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Sources of inoculum and reappearance of spot blotch of wheat in rice–wheat cropping systems in eastern India

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Abstract

A study was undertaken to examine the main source of inoculum of *Bipolaris sorokiniana* responsible for its reappearance in rice–wheat cropping regions of eastern India. Soil samples were collected at monthly intervals during April–October in the years 2000 and 2001 from fields having rice–wheat cropping. *Bipolaris sorokiniana* conidia were isolated and their viability was found to decline sharply with the onset of flooding in the month of August. In contrast to 82% in April, viability was 4% and <1% in August and September, respectively. Viable conidia were multiplied in the laboratory and inoculated on to susceptible cv. Sonalika under controlled conditions for test of pathogenicity. Appearance of symptoms typical to spot blotch were recorded after 7 days. Twenty-two different species (weeds and grasses) normally found to be associated with rice–wheat fields were tested for the presence of *B. sorokiniana* to evaluate their possible role as alternative hosts. Only three species, i.e. *Setaria glauca*, *Echinochloa colonum* and *Pennisetum typhoids*, were found to naturally harbour *B. sorokiniana*. Isolates from these hosts were tested for pathogenicity and also for their possible spread to wheat. When reisolated from these hosts, the pathogen did not infect wheat. Seeds of 25 different wheat genotypes were tested for *B. sorokiniana* infection. All genotypes were infected and the incidence of infection varied from 26% to 86%. Five isolates of wheat and one isolate from each of the three species (*S. glauca*, *E. colonum* and *P. typhoids*) were subjected to RAPD analysis. Two broad clusters were formed, suggesting that the wheat isolates were different from the isolates originating from other hosts. The results indicate that seeds are the most important source of inoculum for the reappearance of spot blotch of wheat in rice-wheat cropping systems in eastern India.

Introduction

Spot blotch caused by *Bipolaris sorokiniana* (syn. *Helminthosporium sativum*, teleomorph *Cochliobolus sativus*) is a serious disease of wheat in the Mega Environment 5 (ME 5) characterized by a warm humid climate (Duveiller et al., 1998a; Joshi et al., 2002). In the humid sub-tropics of south east Asia (which falls under ME 5), rice–wheat is the predominant cropping system, covering around 11–12 million hectares (Hobbs and Morris, 1996). Rice–wheat cropping is a unique system where rice is grown in water-logged anaerobic conditions during the monsoon period of July–October followed by wheat during November–April. The average yield loss caused by leaf blight (spot blotch, tan spot, and *Alternaria* blight) in south Asia is around 20% (Saari, 1998), but yield losses between 20% and 80% have been reported by Duveiller and Gilchrist (1994). Under severe conditions, the yield losses may be as high as 100% (Srivastava et al., 1971; Mehta, 1994).

Resistance to spot blotch in the commonly grown wheat cultivars of south east Asia is generally unsatisfactory (Duveiller et al., 1998b; Joshi et al., 2004a, b) and integrated disease management is accepted as the best way to manage the disease (Joshi and Chand, 2002). Knowledge concerning the survival and reappearance of the spot blotch pathogen is essential for proper implementation of integrated disease management. However, there is lack of clear information on these issues in rice–wheat cropping systems. Some workers (Shaner, 1981; Ries and Forcelini, 1993) have suggested that infected seeds could be the main source of inoculum, whereas others (Chin and Tinline, 1963; Ries and Santos, 1987) have reported the survival of free dormant conidia in soil for more than a year in monoculture systems. It is also possible that grass hosts may harbour the pathogen and act as alternate hosts (Nelson and Kline, 1962; Misra, 1973; Duveiller and Gilchrist, 1994). The objective of this study was to investigate the main source of inoculum of *B. sorokiniana* responsible for its reappearance in rice–wheat cropping regions of eastern India.

Materials and methods

The present study consisted of four experiments: (1) isolation of *B. sorokiniana* from soil and testing its viability; (2) studies of various plant species as possible alternate hosts; (3) studies of the presence of *B. sorokiniana* in wheat seeds and; (4) RAPD analysis of *B. sorokiniana* isolates obtained from different hosts. RAPD was conducted to confirm the results of experiment (2) i.e. whether the isolates of *B. sorokiniana* obtained from wheat and other hosts were the same or different.

Isolation of Bipolaris sorokiniana *from soil and assessment of viability*

Soil samples were collected at monthly intervals during April–October in the years 2000 and 2001 from 10 fields having rice–wheat cropping at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India (located at 25.2° N latitude and 83.0° E longitude) where all field work was performed. Samples were taken in the third week of each month, using a modification of the method of Duczek (1986). Using a soil auger, three soil samples were taken on parallel paths with an inter-path distance of 25 m in each of 10 fields measuring 1–2 ha. Each sample consisted of four cores, each 15 cm deep. Thus, a total of 30 samples and 120 cores were taken in the experiment, with the first core taken 25 m from the border of the field. The remaining three cores were taken at a distance of 20 m, proceeding into the field from the field border. At each sampling time, soil was mixed from all the cores from a single field.

Viability of conidia in each of the soil samples was tested by the modified method of Duczek (1981). Ten grams of soil was mixed with 5 ml of mineral oil in a 25×200 mm screw-capped test tube and 5% NaCl solution was added to make the final volume reach up to the top 50 mm of the tube. Tubes were shaken manually for about 5 min and allowed to stand for 5–10 min. Three milliliter of the upper (oil) phase was mixed with 1 ml of molten potato dextrose agar (PDA) supplemented with 1% molasses and 0.1% Tween 20 in a 9 cm glass Petri dish. For each soil sample, 20 such plates were incubated at 25 ± 1 °C for 16–20 h in each of the three replicates. The plate surface was flooded with cotton blue lactophenol (0.5 ml per plate) and observed at a total magnification of ×100 in a compound microscope (area of the field of vision was ca. 1 mm^2). Percentage germination was determined on 100 spores of each of the 20 plates. Each plate was observed three times at different time points. Thus for each of the 10 fields, 6000 spores were observed in a replicate. Viability count of conidia was taken after 72 h. Conidia were considered viable when the germ-tube was equal to or greater than the width of the conidium. The viable conidia of 10 isolates were multiplied in the laboratory (Chand et al., 2003) and tested for their pathogenicity under the controlled conditions of a poly-house (a plastic tunnel having the facility of partial control of temperature and capability to maintain >90% humidity through automatic sprinklers). The conidia were inoculated onto five plants of the susceptible cv. Sonalika in each of the three replicates following the method given by Joshi and Chand (2002). For each plant about 20 ml suspension having 10^4 spores ml^{-1} was used. Appearances of symptoms typical of spot blotch were recorded after 7 days.

Data on percentage conidial viability and pathogenicity of isolates (%) were analysed using

INDOSTAT software for a randomized block design (RBD) for calculating LSD values. Due to wide range of percent values, arc-sine transformation values were used for analysis (Steel and Torrie, 1960; Snedecor and Cochran, 1968; Gomez and Gomez, 1983).

Study of other plant species as possible alternate hosts

This study consisted of two experiments: (i) isolation of *B. sorokiniana* from weeds and crop plants; (ii) test of spread of inoculum to wheat. In (i) plants normally found to be associated with rice–wheat fields, *Oryza sativa*, *Saccharum officinarum*, *Setaria glauca*, *Avena fatua*, *Bamboo* spp., *Emperata cylindrica*, *Echinochloa colonum*, *Cyperus rotundus*, *Cynodon dactylon*, *Paspalam notatum*, *Pennisetum typhoids*, *Zea mays*, *Sorghum halepense* and *Eleusine indica*, were screened for *B. sorokiniana* infection. Some additional plants growing in surrounding fields, *Phaseolus vulgaris*, *Pisum sativum*, *Vigna radiata*, *Vigna mungo*, *Cocos nucifera*, *Elacis guinnensis* and *Polianthes tuberosa*, were also screened. For each plant species a total of 10 plants were taken from five different locations. *Bipolaris sorokiniana* was isolated from all these species. The presence of *B. sorokiniana* was confirmed by examining infected plant parts under a microscope and by culturing the samples and comparing them with the characteristic features of the fungus (Sivanesan, 1987). Following isolation of *B. sorokiniana* (Chaurasia et al., 2000), colonies were cultured form single-spore isolations and multiplied on PDA (Maraite et al., 1998) (cultures will be referred to as isolates hereafter). Spore suspensions (10^4 ml^{-1}) were prepared from five isolates of each plant species and sprayed on to wheat plants (cv. Sonalika) raised under field conditions to test their pathogenicity on wheat (Chaurasia et al., 2000). Plants of cv. Sonalika were sown on 10 December 2002 under irrigated (five irrigations), high fertility (120N:60P:40K) conditions, with alluvial soil having a pH of 7.0. For each isolate, 10 plants of cv. Sonalika were inoculated at growth stage (GS) 59 (Zadoks et al., 1974); seeds were treated with Thiram (tetramethyl thiram disulphide; 100% active ingredient) 2 g kg^{-1} (Nene and Thapliyal, 1979), and the experiment was isolated from other wheat plots by a 20 m wide crop (lentil) barrier grown along the experimental plot.

In order to test the spread of *B. sorokiniana* from other hosts to wheat, 10 rows each of one accession of *Setaria glauca* and wheat (cv. Sonalika) were planted side by side in a poly-house in three replicates. Each row was 2 m long with plant-to-plant and row-to-row spacings of 10 and 25 cm, respectively. To eliminate seed infection, wheat seeds were treated with Thiram prior to sowing. The poly-house was fully covered and hence eliminated the risk of infection from outside sources. An accession of *Setaria glauca* was inoculated with a conidial suspension (10^4 ml^{-1}) of *B. sorokiniana* isolated from the same species previously, to test the potential spread of inoculum from this species to wheat. The same approach was employed to examine the spread of inoculum from two other species, *Echinochloa colonum* and *Pennisetum typhoids* (one accession of each) to wheat. Another set of these species and wheat was planted in a second poly-house (50 m away from the first) without any artificial inoculation to serve as a control for seed infection. Symptoms that appeared on wheat plants were assessed and the pathogen re-isolated and cultured as described in experiment 2(i).

Presence of B. sorokiniana *in seeds*

One hundred grams of seeds were collected from 25 different wheat genotypes. These seeds were obtained by placing a bag around 120 single spikes of each of the cultivars in the pervious crop season. These seeds were subjected to two tests (i) a laboratory test for the presence of *B. sorokiniana* and (ii) a field and poly-house screening of the wheat plants for spot blotch infection. In the laboratory tests, seeds were placed in sterile Petri dishes (five seeds per dish) on top of two filter papers (Whatman 181), moistened with deionised water and incubated in darkness. The temperature regime was as recommended by ISTA (1985). Thirty seeds were used in each of three replicates (1 replicate = 6 plates). After 5 days, each seed was visually examined under a compound microscope for the presence of spores of *B. sorokiniana*. In the field experiments, seeds from the same lots used for the laboratory tests were sown in a field previously planted with rice which was water-logged for around 60 days. Five rows (3 m long) of each

cultivar was dibble-sown in three replicates with a row-to-row distance of 20 cm. Similarly, for the poly-house test, three rows of 2 m length of each cultivar were dibble-planted in three replicates in the poly-house. Before sowing, the poly-house soil was tested following the method of Duczek (1986) to confirm that *B. sorokiniana* was absent.

Disease severity in the field and poly-house grown plants was recorded at GS 77 using the 0–9 scale of Saari and Prescott (1975). Disease severity (%) was also recorded at three different growth stages, GS 69, 77 and 83, to calculate the Area Under Disease Progress Curve (AUDPC). Each time disease was recorded on 10 randomly chosen plants in each replicate (Joshi and Chand, 2002). Data for different observations were analysed as a RBD for calculating LSD values. For seed infection (%), arc-sine Transformation values were used following Steel and Torrie (1960), Snedecor and Cochran (1968) and Gomez and Gomez (1983).

RAPD analysis of isolates derived from different hosts

Five isolates (one isolate from each of the five different groups) of *B. sorokiniana* isolated from wheat (Chand et al., 2003) and maintained at the Department of Mycology and Plant Pathology, Banaras Hindu University, Varanasi, India, were used for RAPD analysis along with those isolated from *S. glauca*, *E. colonum* and *P. typhoids* (one isolate from each species). Wheat isolates were divided into five classes (Chand et al., 2003). Among these, Group I isolates were most frequent under field conditions. In the case of other hosts grouping was not possible due to the close similarity among isolates. Isolates were multiplied in the liquid minimal medium (MM medium) of Leach et al. (1982) at 28 °C. DNA was extracted from lyophilized, ground and filtered mycelium by the CTAB method (Moller et al., 1992). DNA was quantified using a spectrophotometer at the wavelength of 260 nm. DNA was amplified and RAPD analysis was done following Zinno et al. (1998).

Assays were performed with RAPD analysis beads (kits) containing buffer components, dNTP mix and taq polymerase. Random standard primers were obtained from Operon Technologies, Alameda, California, USA (OPA-1, OPA-2, OPA-5, OPC-3, OPG-2, OPT-3 and OPT-6). Fifty nanograms of fungal DNA, 1 μl primer, 0.8 μl taq DNA polymerase and 0.8 μl of dNTP mix were used for each 25 μl PCR assay. Initial denaturation was performed at 95 °C for 5 min. One PCR cycle constituted 1 min at 94 °C (denaturation), 2 min at 34 °C (annealing) and 2 min at 72 °C (polymerisation). A total of 36 cycles was programmed. Final extension was done at 72 °C for 5 min. Amplification was carried out using a thermo–cycler (Techne, Duxford, Cambridge, UK). The PCR product was separated in a 1.5% agarose gel and stained using 1.0 μg ml^{-1} of ethidium bromide solution. The bands were visualized under UV radiation. Polymorphic bands were recorded to calculate a binary matrix of presence (1) or absence (0) of the bands regardless of their staining intensity. Jaccard's similarity coefficient and un-weighted Pair Group Method with Arithmetic Mean (UPGMA) were used to reveal asso-

Table 1. Conidial viability of *Bipolaris sorokiniana* collected during April–October in 2000 and 2001

Month	Conidial viability (%)*			Mean number of isolates showing pathogenicity (%)*		
	2000	2001	Mean	2000	2001	Mean
April	83.0 (65.7)	81.0 (64.2)	82.0 (64.9)	100.0 (85.0)	100.0 (85.0)	100.0 (85.0)
May	70.0 (55.8)	74.0 (59.4)	72.0 (58.1)	100.0 (85.0)	93.3 (76.0)	96.7 (80.5)
June	75.0 (60.0)	77.0 (61.4)	76.0 (60.7)	76.7 (61.2)	76.7 (61.2)	76.7 (61.2)
July	51.0 (45.6)	53.0 (46.7)	52.0 (46.2)	43.3 (41.2)	43.3 (41.2)	43.3 (41.2)
August	3.0 (9.9)	5.0 (12.9)	4.0 (11.4)	10.0 (18.4)	16.7 (23.9)	13.3 (21.2)
September	0.7 (4.7)	0.7 (4.7)	0.7 (4.7)	0.0 (5.0)	0.0 (5.0)	0.0 (5.0)
October	0.7 (4.7)	0.7 (4.7)	0.7 (4.7)	0.0 (5.0)	0.0 (5.0)	0.0 (5.0)
LSD ($P \leq 5\%$)	1.7	1.5	1.4	5.7	7.1	3.7

*Figures in parenthesis correspond to arc-sine transformation values. LSD values are based on analysis of arc-sine transformed values of replicated data.

ciations among isolates (Gower, 1966; Sneath and Sokel, 1973). Analysis was performed with the computer package NTSYS-PC Version 1.7 (Rohlf, 1992). RAPD analysis was repeated three times.

Results

Viability of conidia in soil: On average, 82%, 72% and 78% of the conidia isolated from soil samples were viable in the months of April, May and June, respectively (Table 1). Significant increases in conidial viability from May to June could be due to saprophytic multiplication of conidia on infected crop residues under favourable conditions. However, a sharp decline in conidial viability was recorded in August where only 4% of the conidia were found viable (Table 1). Conidial viability further declined to 0.7% in September–October, prior to the normal planting time of the wheat crop in eastern India (second 2 weeks of November). Conidia isolated during April–June were pathogenic to wheat, but those isolated after August were not pathogenic (Table 1).

Possible alternate hosts and spread of B. sorokiniana *to wheat*

Out of 25 different species associated with rice–wheat cropping either as weeds or as independent crops growing in adjoining fields, three species, *viz. S. glauca*, *E. colonum* and *P. typhoids*, harboured natural populations of *B. sorokiniana*. In tests of pathogenicity, isolates from these hosts caused spot blotch symptoms on wheat consisting of small light brown spots variable in size and shape, mostly oval to oblong to somewhat elliptical, 0.5–10 mm long and 3–5 mm wide. Well developed lesions showed dark brown margins surrounded by chlorotic zones with a light brown centre. Often these spots coalesced and formed larger necrotic patches. Isolates were therefore regarded as pathogenic to wheat. When these three hosts were planted side by side with wheat and inoculated with their respective isolates, disease severity and AUDPC values of the three hosts were significantly higher than those of wheat plants (Table 2). Wheat grown as the control also showed significantly lower disease severities and AUDPC than the three hosts. Disease severity was high in the poly-house conditions due to the presence of higher humidity and temperature in comparison to the field conditions.

Table 2. Disease severity and Area Under the Disease Progress Curve (AUDPC) observed in different hosts of *Bipolaris sorokiniana* in rice–wheat cropping system

Hosts	Mean disease severity[a]	Mean AUDPC
Setaria glauca	5.3	896.7
Echinochloa colonum	6.2	926.2
Pennisetum typhoids	6.7	925.0
Wheat[b]	3.1	428.2
Wheat (control)[c]	3.0	418.7
LSD ($P \leq 5\%$)	2.3	43.4

[a]According to 0–9 scale of Saari and Prescott (1975).
[b]Inoculated wheat plants grown with three other hosts.
[c]Non-inoculated wheat plants grown with three other hosts.

Presence of conidia in wheat seeds

Bipolaris sorokiniana conidia were found on all 25 lines under study. Seed infection varied from 86% (HD 2329) to 26% (HUW 206) (Table 3). The severity of disease in the non-inoculated plants harbouring *B. sorokiniana* was similar under field and poly-house conditions and varied from 4 to 9 on a 0–9 scale (Table 3). The AUDPC values for these lines varied from 1066.6 to 2391.6 and 1191.6 to 2518.3 in the field and poly-house, respectively. The highest and lowest disease infection was displayed by cv. Sonalika and HUW 206, respectively.

RAPD analysis of isolates from different hosts

RAPD analysis based on Jaccard's similarity coefficient and UPGMA showed that one isolate investigated from each of the three hosts, *Setaria glauca*, *E. colonum* and *P. typhoids*, differed significantly from one wheat isolate of each of the five groups (Figures 1 and 2). Two broad clusters were formed; the five groups of wheat isolates formed one cluster, whereas the other cluster comprised isolates from the other three grass species (Figure 2). Wheat isolates belonging to groups 3 and 5, showed maximum similarity with the OPG-2 primer (1.00). The isolates of Group I showed least similarity (0.36) to the other wheat isolates. The wheat isolates displayed 19% similarity to the isolates collected from the three other hosts. Similarity between isolates from *Setaria* and *Echinochloa* was higher (0.66) than between *Setaria* and *Pennisetum* (0.50).

Table 3. Mean infection (%) of *Bipolaris sorokiniana* in the seeds of different wheat genotypes, disease severity and Area Under Disease Curve (AUDPC) under field and poly-house conditions

Genotype	Seed infection (%)[a]	Mean disease severity[b]		Mean AUDPC	
		Field	Poly-house	Field	Poly-house
Sonalika	82.4 (64.9)	8.6	9.0	2241.6	2450.0
Ginshun 7	68.3 (55.6)	6.6	7.0	1620.0	1766.6
Angostura	80.8 (63.4)	8.6	9.0	2041.6	2148.3
Raj 2535	66.5 (54.5)	6.6	7.3	1891.6	1991.6
VL 421	66.2 (54.3)	7.3	7.3	1891.6	1991.6
SERI 82	64.0 (53.1)	7.3	8.0	1808.3	1916.6
Attila 48	68.3 (55.6)	7.3	8.0	1833.3	1958.3
K9331	82.7 (65.2)	7.6	9.0	1841.6	1958.3
HUW 318	54.2 (47.3)	5.3	7.3	1366.6	1500.0
PBW 154	76.5 (60.7)	8.6	9.3	2098.3	2223.3
WH 147	82.1 (69.9)	8.6	9.0	2091.6	2200.0
HD 2329	86.3 (68.0)	9.0	9.0	2060.0	2518.3
Kalyansona	66.1 (54.3)	6.6	7.3	1650.0	1833.3
HUW 55	64.0 (53.7)	6.6	8.0	1650.0	1750.0
Raj 3855	66.2 (54.3)	7.0	7.0	1900.0	2016.6
HDR 70	80.5 (63.5)	7.6	9.0	1891.6	2025.0
HW147	84.9 (66.5)	9.0	9.0	2391.6	2491.6
HI 617	82.2 (64.9)	8.6	9.0	2200.0	2300.0
Agra local	52.0 (46.2)	5.6	7.0	1516.6	1633.3
UP 2338	76.5 (60.6)	7.6	8.3	1733.3	1850.0
WL 711	74.1 (59.4)	8.3	9.0	2316.6	2416.6
UP 262	76.4 (60.7)	8.0	8.3	2100.0	2183.3
HUW 234	82.6 (64.9)	8.0	8.6	1816.6	1941.6
HUW 206	26.3 (30.6)	4.3	5.0	1066.6	1191.6
KAVZ 34	56.2 (48.5)	6.6	7.0	1366.6	1475.0
LSD ($P \leq 5\%$)	2.5	1.2	1.3	223.9	97.8

[a]Figures in parenthesis correspond to arc-sine transformation values. LSD values for seed infection (%) are based on analysis of arc-sine transformed values of replicated data.
[b]According to 0–9 scale of Saari and Prescott (1975).

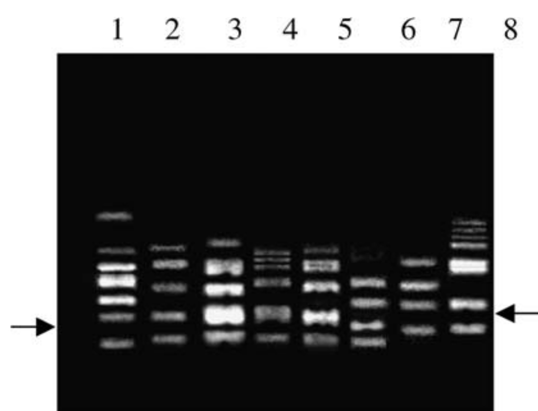

Figure 1. RAPD profile of eight isolates of *Bipolaris sorokiniana* (OPG-2 primer) from four different hosts. (1–5) Five wheat Isolates divided into five different groups (Groups I–V); (6) isolate from *Setaria*; (7) isolate from *Echinochloa*; (8) isolate from *Pennisetum*. Arrows display the polymorphic bands in wheat and the other three hosts.

Discussion

Although there are reports of survival of *B. sorokiniana* conidia in soil for 8 months (Ledingham, 1970) or even for more than a year (Chin and Tinline, 1963; Ries and Santos, 1987), the conditions in the monsoon climate of the Indian subcontinent are quite different. In the rice–wheat cropping region of India, covering 10.5 million ha, the monsoon arrives during late June or early July and from here onwards, water-logged conditions arise in the rice fields. Water-logging causes anaerobic conditions detrimental to the survival of *B. sorokiniana*. A sharp decline in the isolation of viable conidia of *B. sorokiniana* from August onwards supports this view. Furthermore, the conidia isolated from soil in the months of August and beyond were not found to be pathogenic. Thus, the

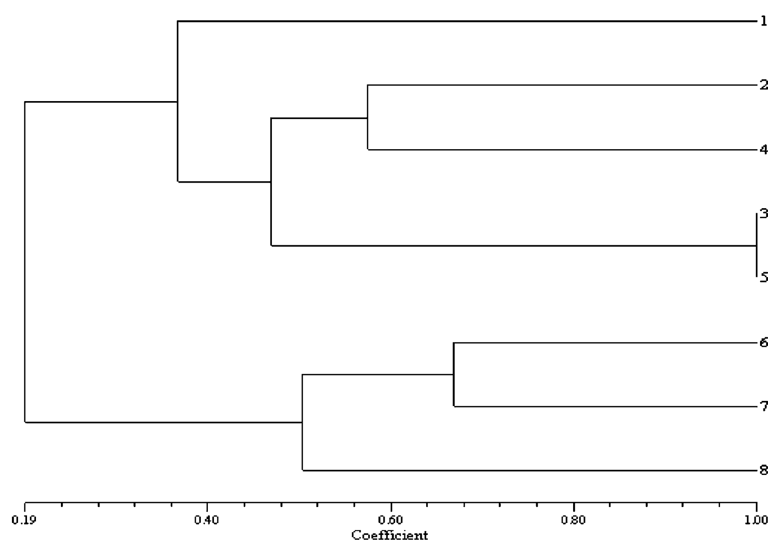

Figure 2. Dendrogram (Rohlf, 1992) for the RAPD profile of eight isolates of *Bipolaris sorokiniana* from wheat and three other hosts. (1–5) Five wheat Isolates divided in to five different groups (Groups I–V); (6) isolate from *Setaria*; (7) isolate from *Echinochloa*; (8) isolate from *Pennisetum*.

few viable conidia were saprophytic and could not be expected to serve as the source of inoculum in the subsequent wheat crop.

Bipolaris sorokiniana is a pathogen of broad host range (Nelson and Kline, 1962; Misra, 1973). Duveiller and Gilchrist (1994) suggested that alternate hosts could act as a green bridge for the survival of *B. sorokiniana* across crop seasons. Rice has also been reported as a host species for the spot blotch and tan spot pathogens in inoculation trials in India (Misra, 1973). In the present study, only three out of 25 plant species associated with rice–wheat cultivation were found to harbour *B. sorokiniana* when investigated in the off-season for wheat. Although *B. sorokiniana* isolates from these hosts were pathogenic to wheat under artificial inoculation conditions, their spread to wheat could not be confirmed under natural conditions. Thus, spread of inoculum from alternate hosts does not appear to be a mechanism for the reappearance of spot blotch in rice-wheat cropping systems after the period of water-logged conditions. RAPD analysis showed that the *B. sorokiniana* isolates from these hosts were not closely related to the wheat isolates and represented separate populations. The five isolates of wheat investigated in this study were obtained after evaluation of large number of isolates collected from five different zones of India (Chand et al., 2003). Even though only a few isolates were studied, these data also support the conclusion that the occurrence of a green bridge is not likely to be responsible for the reappearance of spot blotch in rice–wheat cropping systems of south east Asia.

We have concluded that water-logged conditions in the soil reduce the frequency of viable conidia to an extremely low level. The limited number of conidia (<1%) surviving after flooding carry far less potential to cause an epidemic in comparison to high seed infection (12–86%). The surviving conidia after August were not pathogenic. Thus, these conidia appeared saprophytic and could not be expected to serve as the source of inoculum in the subsequent wheat crop.

Shaner (1981) reported the survival of dormant conidia of *B. sorokiniana* in seed and Ries and Santos (1987) suggested that infected seeds were the only source of inoculum in a new area, even within a season. A small amount of inoculum was sufficient to create an epidemic. In the present study, the percentage infection of *B. sorokiniana* in the seeds of wheat genotypes was high and the occurrence of spot blotch under non-inoculated conditions in the field and poly-house suggested that infected seed is probably the main source of inoculum for the survival and reappearance of spot blotch of wheat in rice–wheat cropping sys-

tems. Although in a developing country like India, the majority of seed used by farmers is their own seed or seed obtained through farmer-to-farmer contact, substantial numbers of farmers purchase seed from distant places which may be several hundreds of kilometers from their villages. Seed produced in the North Eastern Plains Zone of India, which is a hot spot for spot blotch of wheat, is likely to carry a sufficient inoculum load to cause further spread of disease under conducive conditions. This calls for the adoption of suitable measures to prevent the spread of spot blotch infection through seeds. Seed treatment using appropriate chemicals and the sowing of healthy disease-free seed should be practiced for the prevention of this disease.

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References.

Chand R, Pandey SP, Singh HV, Kumar S and Joshi AK (2003) Variability and its probable cause in natural populations of spot blotch pathogen *Bipolaris sorokiniana* of wheat (*T. aestivum* L.) in India. Journal of Plant Disease and Protection 110, 27–35

Chaurasia S, Chand R and Joshi AK (2000) Relative dominance of *Alternaria triticina* Pras. et Pra and *Bipolaris sorokiniana* (Sacc.) Shoemaker, in different growth stages of wheat (*T. aestivum* L.). Journal of Plant Disease and Protection 107: 176–181

Chin SHF and Tinline RD (1963) Spore germinability in soil as an inherent character of *Cochliobolus sativus*. Phytopathology 53: 1109–1112

Duczek LJ (1981) Number and viability of conidia of *Cochliobolus sativus* in soil profiles in summer fallow fields in Saskatchewan. Canadian Journal of Plant Pathology 3: 12–14

Duczek LJ (1986) Populations in Saskatchewan soils of spore-perforating amoebae and an amoeba (*Thecamoeba granifera* s. sp. *minor*) which feeds on hyphae of *Cochliobolus sativus*. Plant and Soil 92: 295–298

Duveiller E and Gilchrist L (1994) Production constraints due to *Bipolaris sorokiniana* in wheat: current situation and future prospects, In: Saunders DA and Halecht GP (eds) Wheat in Heat Stress Environment: In Irrigated Dry Area and Rice–Wheat Farming System (pp 343–352) CIMMYT, Mexico, DF

Duveiller E, Garcia I, Franco J, Toledo J, Crossa J and Lopez F (1998a) Evaluating spot blotch resistance of wheat: Improving disease assessment under controlled conditions and in the field. In: Duvellier E, Dubin HJ, Reeves J and McNab A (eds) *Helminthosporium* Blights of Wheat: Spot Blotch and Tan Spot (pp 171–181) CIMMYT, Mexico, DF

Duveiller E, van Ginkel M and Dubin HJ (1998b) *Helminthosporium* diseases of wheat: Summary of group discussions and recommendations. In: Duvellier E, Dubin HJ, Reeves J and McNab A (eds) *Helminthosporium* Blights of Wheat: Spot Blotch and Tan Spot (pp 1–5) CIMMYT, Mexico, DF

Gomez KA and Gomez AA (1983) Statistical Procedures for Agricultural Research. 2nd edn., Wiley, Las Banos, Philippines

Gower JC (1966) Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 325–338

Hobbs P and Morris M (1996) Meeting South Asia's future food requirements from rice-wheat cropping systems: priority issues facing researchers in the post-Green revolution era. NRG paper 96-01, p 46. CIMMYT, Mexico, DF

International Seed Testing Association (1985) International rules for seed testing. Annexes 1985. Seed Science and Technology 13: 299–355

Joshi AK and Chand R (2002) Variation and inheritance of leaf angle, and its association with spot blotch (*Bipolaris sorokiniana*) severity in wheat (*Triticum aestivum*). Euphytica 124: 283–291

Joshi AK, Chand R and Arun B (2002) Relationship of plant height and days to maturity with resistance to spot blotch in wheat. Euphytica 123: 221–228

Joshi AK, Kumar S, Chand R and Ortiz-Ferrara G (2004a) Inheritance of resistance to spot blotch caused by *Bipolaris sorokiniana* in spring wheat. Plant Breeding 123: 213–219

Joshi AK, Chand R, Kumar S and Singh RP (2004b) Leaf tip necrosis: a phenotypic marker associated with resistance to spot blotch disease in wheat. Crop Science 44: 792–796

Leach J, Lang BR and Yoder DC (1982) Methods for selection of mutants and *in vitro* culture of *Cochliobolus heterostrophus*. Journal of General Microbiology 128: 1719–1729

Ledingham RJ (1970) Survival of *Cochliobolus sativus* conidia in pure culture and in natural soil at different relative humidities. Canadian Journal of Botany 48: 1893–1896

Maraite H, Di Zinno T, Longree H, Daumerie V and Duvellier E (1998) In: Duvellier, E, Dubin HJ, Reeves J and McNab A (eds) Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot (pp 293–300) CIMMYT, Mexico, DF

Mehta YR (1994) Manejo Integrado de Enfermedadas de Trigo-Santa Cruz, Bolivia, 314, CIAT/IAPAR

Misra AP (1973) *Helminthosporium* species occurring in cereals and other Gramineae. U.S.P.L. 480 Project No.A7-CR 133, Grant No. FG-IN-223, Tirhut college of Agriculture, Dholi, Muzaffarpur, Bihar, India. Catholic press, Ranchi, Bihar, India. p 289

Moller EM, Bahnweg G, Sandermann H and Geiger HH (1992) A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies and infected plant tissue. Nucleic Acids Research 20: 6115–6116

Nelson RR and Kline DM (1962) Interspecific variation in pathogenicity in the genus *Helminthosporium* to *Graminacious* species. Phytopathology 52: 1045–1049

Nene YL and Thapliyal PN (1979) Fungicides in Plant Disease Control. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi. pp 35–127

Ries EM and Forcelini CA (1993) Transmissao de *Bipolaris sorkiniana* de sementes para Órgãos radiculares e aéros do trigo. Fitopatologia Brazilaria 18: 76–81

Ries EM and Santos HP (1987) The increased sporulation of *Cochliobolus sativus* on above ground tissue of small grain and its relationship to the origin of inoculum in the soil. Fitopatologia Brazilaria 12: 206–208

Rohlf FJ (1992) NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System Vers 1.70. Exeter software, Setauket, New York, USA

Saari EE (1998) Leaf blight disease and associated soil-borne fungal pathogens of wheat in south and south east Asia. In: Duvellier E, Dubin HJ, Reeves J and McNab A (eds) Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot (pp 37–51) CIMMYT, Mexico, DF

Saari EE and Prescott JM (1975) A scale for appraising the foliar intensity of wheat diseases. Plant Disease Reporter 59: 377–380

Shaner G (1981) Effect of environment on fungal leaf blight of small grains. Annual Review of Phytopathology 19: 273–296

Sivanesan A (1987) Graminicolous species of *Bipolaris, Curvularia, Drechslera, Exerohilum* and their teleomorphs. CAB International Mycological Institute, Mycological Papers No. 158. Wallingford, UK

Sneath PHA and Sokal RR (1973) Numerical Taxonomy. Freeman and Company, San Francisco, USA

Snedecor GW and Cochran WG (1968) Statistical Methods. Iowa State State University Press, USA

Srivastava OP, Luthra JK and Narula PN (1971) Inheritance of seedling resistance to leaf blight of wheat. Indian Journal of Genetics and Plant Breeding 45: 941–943

Steel RGD and Torrie JH (1960) Principles and Procedures of Statistics – With Special Reference to the Biological Sciences. McGraw-Hill Book Company, Inc., New York, USA

Zadoks JC, Chang TT and Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Research 14: 415–421

Zinno TD, Longree H and Maraite H (1998) Diversity of *Pyrenophora tritici-repentis* isolates from warm wheat growing areas: pathogenicity, toxin production and RAPD analysis. In: Duveiller E, Dubin HJ, Reeves J and McNab A (eds) *Helminthosporium* Blights of wheat: Spot blotch and Tan Spot (pp 302–311) CIMMYT, Mexico, DF