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PREVALENCE OF SCLEROTINIA STEM ROT OF MUSTARD IN NORTHERN BANGLADESH

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ABSTRACT
An intensive survey of mustard in eight northern districts of Bangladesh during 2013-14 revealed the occurrence of petal and stem infection by Sclerotinia sclerotiorum. Inoculum was present in all districts and stem infection was widespread over the region. Sclerotinia sclerotiorum was isolated from both symptomatic petals and stems whereas Sclerotinia minor was isolated for the first time only from symptomatic petals. The incidence of petal infection ranged from 23.7% to 48.7% and stem infection ranged from 2.3% to 7.4%, demonstrating that mustard is susceptible to stem rot disease, however the incidence of disease is not dependent on the degree of petal infection. To our knowledge, this is the first report where both petal infection and the incidence of stem rot were rigorously surveyed on mustard crops in Bangladesh.

Key words: Sclerotinia, Stem rot, Mustard, Bangladesh

INTRODUCTION
Mustard (Brassica juncea L.) is the most important source of edible oil in Bangladesh occupying about 0.59 million hectares with an annual production of 0.53 million tonnes (DAE, 2014). The crop is susceptible to a number of diseases. Stem rot is generally caused by Sclerotinia sclerotiorum (Lib.) de Bary, a polyphagous necrotropic fungal plant pathogen with a reported host range of over 500 species from 75 families (Saharan and Mehta, 2008; Boland and Hall, 1994). During favourable environmental conditions sclerotia germinate and produce millions of air-borne ascospores. These ascospores land on petals, use the petals for nutrition and initiate stem infection (Saharan and Mehta, 2008). Sclerotia are hard resting structures which form on the inside or outside of stems at the end of the cropping season. The yield and quality of infected crops can decline gradually resulting in losses of millions of dollars annually (Purdy, 1979).

Sclerotinia stem rot is the second most damaging disease of oilseed rape after blackleg worldwide, with significant yield losses having been reported in China (Liu et al., 1990), Europe (Sansford et al., 1996), Australia (Hind-Lanoiselet and Lewington, 2004), Canada (Turkington et al., 1991) and United States (Bradley et al., 2006). The disease has also been reported from India, Pakistan, Iran and Japan (Saharan and Mehta, 2008). In India, the disease is widespread, particularly in mustard growing regions where incidence has been recorded up to 80% in some parts of Punjab and Haryana states (Ghasolia et al., 2004). Being a neighboring country of India, it would be expected that crops in Bangladesh are also vulnerable to stem rot. Literature regarding the prevalence of the pathogen and incidence of Sclerotinia stem rot, particularly in intensive mustard growing districts, is limited in Bangladesh. To our knowledge this is the first report of Sclerotinia minor in mustard petal and the first survey to determine the petal infestation and incidence of Sclerotinia stem rot of mustard in northern Bangladesh.
METHODS AND MATERIALS

Petal infestation of mustard caused by ascospores of *Sclerotinia* spp. was surveyed in eight districts of northern Bangladesh namely Panchagarh, Gaibandha, Rangpur, Dinajpur, Nilphamari, Kurigram, Thakurgaon and Lalmonirhat (Figure 1).

![Figure 1](http://wikitravel.org/en/File:Map_of_Rangpur_Division.png)

*Figure 1.* Diseased sample collection sites in the mustard growing districts of northern Bangladesh (Map from [http://wikitravel.org/en/File:Map_of_Rangpur_Division.png](http://wikitravel.org/en/File:Map_of_Rangpur_Division.png)).

These districts were located between 25°50′N and 89°00′E near the Himalayan Mountains. The selected fields ranged from 0.1 to a maximum of 1 ha. In total, 200 fields were randomly selected which were from 2 to 10 km apart. Sampling was conducted based on the petal testing protocol for *S. sclerotiorum* (Morrall and Thomson, 1991). An average of 20 healthy or asymptomatic petals from five peduncles with more than six open flowers were collected from five distantly located sites (300 m) in a field, comprising 100 petals from each field and refrigerated at 4°C until assessment in the laboratory. Within 48 hours of collection the petals were placed onto half strength potato dextrose agar (PDA, Oxoid) amended with 100 ppm of streptomycin and ampicillin (Sigma-Aldrich) and incubated at room temperature. After 5 days, *Sclerotinia* species were morphologically identified and scored based on colony morphology, hyphal characteristics and presence of oxalic acid crystals (Hind-Lanoiselet et al., 2003). The average percentage of petal infestation was calculated for each field (Morrall and Thomson 1991).

Colonies of *Sclerotinia* spp. arising from the petals were transferred to half strength PDA and incubated at 25°C until sclerotia had formed. The species of *Sclerotinia* were identified by observing the size of sclerotia (Abawi and Grogan, 1979) and confirmed by sequencing of internal transcribed spacers (ITS) of the ribosomal DNA with the Norgen's Plant/Fungi DNA Isolation Kit (Norgen Corporation) using the ITS1 (TCCGTAGGTAACCTGCGG) and ITS4 (TCCTCCGCTATTGATATGC) primers (White et al., 1990). DNA samples were purified using a PCR Clean-up kit (Bioline) according to the manufacturer’s instructions. The purified DNA fragments were sequenced (ICDDR, Bangladesh) and aligned using...
MEGA v. 6.2 (Tamura et al., 2013) then species were identified through a comparative similarity search in GenBank using BLAST (Altschul et al., 1997).

Pathogenicity tests were conducted in triplicate by inoculating seven healthy, 50 day-old mustard plants cv. BARI Sarisha11. Young, 3 day-old mycelial plugs (5 mm diameter) of *S. sclerotiorum* and *S. minor* grown on PDA were excised from the margins of a culture and attached to the internodes of the main stem of mustard plants with Parafilm® (Sigma-Aldrich). Control plants were exposed to non-inoculated plugs and incubated at 25°C and c.150 µEm−2s−1 for seven days. The incidence of stem rot was assessed before windrowing in the same fields where petal infection was determined. The number of infected plants was determined by placing five quadrates (1 m²) in the same sites as where the petals were collected. The incidence of stem rot was determined by investigating 100 plants per sample. Stem rot symptoms were identified by observing the typical stem lesion (Rimmer and Buchwaldt, 1995) and the disease incidence was calculated using the formula: Disease incidence = (Mean number of diseased cotyledons or stems / Mean number of all investigated cotyledon or stem) × 100%.

RESULTS AND DISCUSSION

Severe petal infestation and symptoms of stem infection were observed throughout northern Bangladesh. Sclerotial morphology arising from petal tests demonstrated that the majority of isolates were *S. sclerotiorum* (Lib.) de Bary, however *S. minor* Jaggar was also present in a couple of samples (Figure 2).

![Figure 2](image)

*Figure 2.* Petal infection (left hand side), *Sclerotinia sclerotiorum* (middle) and *Sclerotinia minor* (right hand side) on ½ strength potato dextrose agar at room temperature.

The phylogenetic analysis of DNA sequences of two samples revealed that KM-1 was closely related to *S. sclerotiorum* while KM-2 was identified as *S. minor* (Figure 3).

![Figure 3](image)

*Figure 3.* Phylogenetic relationship of *Sclerotinia* isolates KM-1, KM-2 from mustard and closely related species based on neighbour-joining analysis of the ITS rDNA sequence data.
The nucleotide sequences of KM-1 and KM-2 were submitted to GenBank as accession KP857998 and KP857999, respectively. The isolates KM-1 and KM-2 were deposited in the Oilseed Research Centre of Bangladesh Agricultural Research Institute under the accession numbers ORC211455 and ORC211456, respectively.

The pathogenicity test revealed that inoculated stems showed typical lesions of stem rot including the formation of white fluffy mycelium and tiny sclerotia on the stem surface. No symptoms were observed on control plants. *Sclerotinia sclerotiorum* and *S. minor* was reisolated from infected plant tissue therefore fulfilling Koch's postulates. *S. minor* has previously been reported on canola in Australia (Hind-Lanoiselet et al., 2001, Khangura and MacLeod, 2013) and Argentina (Gaetan and Madia, 2008). In Bangladesh, *S. sclerotiorum* has been reported on mustard (Hossain et al., 2008). To our knowledge, this is the first report of *S. minor* on mustard in Bangladesh. Although *S. sclerotiorum* is the major causal agent of stem rot in mustard in Bangladesh, *S. minor* has the potential to also cause significant yield losses.

*Sclerotinia sclerotiorum* is becoming an increasingly important pathogen which causes white mould disease throughout a range of host species including mustard. The prevalence of *Sclerotinia* in mustard was observed in all surveyed areas of Bangladesh (Figure 4).

![Figure 4. Typical symptoms of stem rot observed on mustard in the survey areas of Bangladesh.](image)

The incidence of petal infestation was high in Gaibandha (49%) and Panchagarh (47%) suggesting that sclerotinia stem rot is a major threat to mustard production in these districts (Figure 5). This result also suggests that *Sclerotinia* inoculum is widespread throughout the mustard growing areas of Bangladesh. Previous anecdotal evidence suggested that sclerotinia stem rot caused by *S. sclerotiorum* was present in Bangladesh however this is the first report of *S. minor* being present. *Sclerotinia minor* generally infects plants through mycelium as the production of ascospores is scarce (Abawi and Grogan, 1979). However our investigation revealed that *S. minor* may produce ascospores which infect the mustard petals. Abundance in host range and growing other susceptible crops in crop rotation has provided *Sclerotinia* species with the opportunity to survive for long periods of time and therefore they can repeatedly infect available host species including mustard petals.

Following petal testing, 200 fields were revisited and the incidence of stem rot disease determined. Typical stem rot symptoms were observed throughout the mustard field (Figure 4). Stem testing revealed the presence of *S. sclerotiorum* only where *S. minor* was absent in all samples. The maximum stem rot incidence (7%) was observed in Panchagarh while the number of infected stems was comparatively lower (4%) in the highest petal infested district of Gaibandha (Figure 5). These outcomes suggest that the incidence of disease
Figure 5 Percent petal and stem infection by *Sclerotinia* spp in eight districts of Bangladesh.

is not dependent on the degree of petal infestation. The maximum incidence of stem rot disease in Panchagarh district may be due to its close vicinity to the Himalayan mountains where winter is comparatively prolonged and humidity is high. Undesired rainfall (33 mm) was observed in this district at mid-flowering which made the environment favourable to *Sclerotina* infection. Other districts were comparatively dry as no rainfall was recorded and the winter was shorter (BMD, 2014). The wide scale of petal infestation might be due to a combination of relevant factors. Substantial mustard plantings during consecutive years and tight mustard rotations have resulted in increased inoculum pressure. In addition, varietal susceptibility, prolonged flowering, flowering timed with spore dispersal and conducive environmental conditions have initiated petal infections and subsequent stem invasions.

CONCLUSION

This is the first report where both petal infection and the incidence of stem rot were rigorously surveyed in Bangladesh. To validate the survey results it will be necessary to continue surveying for at least the next two consecutive years. However, the results of this preliminary study may assist growers to identify the disease and apply appropriate management strategies. In addition, relevant government agencies may be able to show initiative in managing the problem before the disease becomes an epidemic. The development of a reliable forecasting system by incorporating weather conditions and control measures is crucial for sustainable management of sclerotinia stem rot in mustard in Bangladesh.

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REFERENCES


