Diversity and association of isolates and symptoms of spot blotch caused by Bipolaris sorokiniana of barley (Hordeum vulgare L.)

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ABSTRACT: A three year (2003-2005) survey was conducted to study the occurrence of spot blotch symptoms on barley genotypes grown in the eastern Gangetic plains of India. Nine types of symptoms were recorded. The two most prevalent symptoms were - oval to elongate surrounded by yellow margin (type A) and, narrow, elongated dark brown spots (type D). It was also observed that symptom type D changed to type A after a week from its appearance. The pathogen Bipolaris sorokiniana isolated from these symptom types were categorized into four pathogenic groups viz., 1, 2, 3, and 5. These pathogenic groups were distributed among all symptom types. However, groups 1 and 5 were the most common and comprised of around 74% of the total spot blotch pathogen population recovered from all symptom types.

Key words: Barley, Bipolaris sorokiniana, Cochliobolus sativus, Spot blotch, Symptom

Spot blotch incited by Bipolaris sorokiniana (Sacc. in Sorok.) Shoemaker [Teleomorph: Cochliobolus sativus (Ito & Kurih.) Dreschler ex Dastur.] is one of the most destructive foliar diseases of barley (Hordeum vulgare L.) worldwide because it can cause marked reduction in grain yield and quality of crop (Nutter, et al., 1985; Arabi and Jawhar, 2003). Clark (1979) estimated 16 to 33% grain yield reduction due to spot blotch in Canadian cultivars, while Van Leur (1991) reported 40% yield loss in Syria. In addition to quantitative losses, spot blotch also reduces malting quality of barley (Nutter et al., 1985) which results in the crop being sold for feed at a lower price (Peterson and Foster, 1973). Significant losses in Indian barely lines due to spot blotch have also been reported (Anonymous, 2006).

Spot blotch is characterized by typical oval shaped black to brown necrotic spots on the leaf, generally surrounded by a yellow halo. Many atypical symptoms are also induced by B. sorokiniana which often confuses data recorders with the lesions produced by other pathogens such as Drechslea teres causing net blotch in barley. Consequently, disease screening based on symptoms may reduce the efficiency of evaluation and selection of spot blotch resistant genotypes. Therefore, the objective of the present investigation was to study the type of symptoms produced by different pathogenic groups of B. sorokiniana infecting barley genotypes in different ecological environments and to understand their relationship.

MATERIALS AND METHODS

Collection of symptom types

Spot blotch symptom types were collected from barley cultivars viz., Jyoti, Lakhan, K-560, K-603, Gitanjali and Kanar-16 grown in five villages in each of the four major barley growing districts (Mirzapur, Chandauli, Azamgarh and Varanasi) in the state of Uttar Pradesh during the crop years 2003, 2004 and 2005. These locations were identified as hot spot for natural infections of spot blotch disease in wheat and barley. Disease symptoms were also collected from the barley fields grown at Agriculture Research Experiment Station, Banaras Hindu University, Varanasi, India. Different symptom types were collected separately at heading stage (GS 50-52) (Zadoks et al., 1974). Leaves below penultimate were used for collecting symptom types. Ten diseased samples of each symptom type in each village were examined for the association of B. sorokiniana.

Isolation

To observe the association of B. sorokiniana infecting barley with symptom types, infected areas were washed separately in tap water mixed with surfactant (Sandovit, BASF) (1ml/ L.) and cut into small pieces from individual spots. Leaf pieces of each symptom types were dipped in HgCl₂ solution (0.1%) for 30 seconds followed by four washings with sterile distilled water. Leaf pieces were dried under sterile blotter paper. A piece (1mm²) of diseased portion of each symptom type was inoculated into Petri dishes containing potato dextrose agar medium and incubated at 25 °C for 10 days for the growth of pathogen (Chaurasia et al., 2000). Five replications of each symptom type were maintained. After growth of the pathogen, slides of the recovered pathogen from each symptom type were prepared in cottonblue lactophenol and morphology and measurements of conidia were observed to compare with morphological description of B. sorokiniana given by Sivanesan (1987).
Distribution of symptom types in barley germplasm and their association with fungal groups

One thousand six hundred and eighty seven diverse barley genotypes obtained from Directorate of Wheat Research, Karnal, India and germplasm collections maintained at Banaras Hindu University, Varanasi, India were used. Each genotype was sown in a two meter row length at 23 cm apart under irrigated conditions with recommended doses of fertilizer at the Agricultural Research Station, Banaras Hindu University, Varanasi, India. Ten plants from each of the five genotypes having same symptom types were tagged and monitored for change in the symptom expression on the leaf below penultimate. Days required for change in the symptom were also recorded. Symptom types were counted in all the germplasm lines and the frequency of symptoms was calculated by using the following formula.

\[
\text{% frequency of symptom type in barley genotypes} = \frac{\text{Number of genotypes showing a particular symptom}}{\text{Total number of barley genotypes observed}} \times 100
\]

After screening for symptom types, infected leaves from five different genotypes having similar symptoms were processed for the isolation and characterization of different pathogenic groups of *B. sorokiniana* as reported by Chand *et al.* (2003). Isolation of *B. sorokiniana* from different symptom types was followed as described earlier (Chaurasia *et al.*, 2000). Five plants of each genotype were used for isolation of *B. sorokiniana*. Recovery of pathogen from each symptom type and its grouping in to different category, based on colony colour, was recorded in five replications. Per cent recovery of pathogenic groups isolated from different symptom types was also calculated to understand the population structure of *B. sorokiniana*.

RESULTS AND DISCUSSION

Occurrence of variable symptom types

Isolation of *B. sorokiniana* from symptom types collected from genotypes grown in different villages of Mirzapur, Chandauli, Azamgarh and Varanasi district of Uttar Pradesh and 1687 germplasm lines grown at Banaras Hindu University, Varanasi demonstrated that this pathogen induce variable symptom types in barley. Based on morphological features, 9 type of symptoms were observed (Fig. 1). These were grouped as, A: oval, elongated surrounded by yellow margin; B: elongated similar to net blotch; C: chlorosis in large patches followed by necrosis; D: narrow, elongated dark brown; E: chlorosis from center scattered small pustules; F: small, scattered, oval; G: small dot like growth continuous with vein; H: pinhead size, scattered and, I: necrosis started from margin of leaves (Fig. 1).

Distribution of symptom types

Observation of symptom types of *B. sorokiniana* in different villages in eastern Gangetic plains of India showed that symptom types A and D were the most frequent during 2003-2005 (Fig. 2). Symptom type F that constituted 12% of total
symptoms in the year 2003, was the third most frequent symptom. However, in the year 2004 and 2005, the symptom type E was the third most visible symptom. Its frequency in the year 2004 ranged between 10-16%, while it was only 5% in the year 2005 (Fig. 2). Observations on changes of symptoms in barley germplasm lines showed that symptom type D turned to typical type A category after a week of symptom initiation. Other symptom types remained unchanged.

Symptom types with maximum percentage (71.5%) displayed by the typical spot blotch symptoms (type A and D) (Fig. 3). Narrow elongated type of symptom (type B, Fig. 1B) similar to that produced by net blotch, and symptom showing chlorosis in large patches followed by necrosis (type C, Fig. 1C) were found in very few lines (2.03 and 0.18%, respectively). Infection followed by chlorosis in and around the midrib (type E, Fig. 1E) and small oval scattered spots (type F, Fig. 1 F) were observed in 10.7 and 10.4 % lines, respectively. These lines were generally resistant to the B. sorokiniana. Very small, dot like densely packed necrotic lesions (type H, Fig. 1H) were noted on very few genotypes (0.36%). In some (0.71%) genotypes, dot like necrotic lesions (type G, Fig. 1G) were arranged in linear fashion. Necrosis from the margin and its spread towards the midrib (Fig. 1I) was also noticed in 4 % lines. Our results clearly demonstrated that variability existed for symptom types in barley germplasm and many atypical symptoms were produced. Variability in symptom types may be attributed to the association of different pathogenic groups and their aggressiveness (Chand et al., 2003) and the response of barley genotypes which allow pathogen to establish and induce symptoms (Joshi et al., 2007).

Fig. 3. Distribution of different symptom types of spot blotch in 1687 barley germplasm lines
A: oval, elongated surround by yellow margin; B: Elongated similar to net blotch; C: Chlorosis in large patches followed by necrosis; D: Narrow, elongated dark brown; E: Chlorosis from center scattered small lesions; F: Small, scattered, oval; G: small dot like grow continuous with vein; H: pinhead size, scattered; I: start from the margin of leaves.

Fig. 4. Percent recovery of pathogenic groups from different symptom types of spot blotch in barley germplasm lines
Group 1 = Black colony; Group 2 = Brown/dull brown colony; Group 3 = Gray with small white colony; Group 4 = Dull white/greenish black colony; Group 5 = White colony

were recovered from all the symptom types. Group 3 was isolated from by symptom types A, C, D, F, H and I while Group 2 was recovered form symptom types B and E only. Group 4 was not isolated from any symptom types (Fig. 4). These results suggested that black (group 1) and white (group 5) were most frequently associated with symptom types followed by gray with white (group 3) and brown/dull brown (group 2). These results also suggested that pathogenic groups were not associated with a particular symptom type. Absence of group 4 from all the symptom types suggested that this group might be least pathogenic and dominated by other pathogenic groups. The spot blotch pathogen recovered from all the symptom types demonstrated that group 1 was the most common followed by group 5, 3 and 2 (Fig. 5). This indirectly suggested that group 1 could be the most aggressive isolate for barley.

Chand et al. (2003) while working on the natural population of B. sorokiniana in wheat also reported that group 1 was the most frequent as well as aggressive compared to other pathogenic groups.

Fig. 5. Percent recovery of different pathogenic groups from all the symptom types produced on barley germplasm lines
Group 1 = Black colony; Group 2 = Brown/dull brown colony; Group 3 = Grey with small white colony; Group 4 = Dull white/greenish black colony; Group 5 = White colony

Association of pathogenic groups with symptom types and population structure

The pathogen B. sorokiniana was recovered from 90% of samples. Based on colony colour, isolates were grouped in different categories - black (group 1), brown/dull brown (group 2), gray with white spots (group 3), dull white/greenish black (group 4) and white (group 5) as reported by Chand et al. (2003). The pathogenic groups 1 and 5
Table 1. Genotypes representing different pathogenic groups of Bipolaris sorokiniana from variable symptom types

<table>
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<tr>
<th>Symptom type</th>
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Group 1 = Black colony; Group 2 = Brown/dull brown colony; Group 3 = Gray with white spots colony; Group 4 = Dull white/greenish black colony; Group 5 = White colony; — = Pathogen not recovered.

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REFERENCES


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