Pink         | Short      | 78             | MR       | BD4188   | PGRC, BARI | Pink         | Medium     | 75             | S        |
| BD7218   | PGRC, BARI | Pink         | Medium     | 78             | MR       | BD4135   | PGRC, BARI | Pink         | Short      | 79             | S        |
| BD7208   | PGRC, BARI | Pink         | Medium     | 77             | MR       | BD4167   | PGRC, BARI | Pink         | Medium     | 78             | S        |
| BD7214   | PGRC, BARI | Pink         | Medium     | 81             | MR       | BD4195   | PGRC, BARI | Pink         | Short      | 77             | S        |
| BD7215   | PGRC, BARI | White        | Large      | 74             | MR       | BD11209  | PGRC, BARI | Pink         | Medium     | 79             | S        |
| BD4230   | PGRC, BARI | Pink         | Medium     | 81             | MS       | BD11204  | PGRC, BARI | Pink         | Medium     | 80             | S        |
| BD4186   | PGRC, BARI | Pink         | Short      | 75             | MS       | BD4137   | PGRC, BARI | Pink         | Medium     | 74             | S        |
| BD4220   | PGRC, BARI | White        | Large      | 74             | MS       | BD4168   | PGRC, BARI | Pink         | Medium     | 78             | S        |
| BD7216   | PGRC, BARI | Pink         | Medium     | 78             | MS       | BD4141   | PGRC, BARI | Pink         | Medium     | 78             | S        |
| BD4179   | PGRC, BARI | Pink         | Medium     | 80             | MS       | BD4149   | PGRC, BARI | Pink         | Medium     | 75             | S        |
| BD4143   | PGRC, BARI | Pink         | Medium     | 76             | MS       | BD11205  | PGRC, BARI | Pink         | Short      | 80             | S        |
| BD4222   | PGRC, BARI | White        | Large      | 75             | MS       | BD4162   | PGRC, BARI | Pink         | Medium     | 81             | S        |
| BD7213   | PGRC, BARI | Pink         | Medium     | 81             | MS       | BD4165   | PGRC, BARI | Pink         | Medium     | 77             | S        |
| BD4173   | PGRC, BARI | Pink         | Medium     | 77             | MS       | BARI Motorshuti 1 | Pulse Division, BARI | White         | Medium     | 74             | S        |
| BD11208  | PGRC, BARI | Pink         | Medium     | 79             | MS       | BD4163   | PGRC, BARI | Pink         | Medium     | 77             | S        |
| BD4196   | PGRC, BARI | Pink         | Short      | 78             | MS       | BARI Motorshuti 2 | Pulse Division, BARI | White         | Medium     | 74             | S        |
| BD4180   | PGRC, BARI | Pink         | Medium     | 76             | MS       | BD4153   | PGRC, BARI | Pink         | Short      | 75             | S        |
| BD4492   | PGRC, BARI | Pink         | Short      | 78             | MS       | BARI Motorshuti 3 | Pulse Division, BARI | White         | Medium     | 67             | S        |
| BD4173   | PGRC, BARI | Pink         | Medium     | 77             | MS       | BD4175   | PGRC, BARI | Pink         | Medium     | 75             | S        |
| BD4219   | PGRC, BARI | White        | Medium     | 74             | MS       | BD4158   | PGRC, BARI | Pink         | Medium     | 81             | S        |
| BD4177   | PGRC, BARI | Pink         | Medium     | 75             | MS       | BD4160   | PGRC, BARI | Pink         | Medium     | 77             | S        |
| BD4191   | PGRC, BARI | Pink         | Medium     | 77             | MS       | BD4161   | PGRC, BARI | Pink         | Medium     | 78             | S        |
| BD4182   | PGRC, BARI | Pink         | Medium     | 77             | MS       | IPSA Motorshuti 1 | BSMRAU | White         | Short      | 58             | S        |
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Reaction under laboratory conditions following inoculation on four detached leaves of four plants (each of two trials) of each genotype with *Sclerotinia sclerotiorum* isolate PSP-1 (see text). All genotypes were susceptible (S). Reaction: I-Immune, R-Resistant, MR-Moderately Resistant, MS- Moderately Susceptible, S-Susceptible. \( I = 0.0\% \), \( R \leq 4.99 \), \( MR=5.0-19.99\% \), \( MS= 20.00-49.99\% \) and \( S \geq 50.0\% \) disease severity.